TREATMENT STRATEGIES
HEMATOLOGY

Volume 3 Issue 2

- Chronic Lymphocytic Leukaemia
- Chronic Myelogenous Leukaemia
- Haematological Malignancies
- Leukaemia
- Lymphoma
- Myeloma
- Thrombosis
- Veno-occlusive Disease

Articles include:

New Insights into the Biology of Multiple Myeloma Bone Disease and Future Treatment Targets

Primary and Secondary Prophylaxis of Pregnancy-related Venous Thromboembolism

Which is the Objective of CML Treatment in Elderly Patients in the TKI Era? To "Cure" or to "Take Care"?

Includes a Review of the 39th Annual Meeting of the EMBT and the 18th Congress of the EHA

CellGenix

Your Partner for Clinical Cell Therapy

Maximum quality and safety
for your state-of-the-art cell therapy:

CellGro® Serum-free Media
CellGro® GMP Cytokines

- Compliance with international guidelines
- Proven in clinical trials throughout the world
- Expertise of over 15 years in clinical cell therapy

Optimal cell culture of
- Dendritic Cells
- T-Cells
- NK-Cells
- Hematopoietic Stem and Progenitor Cells
- Mesenchymal Stem Cells
- Chondrocytes
- ESC and iPS Cells

CellGenix is the leading manufacturer of high quality GMP reagents for ex vivo cell cultures

Discover more, call us at +49 761 888 89 330
CellGenix GmbH, Germany

CellGenix GmbH · www.cellgenix.com
Am Flughafen 16 | 79108 Freiburg | Germany

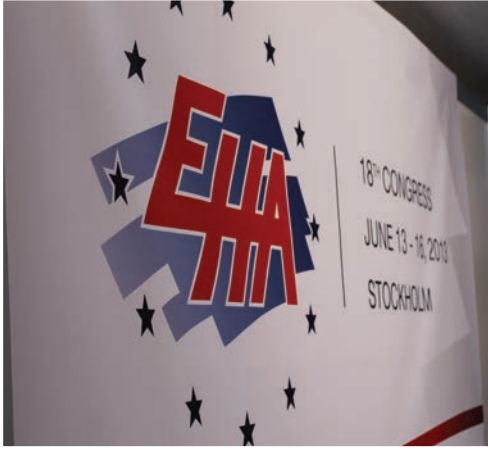
TREATMENT STRATEGIES HEMATOLOGY

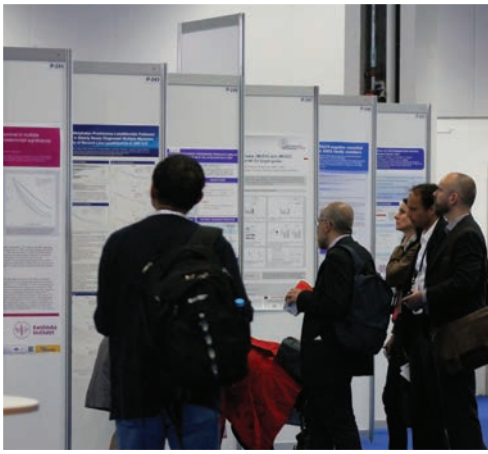
Treatment Strategies - Hematology
The Cambridge Research Centre
Coppergate House
16 Brune Street
London
E1 7NJ

Managing Director **Nigel Lloyd**
nigel@cambridgeresearchcentre.co.uk
Director **Yunus Bhatti**
yunus@cambridgeresearchcentre.co.uk
Publishing Manager **Sara Taheri**
sara@cambridgeresearchcentre.co.uk
Chief Sub-editor **Hannah Corby**
hannah.corby@cambridgeresearchcentre.co.uk
Medical Writer **Vanessa Lane**
Filming **Martin Janes**
video@cambridgeresearchcentre.co.uk
Credit Control Manager **Emma Jones**
emma@cambridgeresearchcentre.co.uk
Accounts **David Mansell**

Published by The Cambridge Research Centre
info@cambridgeresearchcentre.co.uk
www.cambridgeresearchcentre.co.uk
T: +44 (0) 20 7953 8490

Printed by Printech (Europe) Limited





Welcome...

I am delighted to welcome you to the latest edition of *Treatment Strategies – Hematology*. This edition will address the key topical areas in the field of haematology, and features an exciting collection of papers from esteemed haematologists covering subjects such as chronic myelogenous leukaemia, lymphoma, myeloma and thrombosis.

This edition also features two in-depth, independent reviews of the latest developments and key findings from the 39th Annual Meeting of the EBMT, held this year in London, UK, and the 18th Congress of the EHA, held in Stockholm, Sweden.

The EBMT aims to facilitate the sharing of new information and findings in the area of stem cell transplantation. This is certainly achieved at the Annual Meeting, where more than 4,000 participants gather to share their own knowledge and learn from doctors and professionals from all over the world. It is one of the most important events in this area, and we're thrilled to bring you the most important news from the show. The EHA Congress is one of the most important meetings for haematologists in Europe, and attracts over 10,000 participants each year. It has become an unmissable event for discovering the latest techniques, updates and advances in the field, and we aim to bring you the most exciting developments from the show.

We hope that you find that content of this issue informative, and enjoy the range of interesting and thought-provoking perspectives on what is important in the field of haematology today. We would welcome your feedback on this edition as, with your contributions, we will endeavour to ensure that the *Treatment Strategies* series becomes one of the most useful publications in the healthcare industry.

We look forward to joining you next year in Milan for the 40th Annual Meeting of the EBMT and the 19th Congress of the EHA.

Hannah Corby, Chief Sub-editor

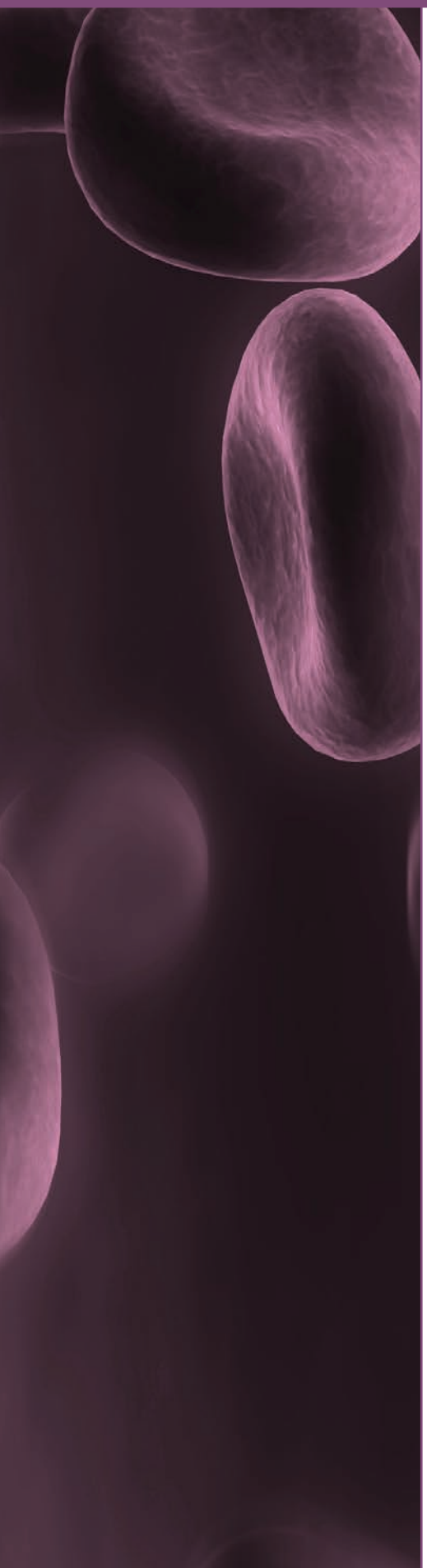
The Cambridge Research Centre wishes to thank the following societies for their assistance and involvement in this publication.

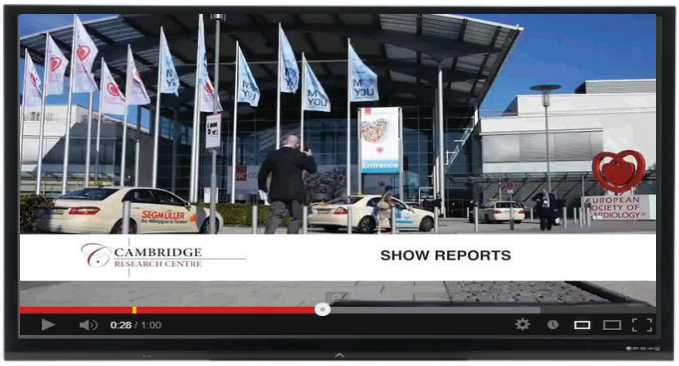
CAMBRIDGE
RESEARCH CENTRE

Sign-up now for our FREE monthly eNewsletter

www.cambridgeresearchcentre.co.uk

CONTENTS...



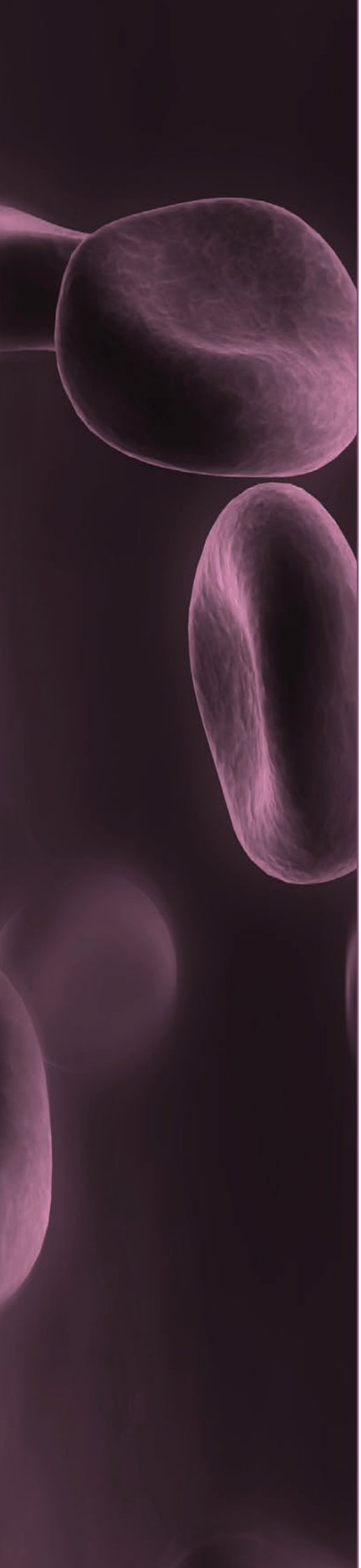


LIGHTS... CAMERA... ACTION!!!

Tune into the latest videos, interviews, symposium reviews, show reports and round-table discussions.

Be sure to look out for our 10 post-congress videos.

WWW.CAMBRIDGERESEARCHCENTRE.CO.UK



EDITORIAL ADVISORY PANEL

Foreword

Anders Waage
Professor and Head of Department, Department of Hematology, St. Olavs Hospital, Norwegian University of Science and Technology and K. G. Jebsen Center for Myeloma Research, Trondheim

Welcome to the latest issue of T*reatment Strategies – Hematology*. T*reatment Strategies - Hematology* invites readers to take a bird's eye view of many aspects of haematology based on detailed basic and clinical documentation, as well as the experience that its contributors have. Strategy is, of course, very different for every disease due to their basic biological characteristics. To be able to be more precise, I will move to discuss the disease that I know best: multiple myeloma. In principle, for 35 years there was only one treatment for multiple myeloma, melphalan-prednisone, until high dose treatment with autologous stem cell support was introduced in the beginning of the 1990s. Since 2000 three new drugs have been introduced: thalidomide, bortezomib and lenalidomide. Seemingly, median overall survival has increased from 3 to 5-6 years during this period. A number of promising drugs are in the pipeline and in 10 years we may have further 5-10 new drugs available. A general evaluation after years of clinical trials and predictions of up and coming drugs is that their efficacy is quite similar, although there are differences regarding side effects. The situation today is that the new drugs are extending survival, although multiple myeloma is still an incurable disease. However, cure is not the only valuable goal for a cancer disease with median age of 70 years. Most patients will be happy to change the course of the cancer disease into a chronic disease with expectation to extend survival with 15-20 years. From this perspective, a substantial improvement has been achieved.

There are two competing treatment strategies for multiple myeloma. Should we combine all drugs in an upfront treatment schedule to achieve the best possible response or should we rely on a sequential schedule, i.e. give one or a few drugs at a time, repeat at relapse and move to the next drug when the patient is resistant to the first drug? The sequential schedule has been the conventional course of action for multiple myeloma as well as CLL and indolent lymphoma. However, there is an emerging attitude that a more AML- or ALL-like treatment schedule (but with other drugs) may also be advantageous for multiple myeloma. If the eventual goal is eradication of the last cancer cell leading to cure, this should be the preferred approach.

However, this dichotomised treatment consideration is too simple, as it has become increasingly evident that myeloma is not one, but many diseases. This can be corroborated by the great diversity within several disease characteristics: clinical behaviour, proliferative index of the myeloma cells, aggressiveness of bone disease, lack of consensus mutations and other molecular events in early stages, extramedullary plasmacytoma versus diffuse intramedullary growth etc. A treatment strategy that truly reflects this variation is lacking today and will be an obvious area of research in the coming years.

Finally, a concern which many fields have in common is that the generation of new drugs is rapidly increasing due to effective tools in molecular biology and high throughput screening methods. The scientific clinical documentation to find the right place for new drugs is a much slower process which lags far behind. Consequently, a queue for clinical documentation is forming. Many clinical researchers as well as authorities feel this pressure. In this situation we should take care not to bypass the scientific documentation.

We hope that you enjoy the latest edition of *Treatment Strategies – Hematology* and the papers that have been included. Haematology is one of the most dynamic areas of medicine, in which new discoveries and developments are constantly being made. We hope that the publication gives an in-depth overview of some of the most important and interesting topics within the field today.

Anders Waage is a professor in Hematology at the Norwegian University of Science and Technology. He is a specialist in Internal Medicine and Hematology and Head of the Department of Hematology, St Olavs Hospital, Trondheim. This clinical practice covers all aspects of haematology, with a special interest in multiple myeloma. This disease has been the main research focus for about 20 years and is now carried out in the newly established K. G. Jebsen Center for Myeloma Research. Dr. Waage has participated in a number of clinical studies focusing on the new emerging drugs used in multiple myeloma, and a long-lasting translational research interest has been Hepatocyte growth factor (HGF), which seems to play a role in several facets of multiple myeloma. He has been chairman in the Nordic Myeloma Study Group and he has been a member of the Board of this organisation for many years. He has also been Chairman in the Norwegian Society of Hematology and a member of the Scientific Advisory Board of EHA.

TREATMENT STRATEGIES
HEALTHCARE PUBLISHER

Visit the publications online and view in our e-Book format

Submit manuscripts to *editor@cambridgeresearchcentre.co.uk*

Advertise your products and services within the Treatment Strategies series and appeal to today's marketplace

All articles included in Treatment Strategies are available as reprints

www.cambridgeresearchcentre.co.uk

EBMT Meeting 2013 Review

07 - 10 April 2013 - London

39th Annual Meeting of the European Group for Blood and Marrow Transplantation

INSIDE...

Hannah Corby, *Treatment Strategies*, reports from the 39th Annual Meeting of EBMT, and brings you the breaking news, research and drug developments from the show. This review also highlights the achievements of several prizes and award winners, and as well as the most important symposia of the event, the results of which will have a direct impact upon clinical practise.

The European Group for Blood and Marrow Transplantation (EBMT) is a non-profit organisation which aims to advance the fields of blood and marrow transplantation and cell therapy worldwide through science, education and advocacy. Indeed, the EBMT Annual Meeting has become the most important annual event for research and education in the field of bone marrow transplantation in Europe. The meeting now attracts over 4,000 participants, including international experts, physicians, clinical research technicians and patients.

The scientific programme opened with the prestigious EBMT lecture, which this year was given by Peter Parham, and was followed by the Van Bekkum Award, which was won by the abstract 'Long-term outcomes after autologous haematopoietic cell transplantation for multiple sclerosis: A joint study from the Center for International Blood and Marrow Research (CIBMTR) and from the European Group for Blood and Marrow

***Introduction** continues on page 12*

ExCel London was the host venue of this year's exciting Meeting.

continued from page 11

Transplantation (EBMT)'.

The traditional joint sessions, held with WMDA, ASBMT and WBMT form a key element of the Meeting, and this year included a celebration of the one millionth transplant, performed in 2013, and a tribute to the legacy of E. Donnall Thomas, who developed bone marrow transplantation as a treatment for leukaemia. Individual sessions were dedicated to the interest of attendees in cell processing and paediatrics on Monday 8th April and Tuesday 9th April respectively.

The Working Party sessions formed the cornerstones of the meeting, and provided delegates with an overview of various study developments and projects. Together with the Meeting chairpersons, active members of the eleven EBMT working parties helped to shape the programme around areas such as acute leukaemia, immunobiology and lymphoma. Oral and poster sessions also offer a furthered opportunity to discuss the findings of EBMT members. The Basic Science Elite sessions were new to the 2013 Meeting, and allowed extra time and discussion to be give to the best of the more scientific submissions.

Controversy sessions also formed an important part of the congress where two opposing arguments were put forward on topics such as 'Chemotherapy versus HSCT in myelfibrosis' and 'Immunotherapy versus HSCT in aplastic anaemia' . Furthermore, Workshop sessions gave attendees the opportunity to discuss technical procedures and clinical management issues such as the ethics of stem cell donation and gene therapy for inmmuno-deficienies and metabolic disease, as well as the opportunity for questions to be put to experts in the field.

The Meeting also had a wealth of symposia, which explored a range of different issues and developments within the field. Key symposia included 'Making personalised cell therapy a reality' and 'Intergrating targeted therapies in the management of haematologica; malignancies – focus on chronic myeloid malignancies.' Plenary sessions also played a key role in proceedings, and were a mixture of clinical management, basic and translational science and health economics. These sessions aimed to challenge delegates current understanding and push the way in which they think about their area of expertise. The scientific programme was also accompanied by specialist meetings of the nurses, data managers, quality managers and statistians, which included a range of relevant topics such as, 'The role of nutrician in HSCT,' 'Complexities of the MED-A' and 'FACT-JACIE standards – current experience and the next editions'.

The Opening Session of the 29th Meeting of the Nurses Group was held on Sunday 7th April 2013. Elisabeth Wallhult, the EBMT Nurses' Group President, welcomed the attendees. During this event the 5th Distinguished Merit Award was presented to Mairead Ni Chonghaile, in recognition for the energy and passion she has shared over the years and is still offering in educating and supporting colleagues, patients and donors of HSCT.

Additionally, the 7th EBMT Patient & Family Day was held on Saturday 6th April with around 200 attendees. The day included a series of patient related educational sessions from experts in the field of bone marrow transplants, as well as workshops and informal discussions. Topics included, life after transplant, what's new in transplantation, state of the art treatment for late effects and how do we find the right donor at the right time.

Awards & Prizes EBMT 2013

Outstanding Achievement Award

Rosi Oneto was honored with the Outstanding Achievement Award for her long-term contributions and support to the EBMT, in particular her efforts in ensuring the success of the Severe Aplastic Anaemia Working Party database.

Basic Science Award Sponsored by Gentium

This award aims to increase the level of basic science and encourage submissions to EBMT by young investigators. It was supported by Gentium with an educational grant of 2,500€.

A Scientific Committee headed by the Chairs of the EBMT Developmental Committee selected the best basic science abstract submitted to the physicians´ programme as the winner of this award.

Abstract title: Engineering a Bone Marrow stem Cell Niche through Endochondral Osification.

Authors: Elia Piccinini, Celeste Scotti, Hitoshi Takizawa, Atanas Todorov, Paul Bourgine, Adam Papadimitropoulos, Andrea Barbero, Markus G. Manz, Ivan Martin (Basel, Zurich, CH).

Van Bekkum Award Sponsored by EBMT

The most prestigious EBMT award, The Van Bekkum Award was presented to the lead author of the best abstract submitted to the physician's programme and was selected by the EBMT Board. The award of 2,500€ was supported by the EBMT.

Abstract title: Long-term Outcomes after Autologous Haematopoietic Cell Transplantation for Multiple Sclerosis: A Joint Study from the Center for International Blood and Marrow Research (CIBMTR) and the European Group for Blood and Marrow Transplantation

Authors: Paulo Muraro, Marcelo Pasquini, Harold Atkins, James Bowen, Dominique Farge, Athanasios Fassas, Mark Freedman, George Georges, Nelson Hamerschlak, Eva Havdrova, Tomas Kozak, Gian Luigi Mancardi, Daniela Morais, Richard Nash, Steve Pavletic, Jian Ouyang, Albert Saiz, Manuela Badoglio, Xiaobo Zhong, Maria Pia Sormani, Riccardo Saccardi

Clinical Service Award Sponsored by EBMT

The Clinical Service Award was awarded to Hele Everaus, Professor of Haematology and Oncology. Her outstanding life-time achievement in EBMT and her major contribution to the training of her colleagues and young medical doctors made her a clear candidate for this award.

Nurses Group Awards and Prizes

Each winner of the Nurses Group Awards received 1,500€.

5th Distinguished Merit Award was presented to Mairead Ni Chonghaile.

Best Poster Prize was awarded to R. Johnston, S. Pike and C. Duran from Great Ormond Street Hospital, London, UK.

Best Oral Presentation was awarded to J. Beckerson, N. Jones, S. Lodhia, K. Montanheiro, J. Pickard, R. Prior, E. Ripley and N. Scott.

Honorary Membership

In recognition of their outstanding contribution in the field of Stem Cell Transplantation and to the EBMT, three new Honorary Members were welcomed by EBMT.

Professors Boris Labar
Shaun McCann
Wolfgang Hinterberger

Jon van Rood Award sponsored by Fresenius Biotech GmbH

This recognises outstanding contributions in the field of haematopoietic transplantation immunology, immunogenetics, tolerance and graft-vs-leukaemia/tumour effects.

Prof. Hans-Jochem Kolb organised the 5th edition of this Award. The winner was selected by a distinguished International panel under the supervision of Prof J.J. van Rood. Fresenius Biotech GmbH generously supported this award with an unrestricted educational grant of 10,000€.

This year, two papers were selected as the winners:

IL-7 and IL-15 Instruct the Generation of Human Memory Stem T Cells from Naive Precursors.
N. Cieri (Milan, IT)

Endogenous HLA Class II Epitopes that are Immunogenic *in vivo* Show Distinct Behavior toward HLA-DM and its Natural Inhibitor HLA-DO.
A.N. Kremer (Leiden, NL)

Nature Publishing Poster Awards Sponsored by Nature Publishing Group

The Nature Publishing Group recognises both the best clinical and the best science poster presentations by physicians. The awards were announced during the Closing Ceremony and given by John Goldman. Each Award is £1,000.

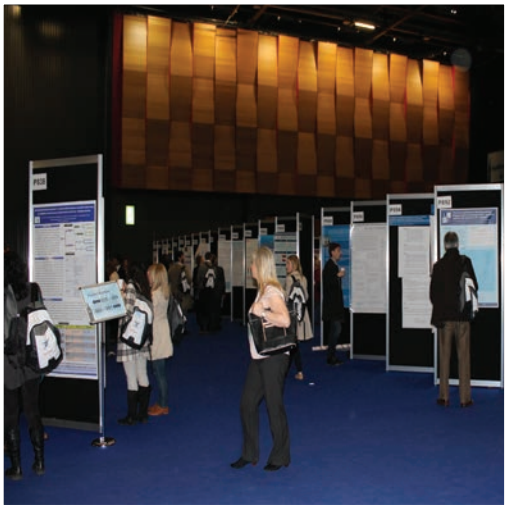
Thomas Luft (Heidelberg, Hannover, DE) was awarded with The Best Clinical Poster Award.

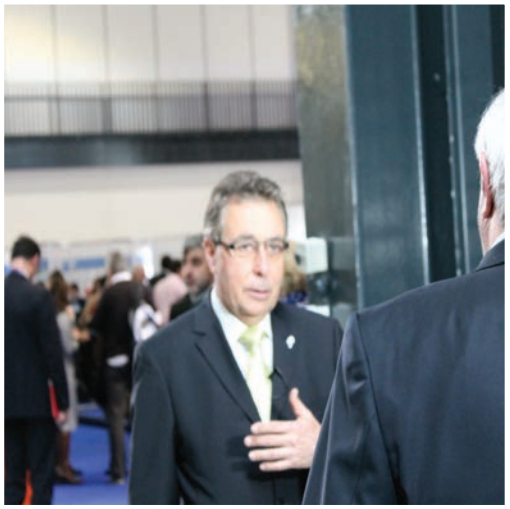
Two Posters were presented with the Best Science Poster Award; Evelyn Ullrich and Lara Crucitti.

Jian-Jian Luan Award for Lymphoma Transplant Research Sponsored by the EBMT LWP

A scientific committee headed by the Chairperson of the EBMT LWP presented the best abstract in the topic of 'lymphoma' with this Award, with 1,000€ donated by the EBMT LWP.

Abstract title: Post-transplantation Cyclophosphamide is Safe and Effective to Prevent Immunological Reaction after Unmanipulated Haploidentical BMT Following Nonmyeloablative Conditioning for Advanced Lymphomas.
Authors: Luca Castagna, Stefania Bramanti, Sabine Fürst, Laura Giordano, Roberto Crocchiolo, Barbara Sarina, Elisa Mauro, Lucio Morabito, Reda Bouabdallah, Monica Balzarotti, Antonella Anastasia, Elisabetta Todisco, Carmelo Carlo Stella, Ercole Brusamolino, Didier Blaise, Armando Santoro (Marseille, FR; Milan, IT)





PATIENT AND FAMILY DAY EBMT LONDON 2013

Date: Saturday, 6th April 2013
Time: 9:00 am
Location: The Mermaid Theatre, Puddle Dock Blackfriars, London

The EBMT Patient and Family Day is an event which is arranged to run just ahead of the main EBMT meeting. Now in its seventh year, this annual event provides an important opportunity for patients, donors and carers, as well as family members, to meet with leading international transplant clinicians.

A series of plenary sessions and workshops were arranged throughout the day which enabled both formal and informal interactions between delegates and speakers. Patients who have had, or expect to have, a bone marrow transplant were given the opportunity to learn about the latest developments in the field, to understand how best to manage any complications and how to adjust to life after transplant. Previous and prospective stem cell donors also had the chance to gain a deeper understanding of the importance of their donation.

EBMT believes that the best way to empower patients is to ensure that they are well-informed. In order to achieve this, EBMT works hard to make this an exciting and educational day that patients and families will enjoy and find beneficial in building relationships of trust and confidence with their own clinical teams.

Patient and Family Day Chair

Dr Maria Gilleece
Consultant Haematologist
Leeds Teaching Hospitals

Organising Committee for Patient & Family Day

Professor Jane F Apperley, Hammersmith Hospital, London.
Jess Ridout, The Anthony Nolan Trust
Richard Davidson, The Anthony Nolan Trust
Tuula Rintala, King's Hospital London
Catherine Foggo, EBMT
Dan Wilde, EBMT

SPECIAL BONE MARROW TRANSPLANT DAY

Taking place on 6th April 2013, and organised by a team from Imperial College, London, EBMT and the Anthony Nolan, this event was supported by a number of blood disease charities. The day gave people with blood disorders the chance to meet other patients, talk to medical experts and hear about the latest developments in blood and marrow transplantation. The event covered topics such as, 'What's new in transplantation', 'Treatment of Graft vs Host Disease' and issues like 'Life after transplant' and 'Fertility and later effects'.

Professor Jane Apperley, President and Chair of the Centre for Haematology at Imperial College, underlined the importance of the event: "These patient and family topics have become an integral part of the EBMT's activities." She added: "I want to thank the organising committee for their outstanding work and our sponsors for their generosity. They have made this day possible."

This event comes soon after the announcement by the WMDA (the World Marrow Donor Association) of the 1 millionth transplant worldwide, a testament to the growing success of blood and marrow transplantation as a potential cure for many diseases. Last year alone, UK charity and donor register Anthony Nolan gave 1,000 people the chance of life through unrelated bone marrow matches.

Alejandro Madrigal, EBMT President and Scientific Director at Anthony Nolan, highlights: "The mission of the EBMT is to save the lives of patients with life-threatening diseases blood diseases by advancing the fields of blood and marrow transplantation and cell therapy worldwide, through science, education and advocacy. We do hope that patients and their families enjoyed this fantastic and informative day."

Maria Gilleece, Chair of the EBMT Patient & Family Day and Consultant Haematologist, Leeds Teaching Hospitals, added: 'Progressive breakthroughs in blood and marrow transplantation over the last forty years, have led to greater numbers of patients undergoing and surviving treatment. This day provided patients with a wonderful opportunity to hear about the latest developments in blood and marrow transplantation - whether the transplant is behind them, or ahead of them, they will have found this day useful. An international panel of specialists talked about how volunteers and family donors are chosen, how a transplant is performed and how to get back to a healthy and great quality of life after transplant. We want to inform and empower our patients so that they can work with their transplant teams to achieve the best possible result.'

Temperature Specialists Planer PLC Showcase New Monitoring Systems at EBMT

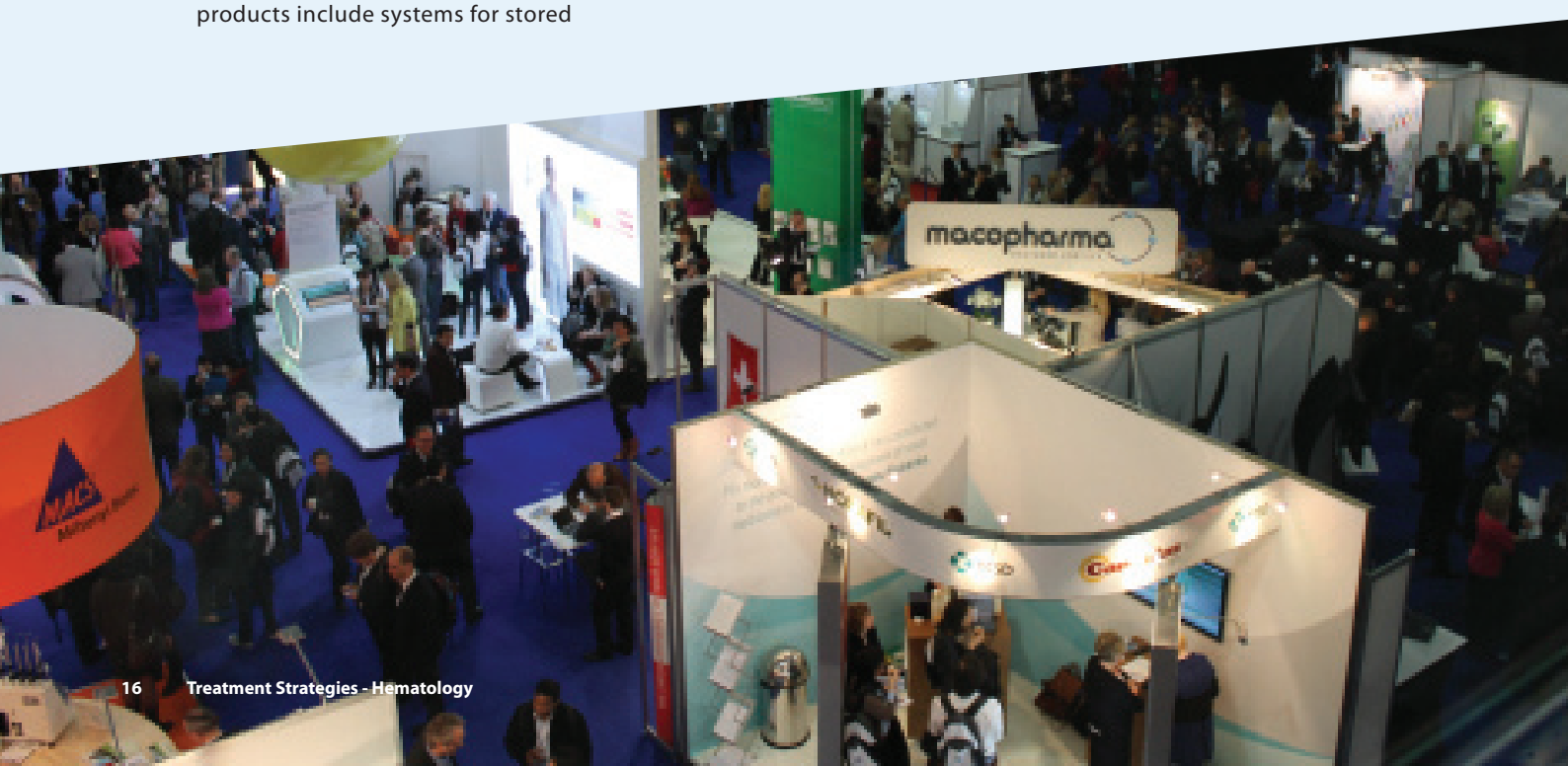
Specialising in the control of temperature and other parameters, Planer PLC help their customers to achieve scientific breakthroughs in biology, medicine and industry. Over the last thirty-five years they have also assisted customers in managing processes, records and data. They pioneered the development and use of many controlled temperature products as well as monitoring and logging of other key lab parameters. Planer hold a number of patents and have received the Queen's Award for Technology and awards from the DTI for Innovation and Good Practice in Microelectronics. The company is also approved and assessed to the demanding standards of medical device manufacture: ISO13485:2003 & Medical Devices Directive, Annex 11 93/42/EEC: LRQA. ISO 9001:2000, ISO13485:2003 and the Medical Devices Directive, Annex 11 93/42/EEC standard.

Customers who depend on the viability of their stored samples use Planer's freezers, incubators and software products: including bench top incubators, programmable freezers, ancillaries and consumables for the storage and preservation of medical and biological specimens - cells, cord blood, bone marrow, embryos, botanical matter, semen, oocytes, seeds, skin, ovarian tissue, heart valves, blood vessels and others. Planer's software products include systems for stored specimen location, laboratory alarm systems, environment control and web based network products for sample and equipment monitoring.

There are users of Planer software and temperature equipment in most developed countries of the world - from Australia, Azerbaijan and Austria through China, Croatia and Cyprus, India, Italy, Japan, Rwanda, Russia, Singapore to the USA, Turkey and Zambia. Planer have produced in excess of 10,000 standard equipments for use around the world. In addition, they have built and installed many large custom made equipments and systems.

Planer exhibited at the 39th Meeting of EBMT and showcased the new DATAcentre monitoring and alarm System, the ReAssure monitoring and logging software suite, controlled rate freezers and their ShipsLog temperature data logger for vapour shippers.

For more information please visit
www.planer.com



The Next Generation Chimerism Analysis for Higher Sensitivity Patients

During this year's EBMT Meeting held in London, Biotype Diagnostic launched a new high-resolution diagnostic strategy for the area of stem cell transplantation and leukaemia diseases. Combining two established methods of DNA-analysis, Biotype has developed a product which allows the detection of only 5 diseased blood cells in 10,000 healthy cells. When compared to the currently used standard methods, this is in accordance with an increased sensitivity by a factor of 20. Physicians may notice signs that signify imminent relapse earlier and, therefore, can immediately interfere therapeutically. Patients will benefit from a considerably better healing opportunity as well, and their health-related quality of life will be increased tremendously.

Biotype's new diagnostics for quantitative chimerism monitoring to assess the risk of threatening relapse is a combine strategy comprising two DNA-based approaches. Mentype® DIPscreen is the multiplex-PCR screening tool developed to identify insertion/deletion DNA-polymorphisms (DIPs/INDELs) that occur individually in donor or recipient, and thus constitute informative loci. A single multiplex-PCR simultaneously screens 33 DIP-markers together with the gender specific locus amelogenin. Representing the entrance of the combined strategy, this approach mediates the switch from conventional PCR methods towards the highly sensitive real-time PCR analysis.

In the results of the screening, Mentype® DIPquant kits quantitatively address identified DIP-loci and represent the quantitative part of the combined strategy when choosing out of a pool of 57 allele-specific real-time PCR (qPCR) monoplex assays. These monoplex-assays were shown to accomplish a detection limit lowered to 0.05% sensitivity. Moreover, all assays were designed to run with the same qPCR parameters to allow parallel analysis of several DNA-loci at one time in one run.

Poster Displays at EBMT 2013.

Combining multiplex-PCR with real-time PCR analysis reflects a comprehensive strategy to reliably and individually monitor the post-transplant progress. As a result, a threatening relapse following stem cell transplantation, which is by means a highly stressful and risky treatment for patients, can be recognised earlier and allows for a fast and targeted therapy.

For more information, please visit:

www.biotype.de

Biosafe Present Multi-centre Cell Washing Poster at EBMT 2013

As in previous years, EBMT 2013 was a success for Biosafe, who presented a poster on Multi-centre Cell Washing at the Meeting.

On 8th April 2013 Dr. Saccardi presented the poster, which summarised the results of a multicentric, prospective trial run in four European Transplant Centres. This trial aimed to assess the safety and reproducibility of the automated, clinical grade washing system SmartWash™.

The Meeting had a host of interesting and informative stands.

Biosafe are convinced that it's important to:
- Reduce cryoprotectant toxicity
- Remove ABO-incompatible RBC stroma and free haemoglobin
- Prevent/Reduce transfusion reactions

Smartwash ™ ensures:
- The removal of cellular debris, DMSO and free haemoglobin
- A high CD34+ recovery
- That the final product remains viable long after washing procedure
- A fully automated and safe procedure

The SmartWash™ protocol is designed for washing peripheral blood stem cells, specifically the automated washing of thawed leukaphaeresis products. Typically, the protocol washes out 90% of DMSO and free haemoglobin. The cellular fraction is collected in a transfer bag ready for transplantation. The washed cells are viable long after the washing procedure, allowing further processing with no time constraints.

The thawed product is first diluted in a washing buffer solution, then centrifuged and finally re-suspended in fresh medium. The protocol allows the processing of thawed units from 50 ml to 880 ml with an output volume between 50 ml and 200 ml. Up to 4 procedures can be performed sequentially with the same separation.

The poster concluded that the SmartWash™ protocol is an efficient and safe automated method to wash thawed PBSC grafts in a closed, clinical- grade system. Benefits for clinical staff include a lower frequency of infusion-associated adverse events as well as a reduced burden as compared to management of thawed products at the bedside.

For more information, please visit:

www.biosafe.ch

Satellite Symposia at the 39th Meeting of the European Group for Blood and Marrow Transportation

Technology Meets Biology in the Fight Against ALL and Other Haematological Malignancies

David Marks (University Hospitals Bristol, UK) chaired the Amgen sponsored satellite symposium at the 39th Annual Meeting of EBMT. This symposium explored bilogicals and biosimilars and their potential impact upon haematology and transplant practice today and in the future.

The symposium "Technology meets biology in the fight against ALL and other haematological malignancies" began with the Chair's introduction from David Marks. The focus was on the changing landscape of haematological malignancy treatment and the potential role of innovative technology in the treatment paradigm.

In the first session, "Biologicals, biosimilars and the changing landscape of therapy", Professor Hakan Mellstedt (Karolinska University Hospital, Sweden) focused on and explored the evolution of biologicals and the potential role of biosimilars in haematology.

In the session "Current challenges in ALL management and innovative therapies of the future" David Marks discussed the potential of new agents in this challenging malignancy.

Before the Chair's summary in the closing session, Professor Max Topp (University of Würzburg, Germany) gave a presentation entitled "Blinatumomab: clinical development programme in ALL". This examined the mode of action, current clinical data and future potential of the BiTE antibody, blinatumomab in the treatment of haematological diseases and specifically, ALL.

Managing Infectious Complications in HSCT Recipients

Astellas Pharma Europe Ltd attended the 39th Annual Meeting of EBMT in London and ran an educational symposium chaired by Peter Donnelley of the Radbourg University Nijmegen, The Netherlands. Entitled "Managing infectious complications in HSCT Recipients", the symposium was held on Sunday 7th April 2013.

Peter Donnelley began the symposium with the Chair's welcome and an introduction. This was followed by the first presentation given by Dragana Milojkovic (UK). In this presentation the burden of Clostridium difficile infection (CDI) was highlighted, and the risk factors for CDI in HSCT recipients and its clinical applications were outlined. It also discussed the limitations of current therapeutic options and the need for new management strategies to reduce the risk of recurrence, which remains the key challenge in managing CDI. An update on the clinical trials of treatments for CDI in immunosuppressed adults was also provided.

The topic of the second presentation was the importance of early management of invasive fungal infections in haematology patients with an update on management guidelines in this setting. Presented by Michael von Bergwelt-Baildon (Germany), who discussed patient specific issues including approaches to maintain effective antifungal prophylaxis in patients unable to rely on oral azole agents, due to severe mucositis or concerns relating to drug-drug interactions.

Finally, Paul Griffiths highlighted the burden of Cytomegalovirus (CMV) in the HSCT population. Discussions included the limitations of existing disease management strategies, the need for effective preventative measures and the rationale behind CMV vaccine development.

A Practical Guide to Antifungal Decision Making: Diagnosing Fungal Disease in Stem Cell Transplant

With the support of Gilead, the Continuing Antifungal Research & Education (CARE) steering committee presented a satellite symposium on "A practical guide to antifungal decision making: diagnosing fungal disease in stem cell transplant patients" with a focus on how to best use fungal diagnostic tools to inform the management of stem cell transplant patients.

On Sunday 7th April Catherine Cordonnier opened with the Chair's welcome speech. Here she explained that the CARE programme aims to provide specialists who treat fungal disease with an interactive forum where they can share invaluable insights, opinions and experiences.

In "Introducing the lab – what it can do for you. How can therapies affect diagnostics?", Paul Verweij provided an overview of the tools used for diagnosing invasive fungal disease. This was followed by the second presentation "State-of-the-art diagnostics in haematology", by Johan Maertens. In this session the current thinking on best practice was discussed, illustrating when diagnostics should be used and how they can contribute to clinical decision making. These principles were then applied to two interactive case discussions.

Marcio Nucci, presented the final session "How do diagnostics affect therapeutics? What to do if diagnostics are inconclusive."

Defibrotide and Veno-Occlusive Disease

At the 39th Annual Meeting of the European Group for Blood and Marrow Transplantation and the 29th Annual Nurse Group Meeting of the EBMT Gentium's lead product, Defibrotide, was the subject of two symposia.

Together Professor Selim Corbacioglu (Children's Hospital Regensburg, Germany) and Professor Tony Pagliuca (King's College Hospital, London) chaired the symposia held on the 8th April entitled "Understanding and managing veno-occlusive disease (VOD)". This covered VOD's pathophysiology and case studies as well as the latest clinical information and cost-effectiveness of Defibrotide.

Dr. Enric Carreras' (Hospital Clinic Barcelona, Spain), presentation was entitled "Understanding the pathophysiology of VOD and the importance of the endothelium". Dr. Carreras presented results of *ex vivo* and *in vitro* studies exploring the disease's pathogenesis and Defibrotide's possible mode of action in preventing endothelial damage.

Possible points for intervention in VOD and the need to move beyond purely supportive care were highlighted in Dr. Paul Richardson's (Harvard Medical School; Dana Farber CancerInstitute, USA) presentation entitled "Protecting the endothelium and restoring the thromboticfibrinolytic balance in VOD to improve patients outcomes". Dr. Richardson also provided an update on Defibrotide's clinical activity.

In his presentation, "Active management of VOD can be cost-effective and affordable," Professor Pagliuca outlined the high cost of post-transplant complications, and explained how Defibrotide's clinical effectiveness translates into practical cost-effectiveness in this setting.

To close the session, Professor Corbacioglu and Dr. Victor Noriega (King's College Hospital, London) presented VOD case studies reviewing the risk factors and indications for prevention and also the importance of early diagnosis and treatment.

The 9th April Nurses symposia, "Veno-occlusive disease: a significant and potentially life-threatening complication of haematopietic stem cell transplantation," was chaired by Elisabeth Wallhult (President of the EBMT Nurses Group, Sahlgrenska University Hospital, Sweden). The focus of this symposia was on how nurses can best identify and then support patients with VOD.

In "VOD: a potentially life-threatening early complication of HSCT", Dr. Sarah Marktel (San Raffaele Scientific Institute, Italy) discussed the disease's clinical presentation, classification, risk factors, and treatment options.

"The importance of active management of patients with VOD ," was the title of Jennifer Cooper's (The Great North Children's Hospital, Royal Victoria Infirmary, Newcastle upon Tyne, England) presentation. In this presentation the nursing assessment and nursing intervention for VOD patients in her centre was also explained.

Elisabeth Wallhult and Jennifer Cooper brought the central themes together in the final presentation of this symposia. They presented case studies about the challenges and importance of early VOD diagnosis, and the role that Defibrotide can play in the prevention and treatment of this disease.

Improving Long-Term Outcomes and Managing Complications in APL - From Clinical Trials to Clinical Practice

Francesco Lo Coco (University Hospital Tor Vergata, Italy) chaired Teva's satellite symposium "Improving long-term outcomes and managing complications in acute promyelocytic leukaemia (APL) - from clinical trials to clinical practice" at the EBMT Meeting on Sunday 7th April. He went on to consider whether it is possible to further improve the success in the first-line treatment of APL.

This was followed by a presentation from Miguel Sanz (University Hospital La Fe, Spain) on how to select the appropriate treatment for patients who do relapse.

Michael Schmitt (University Clinic and University of Heidelberg, Germany) then discussed the role of biosimilar granulating colony-stimulating factor (G_CSF) in stem cell mobilisation in patients undergoing stem cell transplantation (SCT).

In the final presentation of the symposium, Javier Lopez (University Hsopital Ramon y Cajal, Spain) suggested ways in which strategies for preventing and treating invasive fungal infections can be optimised.

By transferring some of the lessons learned from the results of clinical trials into daily clinical practice it is hoped that long-term outcomes in APL can be improved and better management of complications can be achieved.

Integrating the New Targeted Therapy ADCETRIS in the Management of Relapsed/Refractory CD30+ Lymphomas

On Sunday 7th April, Graham Collins opened this symposium with a welcome speech for Takeda pharmaceuticals International GmBH.

Despite good advances in front-line management, the prognosis for patients with relapse/refractory (R/R) Hodgkin lymphoma (HL) and R/R systemic anaplastic large cell (sALCL) can be poor. Autologus and allogeneic stem cell transplantation (ASTC; allo-SCT) can play a role in management but do not always lead to a long-term disease-free future.

Providing effective therapies for patients who relapse following transplant, or who are ineligible or unsuited for transplant remains a clinical challenge.

Therapies that target cell surface receptors - such as CD30 - are an emerging class with the potential to greatly improve patient outcomes. This symposium reviewed current therapies and treatment guidelines, highlighting the unmet needs in R/R HL and R/R sALCL, as well as the latest clinical data for brentuximab vedotin in post-transplant and transplant-naive/ ineligible patients with R/R HL or R/R sALCL.

Chimerix Presents at EBMT: Antiviral Activity of CMX001 in Patients With Life-Threatening Viral Infections

CMX001 demonstrated positive clinical activity against life threatening adenovirus infection in patients enrolled in the CMX001 Compassionate Use Program.

A significant proportion of patients in the CMX001 Compassionate Use Program had evidence of active infection with more than one virus.

Chimerix, Inc., a biopharmaceutical company developing novel, oral antivirals in areas of high unmet medical need, presented the first clinical outcomes data on the use of CMX001 in patients with life-threatening infection with adenovirus (AdV), a disease with no approved therapy at the 39th Annual Meeting of European Group for Blood and Marrow Transplantation's (EBMT). CMX001 is an investigational oral nucleotide analog lipid-conjugate that has a broad spectrum of antiviral activity against double-stranded DNA (dsDNA) viruses.

These data were presented during the infectious diseases oral session on Wednesday, 10th April, and the infectious diseases poster session on Tuesday, 9th April.The presentations included:

• CMX001 is a potential treatment for AdV infection: Preliminary antiviral activity results from an open label, expanded access study of CMX001 for the treatment of serious or life-threatening diseases caused by dsDNA viruses.

This presentation featured preliminary clinical and viral outcomes data from 57 patients infected with AdV who were treated with CMX001 in an open label, expanded access study. Patients received oral CMX001 twice weekly. The median duration of CMX001 treatment was 12 doses over seven weeks, with the longest duration of treatment of 43 weeks. The level of AdV in the patient's blood was measured at the beginning of therapy, regularly during the treatment period, and at one and four weeks post-treatment. Thirty-four of the 57 patients (79%) had at least a 90% decrease in the amount of virus in the blood at the end of treatment. Patients who had a 90% decrease in AdV blood levels had overall better outcomes, with 9% AdV-associated deaths, compared to a 27% mortality rate for patients who had less than a 90% decrease in viral levels. Patients who began CMX001 therapy earlier in the AdV infection, as soon as virus was detected in the blood and before symptoms were present, had a lower mortality rate of less than 7%, while patients who were treated after virus was detected in the blood and symptoms had developed had a mortality rate of 37% during the one-month follow-up period. Untreated AdV infections in the respiratory tract can have a mortality rate of 80% in children who have received a bone marrow or stem cell transplant.

There are currently no approved therapies for AdV infection. CMX001 is in a Phase 2 study as an early treatment for AdV infection in young adults and children who have received a stem cell or bone marrow transplant.

• Demographic/baseline characteristics of patients treated with CMX001 for serious or life threatening dsDNA virus infections: predictors of multiple dsDNA virus infections.

Demographic and baseline characteristics from 320 patients enrolled in the CMX001 Compassionate Use Program were analysed using a logistic regression model. To qualify for the programme, patients must have had a life-threatening infection with at least one dsDNA virus. This analysis assessed the predictability of demographic and baseline characteristics (gender, age, race/ethnicity, transplant type) on specific viral infections and the likelihood of having more than one viral infection. Approximately one-third of patients enrolled in the Compassionate Use Program had evidence of more than one active infection with a dsDNA virus. The most common co-incident infections in the same patient were CMV and BK virus (BKV), AdV and BKV, and AdV and CMV. Patients enrolled following stem cell transplant, particularly children, had an increased likelihood of multiple dsDNA virus infections. The potential of CMX001 as a broad-spectrum prevention for multiple dsDNA viral infections will be explored in a large Phase 3 study beginning in mid-2013.

"These data from our Compassionate Use Program show that CMX001 has the potential to treat life-threatening infections with AdV, a disease which can be 80% fatal in young adults and children who have undergone a stem cell or bone marrow transplant. CMX001 is being developed as a potential prevention or early treatment for AdV and other viral infections such as cytomegalovirus (CMV), which threaten patients with a weakened immune system. CMX001 is the only investigational antiviral with the potential to treat or prevent viral infections caused by several dsDNA viruses."

M. Michelle Berrey, MD, MPH, Chief Medical Officer of Chimerix

40th EBMT Annual Meeting

30 March - 2 April 2014
Milan, Italy

EBMT invite you to the 40th EBMT Annual Meeting, including the 30th Meeting of the EBMT Nurses Group, the 13th Meeting of the EBMT Data Management Group, the 6th Meeting of the Quality Management Group, and the 8th EBMT Patient & Family Day, which in turn will incorporate the 1st Donor Day.

TREATMENT STRATEGIES SERIES

Visit the publications online to view our eBooks, subscribe to the series and e-mail the papers in PDF format.

Submit your papers to our forthcoming publications within the series - AIDS, Cardiology, Diabetes, Gastroenterology, Oncology, Paediatrics, Respiratory, Dermatology, Hematology and Interventional Cardiology.
E-mail: editor@cambridgeresearchcentre.co.uk

All articles included in Treatment Strategies are available as reprints.
E-mail: reprints@cambridgeresearchcentre.co.uk

Advertise your products and services within the Treatment Strategies series and appeal to today's marketplace. We provide an excellent base for advertising to targeted key audiences.
E-mail: sales@cambridgeresearchcentre.co.uk

www.cambridgeresearchcentre.co.uk

18th Congress of EHA Review

13 - 16 June 2013 - Stockholm, Sweden

EHA Congress - Europe's Leading Haematology Congress

INSIDE...

Sara Taheri, *Treatment Strategies*, takes an in-depth look over the 18th Congress of EHA, highlighting the most important findings and products, breaking news, symposia, awards and more. This review aims to deliver the key messages and news stories from the event, and will also cover important sessions, the content of which will directly influence and aid in the development of clinical practise.

As Europe's leading haematology meeting for laboratory and clinical haematologists, the EHA congress attracts participants from European and non-European countries alike, and over the years the number of attendees has swelled. At the 18th Congress of the EHA, around 9000 participants and 66 exhibiting companies were in attendence.

The Congress aims to improve patient care by supporting the development of research and education, and aims to promote research in the field of haematology. Various educational activities were available at the Congress including scientific workshops, symposia, round-table discussions, tutorials, distance learning tools and blended learning in the new EHA Master Class. The EHA has also recently revised, and has now published, their European Hematology Curriculum, the CV Passport, and a mobility tool for trainees, which forms the core of their expanding education. The topics offered range from stem cell physiology and development, to leukaemia, lymphoma, diagnosis and treatment, red blood cells, white blood cells and platelet disorders, haemophilia and myeloma, thrombosis and bleeding disorders, transfusion and stem cell transplantation.

A novel feature of this year's congress is the addition of a dedicated advocacy track, which combined all of the patient and policy related sessions into one full day on Saturday, 15th June. Some of the topics that were discussed in these sessions included; Patients' involvement as partners in clinical research; explaining unavailability of treatment and Personalised medicine.

In June 2012, the EHA introduced an annual theme to link all of their initiatives on a specific item. These would be at the heart of their publications, the EHA Newsletter and

This year's EHA meeting was held in Stockholm, Sweden.

the EHA Congress with a special Scientific Working Group Session and Patient Advocacy Session.

The theme for June 2013 - June 2014 is dedicated to "Age and Aging in Blood Disorders" and throughout the year various aspects of this broad theme will be debated and reported on. This issue has various and far reaching implications, which will only increase with time as the general population begins to live longer and longer. As a common issue in today's society this theme, "Age and Aging in Blood Disorders" is worthy of further research.

For the 18th Annual Congress of the EHA, an extensive selection of sessions were developed by the Scientific Program Committee under the leadership of Jorge Sierra, most notably the Updates-in-Hematology format, which was presented on Wednesday 12th June 2013. This format included three programmes organised by pharmaceutical and medical educational companies, which focussed upon medical education and updated delegates on the recent advances in diagnosis and treatment in different areas of haematology; chronic myeloid leukaemia, myeloproliferativeneoplasms and myelodysplastic syndromes. These sessions were set in a more intimate setting, which allowed optimal discussion and debate.

During the Opening Ceremony on Friday, 14th June, Professor Dr Klaus Rajewsky was announced as the winner of the this year's José Carreras Award, which was presented to him in the José Carreras Lecture.

Additionally, Professor Tiziano Barbui was awarded with the 6th EHA Jean Bernard Lifetime Achievement Award for his outstanding contributions to the advancement of haematology.

The Plenary Sessions included some exciting presentations, including 'Natural killer cells: From biology to clinical use' and 'Splicing mutations in hematology: Influence on prognosis and risk stratification', and on Saturday, 15th June the best of the abstracts submitted for the Congress were presented.

The congress also provided some specialist sessions and events for haematologists in training. The Early Career Club was a lounge where these individuals could access information about EHA's programs, use computer facilities and relax. On Thursday, 13th June the newly formatted annual Early Career Reception was hosted. On Friday and Saturday, 14th and 15th June, two special Early Career Sessions were held which focused on career issues and innovation in clinical trial design.

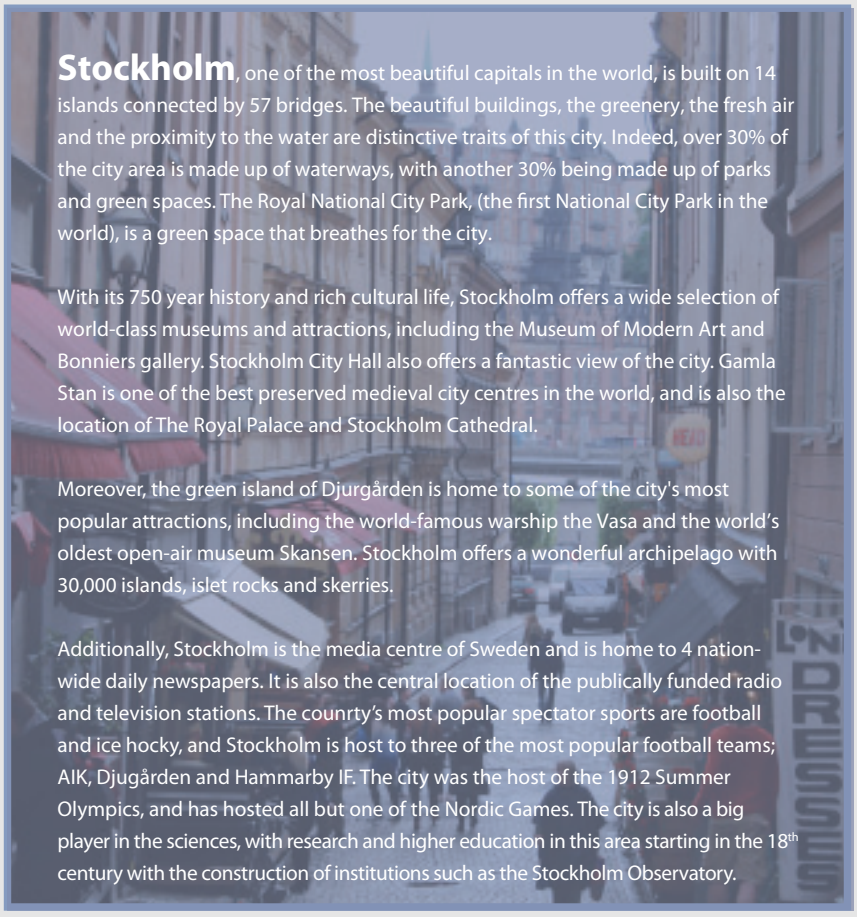
Additionally, the Scientific Working Group sessions took place on Thursday, 13th June and highlighted new research advances within the society. This year the 'Red cells and iron' and the 'European Research Initiative on CLL' Scientific Working Groups were selected to organise the Scientific Working Group 'Hematology-in-Focus' sessions, which concentrated on hot scientific or clinical topics.

Highlights of the Scientific Working Group sessions included the acknowledgement of work by Doctors John B. Gurdon and Professer Shinya Yamanaka, the winners of the 2013 Nobel Prize for Medicine or Physiology by the Scientific Working Group for Stem Cells.

Awards presented at this year's EHA Congress

José Carreras Award
Professor Dr Klaus Rajewsky

Jean Bernard Lifetime Achievement Award
Professor Tiziano Barbui



Stockholm, one of the most beautiful capitals in the world, is built on 14 islands connected by 57 bridges. The beautiful buildings, the greenery, the fresh air and the proximity to the water are distinctive traits of this city. Indeed, over 30% of the city area is made up of waterways, with another 30% being made up of parks and green spaces. The Royal National City Park, (the first National City Park in the world), is a green space that breathes for the city.

With its 750 year history and rich cultural life, Stockholm offers a wide selection of world-class museums and attractions, including the Museum of Modern Art and Bonniers gallery. Stockholm City Hall also offers a fantastic view of the city. Gamla Stan is one of the best preserved medieval city centres in the world, and is also the location of The Royal Palace and Stockholm Cathedral.

Moreover, the green island of Djurgården is home to some of the city's most popular attractions, including the world-famous warship the Vasa and the world's oldest open-air museum Skansen. Stockholm offers a wonderful archipelago with 30,000 islands, islet rocks and skerries.

Additionally, Stockholm is the media centre of Sweden and is home to 4 nation-wide daily newspapers. It is also the central location of the publically funded radio and television stations. The counrty's most popular spectator sports are football and ice hocky, and Stockholm is host to three of the most popular football teams; AIK, Djugården and Hammarby IF. The city was the host of the 1912 Summer Olympics, and has hosted all but one of the Nordic Games. The city is also a big player in the sciences, with research and higher education in this area starting in the 18th century with the construction of institutions such as the Stockholm Observatory.

EHA 2013 - STOCKHOLM

MARCELL at the EHA 2013

Since it was founded over 20 years ago, the Congress of the European Hematology Association (EHA) has been an important date for European doctors, specialists and scientists working in the area of haematology. At this year's annual Congress, PENTAG Informatik AG presented the current version of MARCELL (Management And Resource System for Stem CELL therapy).

MARCELL brings savings potential to stem cell transplantation programmes. With its integrated ISBT 128-Code, JACIE 5 compatibility and the individual adaptability of the software combined with PENTAG Informatik AG's special emphasis on customer service, MARCELL is a very interesting solution for hospitals and clinics throughout Europe.

MARCELL is a complete software solution covering all phases of stem cell transplantation. It allows centres for stem cell transplantation to carry out data capture and quality control according to JACIE standards. With this software solution, data is held centrally so that it can be used in all phases of stem cell transplantation as well as in the creation of MED-A forms and statistics.

MARCELL shows each individual phase in a workflow, guiding clinicians through the different steps. Phases are divided into tasks, and each step can only be performed once the authorised person has released it for processing. The patient's current therapy status is available at a glance. MARCELL is a modular system which makes it possible to acquire additional modules based on your individual needs.

MARCELL is a web-based solution, which means that software can be accessed regardless of location and doesn't have to be installed on designated computers.

MARCELL - Performance Characteristics

- Quality assurance in accordance with JACIE
- Central data storage
- Up-to-date information accessible from anywhere at any time
- No workflow interruption between paper and digital data
- No redundant data copying
- Error reduction due to input validation
- Quick overview
- Simple analysis
- Seamless tracking of input
- Intuitive user guidance

MARCELL - Basic Design Features

- Release through digital signature
- Storing/saving of pre-defined treatment protocols
- Historicisation of data
- Storage of electronic documents and patient's treatment data
- Document generation using templates and relevant data
- Access authorisation for users/groups
- Single Sign-on

EHA Honorary Awards at the 18th Congress of EHA

Stockholm, 14th June 2013. At the 18th Congress of EHA in Stockholm, the EHA Jean Bernard Lifetime Achievement Award was handed to Professor Tiziano Barbui and the EHA Josè Carreras Award to Professor Klaus Rajewsky.

This year, the EHA Board was pleased to award the 6th EHA Jean Bernard Lifetime Achievement Award to Professor Tiziano Barbui. This prestigious award has been established to honour outstanding physicians and scientists for their lifetime contribution to haematology, and was presented to Professor Barbui at the 18th Congress of EHA.

Tiziano Barbui graduated at the University of Padua, Italy, where he later became Professor of Hematology. He was also a Consultant in Hematology at San Bortolo Hospital in Vicenza and founded the Department of Hematology at the Ospedali Riuniti di Bergamo, where he was Director from 1981 to 2008. Professor Barbui is currently the Scientific Director of the Research Foundation at Ospedali Riuniti di Bergamo, Italy.

Tiziano Barbui has also served as President of the Italian Society of Hematology and as Chairman of the Subcommittee on Lupus Anticoagulant of the International Society of Thrombosis and Haemostasis. Professor Barbui leads the European-Leukemia-Net WP-9 on Myeloproliferative Disorders and the Italian GIMEMA group on Philadelphia negative myeloid neoplasms, and is one of the founders of the NIH Myeloproliferative Disorders Consortium.

Tiziano Barbui is also a member of the EHA Governance Committee, and has been principal investigator for several academic clinical trials. Professor Tiziano Barbui has published more than

700 scientific articles in international peer reviewed journals (total citations 18194, h-index 68) and is among the top 50 Italian scientists recognised by the Virtual Italian Academy. He has contributed to many fields of haematology, but is particularly known for his work on the diagnosis, prognosis and treatment of myeloproliferative neoplasms and lupus anticoagulant.

The EHA Board has selected Professor Dr Klaus Rajewky for the Josè Carreras Lecture at the 18th Congress in Stockholm. Klaus Rajewsky is a German immunologist, renowned for his work on B- cells.

Professor Dr Klaus Rajewky studied medicine in Frankfurt, Munich and at the Pasteur Institute, Paris. In 1964, he started working at the Institute of Genetics in the University of Cologne, and here he became Professor for Genetics.

He has researched Hodgkin disease and the role of B-cells within the immune system. He also developed conditional knockout mice based on Cre-Lox recombination. He is one of the founding fathers of the German Society for Immunology (1967). Since 1994, he has been a member of the United States National Academy of Sciences. From 1995 to 2001 he was head of the Monterontondo station of the European Molecular Biology Laboratory near Rome.

In 1996, he was awarded the Robert Koch Prize, which he shared with Fritz Melchers. In 1998, together with Christiane Nüsslein-Volhard and Peter Stadler, he founded a company whose activities are dedicated to the provision of transgenic mouse generation services, tools and related licenses to both the industrial and academic community.

"The European Hematology association (EHA) aims to promote excellence in clinical practice, research and education in European haematology"

In 2001, Professor Dr Klaus Rajewky started working at the Center for Blood Research at Harvard Medical School, Boston, where an additional focus of his work concerns RNAi, especially icroRNAs, in conjunction with immune development and control. Since the start of 2012 he has worked at Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin, Germany.

The Wallace H Coulter Foundation at the EHA 2013 in Stockholm

Since 2010, the European Hematology Association (EHA) and the Wallace H Coulter Foundation (WHCF) have been in a close partnership, in which EHA is the beneficiary of generous donations for its projects. The support of the WHCF has been the prime mover for the setup of the EHA-ASH Translational Research Training in Hematology (TRTH) programme and an important contributor towards the vast expansion of EHA's outreach programme. Both programmes have had a major impact on the Association's activities and global network.

The importance of the achievements of Wallace Coulter, the inventor of the Coulter Counter - the most valuable tool in the haematology laboratory, as a person and his organisation cannot be underestimated for the haematological community as well as for EHA due to the Foundation's support. EHA is glad to be able to pay tribute and commemorate the 100th birthday of Wallace Coulter.

The EHA-theme of the year (June 2013-June 2014) is dedicated to "Age and Aging in Blood Disorders". This theme encompasses not only the aging of patients with blood disorders but also the concept of the aging of cells. Multiple aspects of the theme will be reported on and debated throughout the year. In particular, the definition of "who is old?", as patients may not have the same thresholds as clinicians.

For the first time, the EHA congress programme is complemented by a dedicated advocacy track, which combines all patient and policy related sessions into one comprehensive full-day programme on Saturday, 15th June. Topics that will be discussed in different sessions include patients' involvement as partners in clinical research, personalised medicine and delivering healthcare in times of economic crises.

Addition of Obinutuzumab or Rituximab to Chlorambucil Improves Outcomes for Patients with CLL and Comorbidity

Dr . Valentin Goede presented promising outcomes for elderly CLL patients at the 18th Congress of the European Hematology Association in Stockholm.

CLL is the most common leukaemia in the western world. Many CLL patients are elderly and have comorbidities rendering them ineligible for aggressive standard treatments.

One of the main unresolved questions is whether (i) combination treatment with the chemotherapeutic drug chlorambucil plus a monoclonal CD20 antibody improves outcomes for such patients compared to treatment with chlorambucil alone and (ii) these patients benefit from the use of the novel CD20 antibody obinutuzumab (GA101) relative to the approved CD20 antibody rituximab. For the first time, the CLL11 trial conducted by the German CLL Study Group (GCLLSG) in collaboration with Hoffmann-La Roche directly compares the following treatments in elderly patients with comorbidities: GA101 plus chlorambucil, rituximab plus chlorambucil, and chlorambucil alone. Addition of GA101 or rituximab to chlorambucil both significantly increased the length of time people lived without their disease worsening (progression-free survival) vs. chlorambucil alone. Both combination treatments showed an acceptable safety profile in the investigated patient population.

These findings are a significant step in improving the treatment of elderly CLL patients with comorbidities (even if the results of the direct comparison of the two antibodies will be available only later during the trial).

Merus Presents Preclinical Data on its Novel Bispecific Antibody at EHA 2013

Merus B.V., a biopharmaceutical company focusing upon innovative human antibody therapeutics, presented preclinical data on its antibody MCLA-117 at the Annual 18th Congress of the EHA 2013 in Stockholm. The compound is being developed for the treatment of acute myeloid leukaemia (AML), a disease with very poor long-term prognosis.

MCLA-117 activates the patient's own immune system by simultaneously binding to the CLEC12A molecule expressed by AML tumour cells and the CD3 molecule expressed by T-cells. CLEC12A is a myeloid differentiation antigen that is expressed on 90-95% of *de novo* and relapsed AML cases and is selectively expressed on leukaemic stem cells. Co-incubation of patients' resting T-cells and AML tumour cells via MCLA-117 resulted in efficient tumour cell lysis, i.e. the potent killing of cancerous AML cells. By introducing mutations in the heavy chain constant region CH2 domain, Merus was able to develop an antibody that in peripheral blood mononuclear cell (PBMC) assays prevented the release of non-specific, pro-inflammatory cytokines, while retaining its full capacity to induce T cell-mediated elimination of AML tumour cells.

The MCLA-117 antibody is based on Merus' proprietary Biclonics™ ENGAGE platform. Human bispecific antibodies from this platform can be manufactured and administered like conventional, full-length IgG molecules, thereby providing for high yield, good stability and a long serum half-life.

"The data we have generated with MCLA-117 so far clearly show that the compound can efficiently eradicate AML tumour cells. Most importantly, the compound can also destroy tumour-inducing cancer stem cells, which are the main cause for relapse," said Lex Bakker, Chief Development Officer of Merus. "In addition, we have designed MCLA-117 to prevent undesired side-effects triggered by cytokine release, which makes it a safe candidate for potent, targeted cancer therapy."

"Based on the favourable research and preclinical data, we are looking forward to moving MCLA-117 into clinical development next year," added Ton Logtenberg, Chief Executive Officer of Merus. "As MCLA-117 was selected from large panels of common light chain human monoclonal antibodies that were screened for both functional and developmental characteristics, we are convinced that we have identified the best clinical candidate for AML.

Analyses Show Efficacy with Ibrutinib Monotherapy in Patients with Relapsed or Refractory Mantle Cell or Diffuse Large B-cell Lymphoma

Data presented at the European Hematology Association (EHA) Annual Congress

Janssen Research & Development, LLC (Janssen), announced the results of two separate Phase 2 studies which suggested that ibrutinib, an investigational oral Bruton's tyrosine kinase (BTK) inhibitor, shows efficacy when used as a monotherapy in patients with relapsed/refractory mantle cell lymphoma (MCL) or diffuse large B-cell lymphoma (DLBCL). The studies were presented at the European Hematology Association (EHA) 18th Annual Congress in Stockholm, Sweden. Ibrutinib is being jointly developed by Janssen and Pharmacyclics, Inc.

"Our comprehensive clinical development programme is studying ibrutinib across a variety of B-cell malignancies; these patients, including those with DLBCL and MCL, are in real need of new treatment options," said Peter F. Lebowitz, M.D., Ph.D., Global Oncology Therapeutic Area Head, Janssen R&D.

"It's particularly exciting to observe the differences in response rates now as compared to earlier data from this study that were presented several months ago."

Ibrutinib in Relapsed/Refractory MCL
In relapsed/refractory MCL patients treated with ibrutinib monotherapy, the key results include:

- An overall response rate (ORR) of 68%, including a complete response (CR) of 21% and a partial response (PR) of 47%.
- The estimated median duration of response (DOR) in all responding patients was 17.5 months. Median progression-free survival (PFS) was 13.9 months. Median overall survival (OS) has not yet been reached, but is estimated to be 58% at 18 months.
- Treatment-emergent adverse events (AEs) were seen in greater than 20% of patients and included diarrhoea (50%), fatigue (41%), nausea (31%), peripheral edema (28%), dyspnea (27%), constipation (25%), upper respiratory tract infection (23%), vomiting (23%) and decreased appetite (21%) and were consistent with previously reported data. Only 7% of patients discontinued due to an AE.

"The results of this single-agent study are encouraging. It is exciting to see how active ibrutinib is in the treatment of relapsed and refractory mantle cell lymphoma, particularly as the responses appear to last," said Professor Simon Rule, Consultant Haematologist in the Department of Haematology at the Derriford Hospital in Plymouth, United Kingdom. "What is equally important is that no new safety signals were observed during this study."

The MCL study was a Phase 2 multicentre, open-label study which included 111 patients with relapsed/refractory MCL at 18 sites internationally. Patients had received a median of three prior therapies and were enrolled into two cohorts based on prior bortezomib exposure – either bortezomib-naive (n=63) or bortezomib-exposed (n=48), with both groups receiving 560 mg of ibrutinib orally, once a day. The primary endpoint of the study was ORR. Secondary endpoints included DOR, PFS, OS and frequency and severity of adverse events.

Ibrutinib in Relapsed/Refractory DLBCL
In the second Phase 2 study involving patients with relapsed/refractory DLBCL (n=70), investigators tested the hypothesis that ibrutinib would be more active in the Activated B-cell-like (ABC) subtype compared to the Germinal Center B-cell-like (GCB) subtype, given that the ABC subtype is dependent on the B-cell antigen receptor (BCR) pathway. Ibrutinib targets the BCR pathway by inhibiting BTK, a critical mediator in malignant B-cell growth and proliferation. Results of the study show that:

- Patients with the ABC subtype showed a significantly greater response to ibrutinib monotherapy compared to those with the GCB subtype (ORR = 41% vs 5%, p=0.007, Fisher's exact test).
- Median OS was 9.76 months for the ABC subtype, compared to 3.35 months for the GCB subtype (p=0.05).
- Within the ABC subtype group, only patients who had a CD79B mutation responded to treatment; patients with only an MYD88 mutation did not respond to treatment, suggesting a MYD88-dependent but BCR-independent pathogenesis for some DLBCL tumours.
- Safety data from 70 patients identified no new safety signals. Grade 3 or higher AEs were seen in greater than 3% of patients and included fatigue (9%), hyponatremia (9%), pneumonia (7%), dehydration (4%), and pleural effusion (4%).

"Seeing clinically meaningful responses among the ABC subtype was encouraging, as patients at this stage are challenging to treat. Additional trials among this patient group are ongoing."

"These results indicate the important function BTK plays in the survival of ABC type DLBCL," explained presenting investigator Sven de Vos, M.D., Ph.D., Associate Professor in the Department of Medicine at the UCLA Medical Center, Los Angeles, who reported the results at the EHA Congress.

The DLBCL study was a Phase 2 multicentre, open-label study that included 70 patients with relapsed/refractory DLBCL with a median of three prior therapies. All patients underwent gene expression profiling to determine their DLBCL subtype, 29 patients were identified with the ABC subtype, and 20 with the GCB subtype. Patients received ibrutinib 560 mg orally, once a day, until disease progression or unacceptable toxicity. The primary objective of the study was to assess ORR categorised by subtype, with secondary objectives being to assess the safety and tolerability of ibrutinib in people with DLBCL.

CTI Announces New Data Presented at EHA 2013 Congress Demonstrating the Safety and Tolerability Profile of Pacritinib

Cell Therapeutics have announced results from a pooled analysis of data from completed Phase 1 and 2 studies of pacritinib, an oral JAK2/FLT3 inhibitor, demonstrating the safety and tolerability profile of pacritinib in patients with myelofibrosis. An integrated safety analysis of four completed Phase 1 and 2 studies included 191 patients who were treated with pacritinib for myeloid, primarily myelofibrosis, or lymphoid malignancies to quantify clinical toxicities, with a focus on haematologic effects. Other JAK2 inhibitors have generally been associated with increases in anaemia and thrombocytopenia but this was not observed with pacritinib. This integrated safety data analysis showed that, regardless of initial platelet counts, pacritinib causes minimal further marrow suppression. Even patients with initial platelet counts <50,000/µL, a high-risk population, tolerated therapy, maintained stable blood and platelet counts and did not require dose reductions for thrombocytopenia. Grade 1 or 2 gastrointestinal events, particularly diarrhoea, were the most common adverse events and may be controlled by early administration of standard anti-diarrhoeal agents. The analysis was presented during a poster session at the 18th Congress of the European Hematology Association (EHA) held in Stockholm, Sweden.

Pacritinib is currently being evaluated in a randomised Phase 3 clinical trial, known as PERSIST-1, in patients with myelofibrosis. Because of pacritinib's relative lack of bone marrow suppression, there are no study entry restrictions due to thrombocytopenia or anaemia, and patients with platelet and red blood cell transfusion dependence are allowed to enroll in the ongoing PERSIST-1 trial. More details on this study can be found at www.clinicaltrials.gov. Pacritinib has orphan drug designation in the United States and Europe.

"This pooled safety analysis supports that pacritinib is well-tolerated with limited haematologic side effects that would allow it to be potentially used across all myelofibrosis patients, particularly in those with low blood cell count."

Abstract #P278: Safety Overview of Phase 1-2 Studies of Pacritinib, a Non-Myelosuppressive JAK2/FLT3 Inhibitor, in Patients with Haematological Malignancies.

"The use of JAK2 inhibitors to treat myelofibrosis has been a breakthrough for patients with myelofibrosis; however, there still exists a significant unmet need for less myelosuppressive agents given the nature of this disorder," said Srdan Verstovsek, M.D., Ph.D., Professor, Leukemia Department, Division of Cancer Medicine, Chief, Section for Myeloproliferative Neoplasms, Leukemia Department, and Director, Clinical Research Center for MPNs at the University of Texas MD Anderson Cancer Center.

Integrated Safety Analysis Results

A review of the safety database from four clinical studies included a Phase 1/2 study in advanced myeloid malignancies, a Phase 1 and Phase 2 study in advanced lymphoid malignancies, and a Phase 1/2 study in myelofibrosis. A total of 191 patients were treated with pacritinib: 129 with advanced myeloid malignancies, including 122 patients with myelofibrosis and 7 patients with acute myeloid leukaemia; and 62 patients with advanced lymphoid malignancies, including 38 patients with non-Hodgkin lymphoma and 24 patients with Hodgkin lymphoma. Of the patients with myeloid disorders, 44 percent had baseline platelet counts <100,000/µl. Pacritinib was dosed from 100 to 600 mg daily during Phase 1 and 400 mg during Phase 2. One hundred and forty-six patients were dosed at or greater than 400 mg daily. The median dose delivered was 98% of intended. The median treatment duration was 306 days (range 2-1210) for those with myeloid disorders and 90.5 days (range 1-631) for lymphoid disorders.

"We believe this analysis shows that pacritinib is well-tolerated and could potentially serve to treat these patients over an extended period of time."

Most patients had no decline in haemoglobin or platelet count during the studies. Of the 30 patients with myeloid disorder with baseline platelet counts <50,000/µL from Phase 1 and 2 studies, the median decline in platelet count observed at the end of study was 3,000/µL. In the 11 patients with myelofibrosis with baseline platelet counts <50,000/µL enrolled in Phase 2 studies, no dose reduction were required for worsening thrombocytopenia. The most common adverse events were gastrointestinal, particularly diarrhoea, most of which were grade 1 or 2. Per the protocols, anti-diarrhoeal prophylaxis was not used routinely in these early studies; however, anecdotal data from treating physicians were used suggests toxicity is readily controlled with early administration of standard anti-diarrhoeal agents. Time to onset of diarrhoea was ≤30 days in 89 percent of those affected but rarely caused drug discontinuation (1%). The most common serious adverse events reported (≥2%) included pneumonia (4.7%) and anaemia (3.1%).

Progression-Free Survival Data from Phase 2 Open-Label Study

Bristol-Myers Squibb and AbbVie Announce Progression-Free Survival Data from Phase 2 Open-Label Study of Investigational Agent Elotuzumab in Combination with Lenalidimide and Dexamethasone in Previously-Treated Multiple Myeloma

Bristol-Myers Squibb Company and AbbVie have announced updated efficacy and safety data from a small, randomised Phase 2, open-label study in patients with previously-treated multiple myeloma that evaluated two doses of the investigational monoclonal antibody elotuzumab (10 mg/kg and 20 mg/kg) in combination with lenalidomide and low-dose dexamethasone. In the 10 mg/kg arm (N=36), which is the dose used in the ongoing Phase 3 trials, median progression-free survival (PFS), or the time without disease progression, was 33 months after a median follow-up of 20.8 months (95% CI: 14.9-NA) and the objective response rate (ORR) was 92%. As previously reported, median PFS was 18 months in the 20 mg/kg arm (N=37) after a median follow-up of 17.1 months (95% CI: 12.912-32.361) and ORR was 76%.

The safety data were consistent with previously-reported results for elotuzumab from this trial. In patients receiving elotuzumab 10 mg/kg or 20 mg/kg, most treatment-emergent adverse events occurred within 18 months of initiating therapy. The most common Grade 3/4 adverse events (seen in > 5% of patients) for the 10 mg/kg and 20 mg/kg arms respectively were lymphopenia (26% and 9%), neutropenia (21% and 22%), thrombocytopenia (21% and 17%), anaemia (13% and 12%), leukopenia (8% and 7%), hyperglycemia (5% and 12%), pneumonia (8% and 5%), diarrhoea (10% and 5%), fatigue (8% and 9%), and hypokalemia (8% and 5%). As previously reported at the 2012 American Society of Hematology Annual Meeting, two deaths occurred on study (multiple adverse events [n=1; pneumonia, multiple organ failure and sepsis]).

These data were presented at the 18th Annual Congress of the European Hematology Association (EHA) in Stockholm, Sweden.

"There remains a high unmet medical need for patients with multiple myeloma, the second most common blood cancer, as many may relapse and stop responding to currently available treatments,"

Thierry Facon, MD, Hospital Claude Huriez, Service des Maladies du Sang, Lille, France.

"The Phase 2 data on elotuzumab are encouraging and support further evaluation in Phase 3 trials."

Ambit Presents Data From Phase 2 ACE Study of Quizartinib

Data From Phase 2 ACE Study of Ambit's Quizartinib in Patients With Relapsed or Refractory Acute Myeloid Leukemia (AML) Presented at 18th Congress of the European Hematology Association

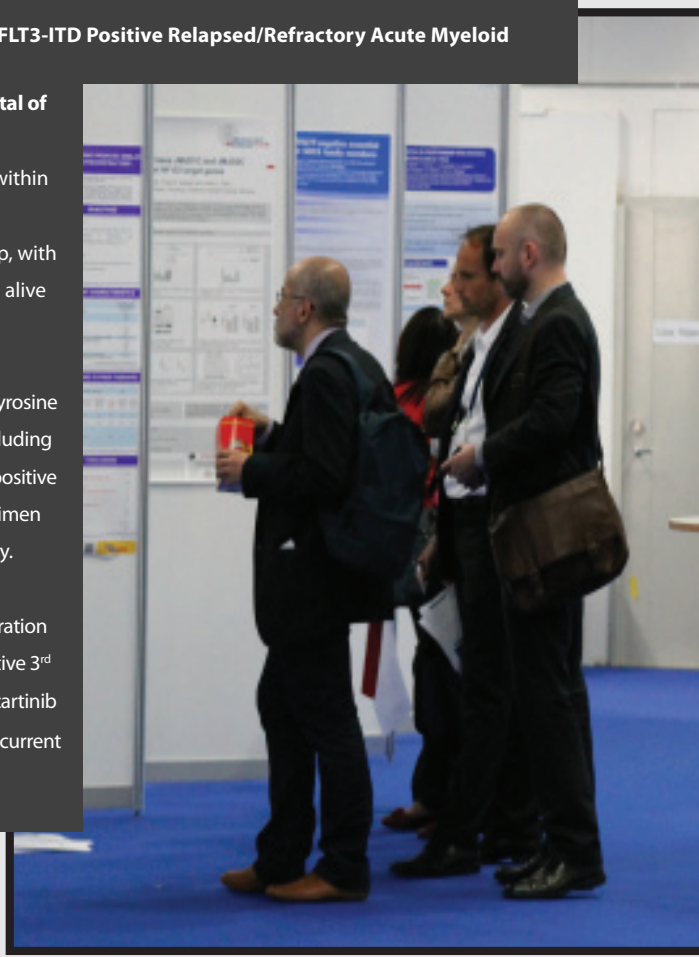
Data from the Phase 2 ACE study of quizartinib (AC220), a FLT3 inhibitor, announced by Ambit Biosciences Corporation were featured in multiple presentations at the 18th Congress of the European Hematology Association in Stockholm, Sweden. Data presented included analyses of patients with relapsed or refractory acute myeloid leukemia (AML) from a Phase 2 clinical trial of quizartinib as monotherapy. In the study, quizartinib was administered orally once a day, in 28-day treatment cycles until disease progression, elective haematopoietic stem cell transplantation (HSCT) or unacceptable toxicity. Based on the positive data from the Phase 2 clinical trial, as well as ongoing discussions with the Food and Drug Administration (FDA), Ambit is planning to initiate a Phase 3 clinical trial in FLT3-ITD positive patients with relapsed or refractory AML in early 2014.

High Response Rate and Bridging to Hematopoietic Stem Cell Transplantation With Quizartinib (AC220) in Patients With FLT3-ITD–Positive Relapsed/Refractory Acute Myeloid Leukemia (AML)
Mark J. Levis, M.D., Ph.D., Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Md.
Data from 136 FLT3-ITD positive patients, aged 18 years or older, with either relapsed disease or who were refractory to second-line chemotherapy or HSCT, were presented. Of the patients treated, 35 percent were successfully bridged to a potentially curative HSCT, with the greatest proportion receiving a HSCT after achieving a CRi (complete remission with incomplete haematologic recovery) with quizartinib. Additionally, 33 percent of patients who were bridged to HCST after achieving a CRi were still alive after one year, with multiple patients alive after more than two years.

Efficacy and Safety of Quizartinib (AC220) in Patients Age ≥60 Years with FLT3-ITD Positive Relapsed/Refractory Acute Myeloid Leukemia (AML)
Hartmut Dohner, M.D., Department of Internal Medicine III, University Hospital of Ulm, Ulm, Germany
Data from 110 FLT3-ITD positive patients, aged 60 years or older, who relapsed within one year or were refractory to first-line therapy were presented. Of the patients treated, 57 percent achieved a CRc, with seven percent having either a CR or CRp, with a median survival of 25.3 weeks. Additionally, 16 patients (15 percent) remained alive for more than 12 months and were classified as long-term survivors.

Quizartinib (AC220) is a novel, potent, highly selective, orally bioavailable FMS-like tyrosine kinase-3 (FLT3) inhibitor currently under evaluation in multiple ongoing studies, including a Phase 2b clinical trial as monotherapy treatment for adult patients with FLT3-ITD positive relapsed or refractory AML and two Phase 1 studies in a combination treatment regimen with chemotherapy, and as a maintenance therapy following transplant, respectively.

On 12th March, 2013, Ambit and Astellas Pharma Inc., announced that their collaboration for the joint development and commercialisation of quizartinib will terminate effective 3rd September, 2013, at which time Ambit will exclusively own worldwide rights to quizartinib and any follow-on compounds. The companies are working on the transition of the current development activities to Ambit.



Novartis Drug Jakavi® Improved Overall Survival of Myelofibrosis Patients

Novartis announced results from a Phase III three-year follow-up study that showed Jakavi® (ruxolitinib) demonstrated improved overall survival and sustained reductions in spleen size compared to conventional therapy.

In a three-year follow-up analysis of the COMFORT-II study, patients treated with Jakavi® demonstrated an overall survival advantage compared to patients receiving conventional therapy. A 52% reduction in risk of death was observed in the Jakavi® arm compared with conventional therapy (HR=0.48; 95% CI, 0.28-0.85; p=0.009),[1] and the estimated probability of overall survival was significantly greater with Jakavi® compared to conventional therapy (81% compared to 61%, respectively) at 144 weeks. Additionally, 51.4% of patients treated with Jakavi® achieved a >=35% reduction from baseline in spleen size. Patients continue to maintain their spleen response, with the median spleen reduction not yet reached in the study. The results are consistent with previous COMFORT-II and COMFORT-I study analyses, which demonstrate that Jakavi® provides significant clinical benefits over conventional therapy and placebo for patients suffering from myelofibrosis, a rare blood cancer.

"Jakavi® is the first drug to demonstrate an improvement in overall survival in patients with advanced myelofibrosis," said Dr. Alessandro M. Vannucchi, Department of Hematology, University of Florence, Italy and lead study author. "Moreover, we are encouraged by these latest study results, which reinforce that the rapid, positive effects of Jakavi in improving patients' symptoms are sustained over the long-term."

Myelofibrosis develops when uncontrolled signaling in the JAK pathway - which regulates blood cell production - causes the body to make blood cells that do not work properly, which scars the bone marrow and results in an enlarged spleen and other severe complications.[2,3] Jakavi® directly targets the underlying mechanism of the disease and significantly reduces spleen size and improves symptoms regardless of JAK mutational status, disease subtype or any prior treatment.[4-8]

Data were also presented from an exploratory analysis of bone marrow morphology from a separate Phase I/II trial of Jakavi®, compared with historical controls from patients treated with conventional therapy. After four years of Jakavi® therapy, bone marrow fibrosis improved in 22% and stabilised in 56% of patients with myelofibrosis. A comparable effect was not seen with long-term conventional therapy.[9]

"For the first time in advanced myelofibrosis, drug therapy showed evidence of bone marrow fibrosis stabilisation or improvement, further supporting that Jakavi® may modify the disease's natural course," said Alessandro Riva, M.D., Global Head, Oncology Development and Medical Affairs, Novartis Oncology.

"These data are of interest because bone marrow transplantation, which carries a high risk of morbidity and mortality, is the only other option proven to impact bone marrow fibrosis in patients with advanced myelofibrosis."

COMFORT-II Three-Year Long-Term Study Background

In the three-year analysis of COMFORT-II (COntrolled MyeloFibrosis Study with ORal JAK Inhibitor Therapy), a total of 45.2% of patients remained on the Jakavi® treatment arm, while all patients randomised to conventional therapy discontinued treatment. For patients on conventional therapy, 61.6% crossed over to the Jakavi® treatment arm, with 48.9% of these patients ongoing in the extension phase of the study. The median duration of Jakavi exposure (randomised and extension phases) was 136 weeks and conventional therapy exposure (randomised treatment only) was 45 weeks. Overall survival was estimated using the Kaplan-Meier method. All AEs were consistent with previous analyses of treatment with Jakavi®. The most common haematologic AEs in either arm (Jakavi®, conventional therapy) were anaemia (50.0%; 16.4%) and thrombocytopenia (50.7%; 13.7%). The most common non-haematologic abnormalities for each arm (Jakavi®, conventional therapy) include peripheral edema (swelling of extremities) (36.3%; 28.8%), diarrhoea (32.2%; 17.8%) and asthenia (weakness) (24.0%; 12.3%).[1]

A total of 191 patients were exposed to Jakavi® by the data cut-off date, 146 patients initially randomised to Jakavi® treatment and 45 patients that eventually crossed over from the conventional therapy arm. Treatment discontinuations in the Jakavi® arm were primarily due to adverse events (AEs) (16.4%) and disease progression (15.1%), while discontinuations in the conventional therapy arm were primarily due to consent withdrawal and other reasons (12.3% each). Only two patients discontinued due to anemia (1%) and seven patients due to thrombocytopenia (3.6%).

References

1. Vannucchi, A, *et al.* Long-Term Outcomes From A Phase 3 Study Comparing Ruxolitinib With Best Available Therapy (BAT) For The Treatment of Myelofibrosis (MF): A 3 Year Update of Comfort II. Abstract #S1111.18th Congress of European Hematology Association (EHA), 2013. Stockholm, Sweden.
2. Leukemia & Lymphoma Society. Idiopathic myelofibrosis. Available at: http://www.lls.org/#/diseaseinformation/myeloproliferativediseases/idiop athic myelofibrosis/. Accessed April 2013.
3. Mesa RA, Schwagera S, Radia D, *et al.* The Myelofibrosis Symptom Assessment Form (MFSAF): an evidence-based brief inventory to measure quality of life and symptomatic response to treatment in myelofibrosis. Leuk Res. 2009;33:1199-1203.
4. Verstovsek S, Mesa RA, Gotlib J, *et al.* A Double-Blind, Placebo-Controlled Trial of Ruxolitinib for Myelofibrosis. New Eng J Med. 2012: March 1;366:799-807.
5. Harrison C, Kiladjian JJ, Al-Ali HK, *et al.* JAK Inhibition with Ruxolitinib versus Best Available Therapy for Myelofibrosis. New Eng J Med. 2012: March 1;366:787-98.
6. Vannucchi A, Kiladjian JJ, Gisslinger H, *et al.* Reductions in JAK2V617F Allele Burden with Ruxolitinib Treatment in COMFORT-II, a Phase III Study Comparing the Safety and Efficacy of Ruxolitinib to Best Available Therapy (BAT). 2012. Abstract #802. American Society of Hematology 2012. Annual Meeting, Atlanta, GA.
7. Harrison C, Kiladjian JJ, Gisslinger H, *et al.* Association of Cytokine Levels and Reductions in Spleen Size in COMFORT-II, a Phase 3 Study Comparing Ruxolitinib to Best Available Therapy (BAT). Abstract # 6625. American Society of Clinical Oncology 2012 Annual Meeting, Chicago, IL.
8. Cervantes, F, *et al.* Long-Term Safety, Efficacy, and Survival Findings From COMFORT-II, a Phase 3 Study Comparing Ruxolitinib with Best Available Therapy (BAT) for the Treatment of Myelofibrosis (MF) Blood. 2012. Abstract #801. American Society of Hematology 2012 Annual Meeting. Atlanta, GA.)
9. Kvasnicka, HM, et al. Long Term Intervention Effects on Bone Marrow Morphology in Myelofibrosis: Patients Treated With Ruxolitinib and Best Available Therapy. Abstract #S591.18(th) Congress of European Hematology Association (EHA). 2013. Stockholm, Sweden.

Auto Hematology Analyzer

Labs today are facing a multitude of challenges, including limited laboratory budgets, increasing requirements for high-quality as well as reliable products, and a shortage of experienced clinicians amongst others. Mindray understands the specific needs of end users, and has used them as the foundation for their new solution BC-3600. Complete with an intuitive operation system, convenient data communication interface, and excellent performance, BC-3600 is a system solution for satellite labs and clinics.

Key features include:

- CBC+3-DIFF, 21 parameters+3 histograms
- Throughput: 60 samples per hour
- Intuitive operation system with TFT touch screen
- Enhanced performance by proven technologies
- Open vial or closed sampling optional
- 40000 results storage with histograms
- Original QC, calibrators and reagents

Mindray was founded in 1991 with the goal of delivering high-quality, competitively priced medical devices to make healthcare more accessible and affordable around the world. In 2006, Mindray listed on the New York Stock Exchange and is now a leading developer, manufacturer and marketer of medical devices worldwide.

The company has three well-established business segments: Patient Monitoring and Life Support Products, *In-Vitro* Diagnostic Products and Medical Imaging Systems. Healthcare facilities equipped with Mindray's products can be found in over 190 countries and regions. A sound distribution and service network has been formed with 31 branch offices throughout China, and overseas subsidiaries in Brazil, Canada, France, Germany, India, Indonesia, Italy, Mexico, Netherlands, Russia, Turkey, the UK and the U.S., Spain, Egypt, Columbia, Thailand and Vietnam. Additionally, the acquisition of Datascope Corporation's patient monitoring business has added significant direct sales and service capabilities on the ground in the U.S. and Western Europe.

Mindray has a global R&D network with research centers in Shenzhen, Beijing, Nanjing, Shanghai, Chengdu, Xi'an, Seattle, New Jersey, Stockholm and Miami. Approximately 10% of total revenue has been consistently re-invested into R&D each year. An average of eight new products has annually been introduced to the market over the past seven years.

Acetylon Pharmaceuticals Presents Encouraging Safety and Clinical Response Data for the Treatment of Multiple Myeloma

Acetylon Pharmaceuticals Inc., presented clinical data of the Company's lead candidate, ACY-1215, for the treatment of relapsed or refractory multiple myeloma at the 18th Congress of the EHA

ACY-1215 is an oral, selective HDAC6 inhibitor currently being evaluated in a Phase 1b clinical trial in combination with the best-in-class drug Revlimid® (lenalidomide, Celgene) and a Phase 1/2 clinical trial in combination with the first-in-class drug Velcade® (bortezomib, Takeda Millennium) for the treatment of relapsed or refractory multiple myeloma.

"We are encouraged by the number of early clinical responses that have been observed in combination therapy. In the Phase 1b study in combination with Revlimid, eight out of ten evaluable patients to date achieved disease response, including one complete response (with marrow confirmation), two very good partial and four partial responses observed. No dose limiting toxicities or severe adverse events, which are common with previous generation non-selective HDAC inhibitors, have been observed in this trial, and dose escalation is continuing. Activity has also been observed in heavily pretreated patients in combination with Velcade in the ongoing Phase 1/2 study, including three partial responses and one minor response to date in thirteen evaluable patients. Responses in both trials have been durable, with patients remaining on study for up to 11 months", said Catherine A. Wheeler, MD, Vice President, Clinical Development of Acetylon.

> ***"ACY-1215 is very well tolerated, both as a single agent and in combination with either Revlimid or Velcade at biologically relevant exposures in patients with relapsed or refractory multiple myeloma,"***
>
> ***Catherine A. Wheeler, MD, Vice President, Clinical Development of Acetylon.***

Preliminary ACY-100 Phase 1/2 Data

ACY-100 is a single arm multicentre open label Phase 1/2 study with dose escalation of ACY-1215 as a monotherapy and in combination with Velcade® (bortezomib, Takeda Millennium) and dexamethasone for the treatment of patients with relapsed or refractory multiple myeloma. At the time of data cut-off, 28 patients were enrolled in Phases 1a and 1b. Patients received single agent ACY-1215 in doses of 40 mg to 360 mg orally on days 1-5 and 8-12 of a 21 day cycle in Phase 1a, and with Velcade administered days 1, 4, 8 and 11 as well as 20 mg of dexamethasone the day of and the day after Velcade dosing in Phase 1b. With ACY-1215 monotherapy, no dose-limiting toxicities were observed and creatinine elevations, anaemia, fatigue, hypercalcemia and respiratory infection, the most common toxicities, were mostly low grade and not attributed to ACY-1215. Possibly related grade 3 toxicities were anaemia and low white blood cell counts. Treatment resulted in six of fifteen heavily pretreated patients achieving stable disease. With ACY-1215, Velcade and dexamethasone combination therapy, treatment was well-tolerated up to 160 mg daily dosing of ACY-1215 (with dose escalation ongoing) with no dose-related adverse events and few grade 3 and 4 related adverse events. Three partial responses, one minor response and four stable disease outcomes were observed out of thirteen heavily pretreated patients evaluable for response assessment (at up to 80 mg daily dosing).

Preliminary ACE-MM-101 Phase 1b Data

The Phase 1b trial is an open-label, multicentre, dose-escalation study to evaluate the safety and efficacy of ACY-1215 in combination with Revlimid® (lenalidomide, Celgene) and dexamethasone in patients with relapsed or refractory multiple myeloma. Patients receive Revlimid, 25 mg after an initial safety cohort at 15 mg, daily on 21 days of a 28 day schedule and 40 mg dexamethasone weekly. In schedule A, ACY- 1215 is being dosed days 1-5 and 8-12 and dose levels of 40 to 240 mg are being explored. If that schedule is well tolerated, a third week of ACY-1215 (schedule B) day 15-19 will be examined. Preliminary results demonstrate that ACY-1215 is well-tolerated in combination with Revlimid and dexamethasone at up to 160 mg daily dosing, and dose escalation is ongoing. No dose-limiting toxicities or serious adverse events have been observed to date. One grade 3 adverse event (neutropenia) was possibly related to ACY-1215, and other adverse events were low grade with no dose relationship observed. Responses have been observed in eight of ten patients evaluable for response, including one complete response, two very good responses, four partial responses and one minor response.

Karyopharm Therapeutics Presents New Data on Selective Inhibitor of Nuclear Export (SINE), KPT-330

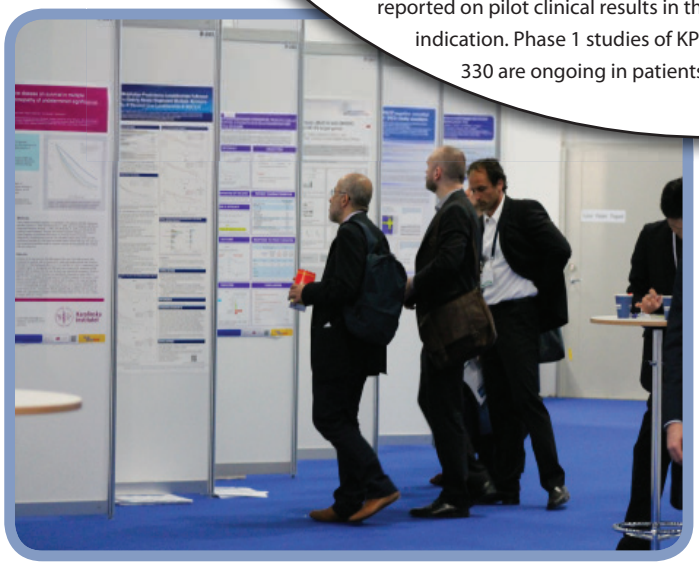
Karyopharm Therapeutics Inc., a leader in the new field of nuclear transport modulators, presented data for its lead compound KPT-330 against advanced chronic myeloid leukaemia (CML) in the CML Biology oral abstract session at the 18th Congress of the European Hematology Association (EHA).

"We are pleased to present these data on our novel XPO1/CRM1 inhibitor KPT-330 in accelerated and blast crisis phase CML in an oral session at the EHA annual congress," commented Karyopharm Chief Scientific Officer and founder Sharon Shacham, Ph.D. "These data, in an important haematologic malignancy, complement our upcoming presentation of KPT-330 in patients with advanced solid tumours at ASCO."

The presentation highlighted preclinical data from blast-crisis CML models and reported on pilot clinical results in this indication. Phase 1 studies of KPT-330 are ongoing in patients with relapsed or refractory haematologic malignancies (NCT01607892) and solid tumours (NCT01607905), whose disease has progressed on currently available treatments.

Dr. Chris Walker from the Ohio State University presented "Preclinical and Clinical Efficacy of KPT-330-Mediated XPO1 Inhibition in Ph+ Leukemias" on Saturday, 15th June, 2013, at 4:30-4:45 PM in Hall C6/7 (Abstract S602).

KPT-330 is one of the company's family of novel selective inhibitors of nuclear export (SINE) compounds that induces cell death selectively in cancer cells through forced nuclear retention and activation of tumour suppressor proteins by blocking Exportin 1 (XPO1/CRM1). It is the first oral, small molecule XPO1 antagonist ever to be tested in humans.



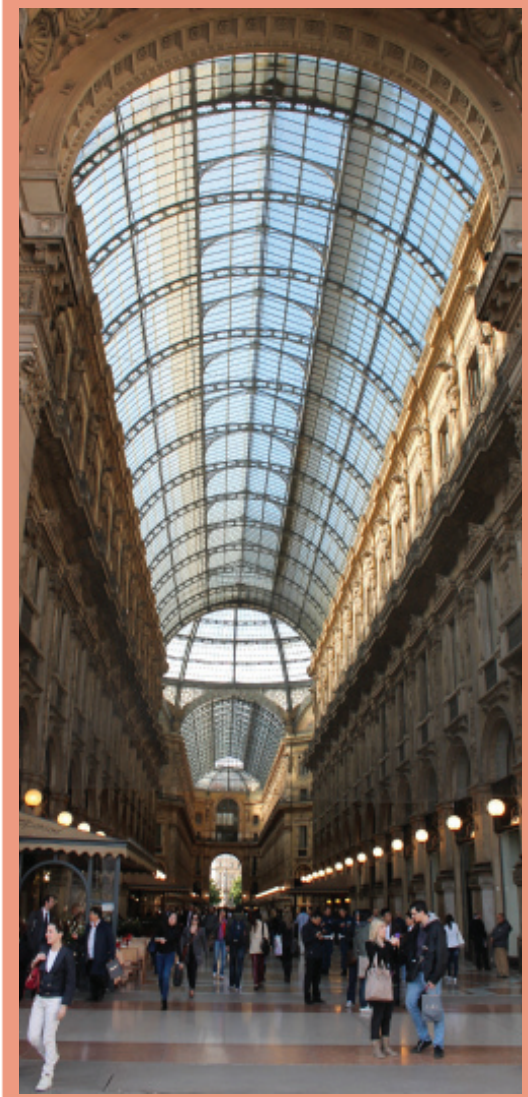
19th EHA Congress
12 - 15 June 2014
International Convention Center
Milano, Italy

After 12 years, the 19th Congress of EHA is returning to Italy. Milan is one of Italy's most fashionable cities and holds several historic and artistic attractions, as well as a congress centre with excellent facilities and a great location.

The Congress aims to present haematology as a comprehensive discipline by hosting a variety of session types covering a diverse range of topics. The education and scientific programme will highlight established clinical practice, recent advances in the field of haematology and views from different stakeholders and international organisations.

The programme committee is also developing selected sessions for various target groups, and combining them into tracks. Besides the topical tracks on, for example, lymphoid malignancies and thrombosis & haemostasis, the committee is also creating an Early Career Track and Advocacy Track with a special focus and interest. Find more information about next year's Congress via the EHA website:

www.ehaweb.org



We look forward to seeing you at next year's congress in Milan!

Defining Optimal Combination Strategies in the Management of Follicular and Mantle Cell Lymphomas

Introduction

The satellite symposium entitled, "Defining Optimal Combination Strategies in the Management of Follicular and Mantle Cell Lymphomas" was held during the 18th Congress of the European Hematology Association (EHA) on Thursday 13th June 2013 in Stockholm, Sweden. The meeting was chaired by Professor Franck Morschhauser, Centre Hospitalier Universitaire de Lille, Lille, France.

Background

Follicular lymphoma (FL) and mantle cell lymphoma (MCL) are two of the more prevalent subtypes of non-Hodgkin lymphoma (NHL), but they possess very different biological characteristics and are associated with dramatically different long-term clinical outcomes. There has been significant progress in recent years in the understanding of the biology underlying both of these diseases and the development of new, more effective treatment options is currently being investigated.

Defining Optimal Combination Strategies in the Management of Follicular and Mantle Cell Lymphomas
Mundipharma Sponsored Satellite Symposium at the 18th Congress of the European Hematology Association (EHA), Stockholm, Sweden, 13th June 2013

Chair: Franck Morschhauser, Centre Hospitalier Universitaire de Lille, Lille, France

Follicular and Mantle Cell Lymphomas: The Current Treatment Landscape
Franck Morschhauser, Centre Hospitalier Universitaire de Lille, Lille, France

State of the Art Treatment for *de novo* Follicular Lymphoma
Mathias Rummel, Hospital of the Justus-Liebig University, Giessen, Germany

Follicular Lymphoma: New Options at Relapse
Fritz Offner, Ghent University, Ghent, Belgium

Treatment Options for MCL Patients Unfit for Intensive Therapy
Carlo Visco, San Bortolo Hospital, Vicenza, Italy

Current and New Approaches for the Younger MCL Patient
Steven Le Gouill, Université de Nantes, Nantes, France

Final Questions and Conclusions
Franck Morschhauser, Centre Hospitalier Universitaire de Lille, Lille, France

Follicular and Mantel Cell Lymphomas: The Current Treatment Landscape

Franck Morschhauser
(Centre Hospitalier Universitaire de Lille, France)

Within the spectrum of NHL, MCL makes up 6% of cases[1, 2] and FL 22% of cases.[3]

FL is an indolent, relapsing/remitting incurable disease, which affects most patients above the age of 50 years, the median age at diagnosis being 60 years old. The median survival is 12-14 years,[4, 5] and the choice of treatment is dependent on the stage of disease and whether patients with advanced stage disease are symptomatic or not.[6]

The ESMO 2013 Consensus Treatment Guidelines have recommended local radiotherapy for limited disease.[6] In patients with a low tumour burden, watch and wait is the standard of care according to Groupe d'Etude des Lymphomes Folliculaires (GELF) criteria for asymptomatic advanced disease. For patients in need of treatment, rituximab with chemotherapy such as CVP, CHOP or bendamustine is recommended with a level of evidence of I,A. Autologous stem cell transplantation (ASCT) is not recommended for consolidation of the first remission but can be considered at first relapse in patients with a poor prognosis.

In contrast, MCL runs a very aggressive course, with a median progression free survival (PFS) of around only 2 years and an overall survival (OS) of 5 years.[7-9] It is also incurable and is typically diagnosed in patients aged 60-65 years; affecting men more than women. For MCL, the ESMO 2013 Guidelines recommend stratified treatment by age, with rituximab-chemotherapy for induction and rituximab maintenance in the elderly (≥65 years), and rituximab-chemotherapy for induction and ASCT as first-line therapy in patients under 60-65 years of age.[10]

GELF criteria	BNLI criteria
• High tumour bulk • Presence of systemic symptoms • Eastern Cooperative Oncology Group (ECOG) performance status > 1 • Serum lactate dehydrogenase (LDH) or β2-microglobulin level above normal values	• Rapid, generalised disease progression in the preceding 3 months • Life-threatening organ involvement • Renal infiltration • Bone lesions • Presence of systemic symptoms: Haemoglobin < 10 g/dL or WBC <1.5 x 109/L, or platelet counts <100 x 109/L; related to bone marrow involvement

Table 1. GELF and BLNI criteria.

State of the Art Treatment for *de novo* Follicular Lymphoma

Mathias Rummel

(Hospital of the Justus-Liebig University, Germany)

Managing patients with FL can be challenging, particularly regarding when to start therapy, which treatment options to consider and balancing efficacy and side effects of treatment.

The GELF and The British National Lymphoma Investigation (BNLI) have proposed and used defined criteria for patients in whom immediate therapy is necessary (Table 1).[11, 12] The Study group indolent Lymphomas (StiL) have also provided indications for treatment (Table 2).[13]

Frederico and colleagues enrolled 942 patients with newly diagnosed FL receiving anti-lymphoma therapy to develop a more accurate prognostic index by using parameters which could not be retrospectively studied before, and by choosing PFS as the principal endpoint.[14] After a median follow-up of 38 months, 261 events for PFS evaluation were recorded. Factors independently predictive for PFS were β2-microglobulin higher than the upper limit of normal, longest diameter of the largest involved node >6 cm, bone marrow involvement, haemoglobin level <12 g/dL and age >60 years. Using these variables, a prognostic model was devised to identify three groups at different levels of risk. The 3-year PFS rate was 91%, 69% and 51% for patients at low, intermediate and high risk, respectively. The 3-year survival rate was 99%, 96% and 84% for patients at low, intermediate and high risk, respectively.

Rituximab plus chemotherapy, most often CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone: R-CHOP), is the first-line standard of care for patients with advanced indolent lymphoma.

Patients with FL can continue for 3-5 years after initial treatment before the disease progresses. The PRIMA Phase III randomised controlled trial, conducted in 223 centres across 35 countries, assessed the potential benefit of 2 years of rituximab maintenance after first-line treatment in

• B symptoms • Haematopoietic failure (Hb <11 g/dL, granulocytes <1,500/µL, thrombocytes <100,000/ µL • Large tumour burden (3 areas >5 cm or 1 area >7.5 cm) • Rapid progression (increase of tumour mass >50% within 6 months) • Complications due to disease (pain, infarction of spleen, hyperviscosity syndrome, etc.)

Table 2. StiL indications for treatment of FL.

patients with FL receiving rituximab plus a chemotherapy regimen.[15] The study included 505 patients assigned to rituximab maintenance and 513 to observation. With a median follow-up of 36 months, PFS was 74.9% in the rituximab maintenance group and 57.6% in the observation group (p<0.0001). Two years after randomisation, 361 patients (71.5%) in the rituximab maintenance group were in complete or unconfirmed complete response (CR/CRu) versus 268 (52.2%) in the observation group (p=0.0001). Overall survival (OS) did not differ significantly between the treatment groups.

A recent study by Frederico and colleagues has confirmed that R-CHOP should be a primary treatment choice for FL patients requiring active treatment. In this study, 3-year PFS rates of 68% were reported for adult patients with previously untreated stages II to IV FL who were treated with R-CHOP.[16]

Bendamustine plus rituximab (BR) has previously been shown to be effective for the treatment of relapsed/refractory disease.[17, 18] A recent German prospective, multicentre, randomised, open-label, non-inferiority trial at 81 centres enrolled patients aged ≥18 years with a WHO performance status ≤2 with newly diagnosed stage III or IV indolent or MCL.[19] Patients were randomly assigned to receive BR or R-CHOP for a maximum of 6 cycles. At a median follow-up of 45 months, median PFS was significantly longer for BR versus R-CHOP (69.5 months versus 31.2 months, p<0.0001). It was concluded that, in patients with previously untreated indolent or MCL, BR could be considered as a preferred first-line treatment approach to R-CHOP because of increased PFS and fewer toxic effects. However, it will be important to wait for the outcomes of the BRIEF study, in order to confirm longer-term benefits. Primary data is expected in 2015.

The MAINTAIN trial is underway to determine if extended maintenance therapy with rituximab in FL has advantages compared to shorter maintenance therapy.[20] Patients with FL will receive induction therapy with BR. If a complete or partial response is achieved, maintenance therapy with rituximab every 2 months for 4 years will be compared to rituximab every 2 months for 2 years. Primary data is expected in 2014.

Follicular Lymphoma: New Options at Relapse

Fritz Offner

(Ghent University, Belgium)

Maintenance treatment with cytotoxic agents has been shown to

improve PFS but not OS.[21-24] This prolongation of PFS is achieved at the cost of increased toxicity, reduced patient well-being and an increased risk of secondary malignancies.

Rituximab-based therapy has been shown to be effective when given for a second time at the time of relapse, after patients had previously responded to rituximab. Whether rituximab maintenance treatment is superior to rituximab re-treatment (at the time of disease progression) has not yet been established.

Data from the EORTC 20981 Phase III study showed that rituximab maintenance strongly improved median PFS, both after induction with CHOP and R-CHOP, and OS when compared with observation.[25] This has been confirmed with long-term follow up to 6 years.[26]

In a number of other randomised studies, the clinical benefit of rituximab and other anti-CD20 maintenance treatments after various remission induction regimens has been shown.[27-30]

Currently, rituximab in combination with chemotherapy (CHOP or fludarabine, cyclophosphamide and mitoxantrone [FCM]) plus rituximab maintenance is the best documented standard of care for patients with relapsed/refractory FL, provided that the initial treatment does not contain rituximab or anthracyclines.[31, 32]

BR has been evaluated in adults with relapsed, indolent B-cell or MCL without documented resistance to prior rituximab and has been found to produce good overall response rates (ORR).[15] When this combination was compared to fludarabine in combination with rituximab over 2 years, it was found to be associated with significantly greater response rates and PFS.[33]

A number of other novel agents are being investigated for the treatment of relapsed/refractory FL. In an initial Phase I/II study of ofatumumab, clinical response rates ranged from 20-63%. Median time to progression for all patients/responders was 8.8/32.6 months at a median/maximum follow-up of 9.2/38.6 months and overall median duration of response was 29.9 months.[23] Ofatumumab was later assessed in a Phase III study that included 116 rituximab refractory patients with FL.[34] Those subjects who received the higher dose (1000 mg) demonstrated an ORR of 10%, including one CR, and PFS of 5.8 months. A number of other studies are ongoing to assess ofatumumab alone and in combination with bendamustine and bortezomib.

Obinutuzumab is also undergoing evaluation as a treatment for NHL.[35] Three Phase III studies are ongoing: GADOLIN (NCT01059630), which will compare bendamustine with the combination of bendamustine and obinutuzumab in patients with rituximab-refractory, indolent NHL (iNHL); GOYA (NCT01287741), which will compare obinutuzumab plus CHOP to R-CHOP in previously untreated patients with CD20-positive diffuse large B-cell lymphoma; and GALLIUM (NCT01332968), which will compare obinutuzumab in combination with chemotherapy versus rituximab with chemotherapy (CHOP, CVP or bendamustine) followed by obinutuzumab or rituximab maintenance in patients with untreated advanced iNHL. The primary completion dates for these studies are January 2015, November 2016 and March 2022, respectively.

CD22 has also been investigated as a target in the treatment of relapsed/refractory NHL. CD22 is a B-cell specific antigen involved in regulating B-cell survival and function. It is an attractive target, in that it is expressed on the majority of B-cell cancers. Studies are ongoing for epratuzumab and inotuzumab ozogamycin, among others. Phase I/II studies have shown that epratuzumab has significant single agent clinical activity across various dose levels in relapsed/refractory FL.[36] Combination with rituximab has demonstrated promising results, with an ORR of 54% (CR 24%) in FL patients.[37] Inotuzumab ozogamycin is a humanised anti-CD22 antibody conjugated to calicheamicin. A Phase II study evaluating the combination of inotuzumab ozogamycin and rituximab reported an ORR of 87% and median PFS of 23.6 months.[38]

Some initial studies have also investigated CD19 as a target in different NHL subtypes, as well as BCR signalling and it's targeting by small molecule inhibitors.

In a Phase II, single arm efficacy and safety study, lenalidomide in combination with rituximab was found to result in an ORR of 98% in previously untreated patients with FL.[39] In addition, CR/CRu was 87%. Responses were high regardless of FLIPI score, tumour bulk or GELF criteria at study entry. In relapsed/refractory patients, lenalidomide monotherapy demonstrated an ORR of only 23%. However, responses were quite durable (>16 months).[40]

Bortezomib is a first-in-class drug designed to target the ubiquitin-proteasome complex. It results in impairment of apoptosis, which is considered one of the most involved pathways in the pathogenesis of iNHL. For this reason bortezomib has been evaluated with great interest in FL. A large international randomised Phase III trial (LYM-3001) reported a median PFS of 12.8 months in patients treated with bortezomib and rituximab versus 11 months with rituximab only.[41] This coincided with a better ORR (63% versus 49%). However, the clinical benefit did not reach the anticipated pre-specified improvement of 33% in PFS. Recently, two Phase II trials investigating the combination of bortezomib, bendamustine and rituximab (VBR) have been completed. In the larger VERTICAL trial, 73 patients with relapsed/refractory FL were enrolled to receive up to five cycles of VBR.[42] The ORR was 88% (53% CR) and the median PFS was 14.9 months.

The t(14;18) chromosomal translocation, which occurs in the majority of FL, results in the juxtaposition of the Bcl-2 gene next to the immunoglobulin heavy chain gene, leading to its constitutive

expression and to signalling unbalance in favour of survival of malignant cells.[43] A number of novel Bcl-2 inhibitors are currently undergoing clinical investigation in combination with other agents in FL, and results are expected shortly.

Several studies are also underway to investigate the potential role for therapies that target phosphatidylinositol-3-kinase (PI3K), such as CAL-101; hystone deactylase (HDAC), such as vorinostat; and Bruton tyrosine kinase (BTK), such as ibrutinib, in the treatment of FL.

There have been many advances in the search for new targeted therapies for NHL. However, rituximab remains a key component in the treatment of relapsed/refractory FL. R-CHOP is recommended first-line. BR is a second-line option, but in light of Professor Rummel's data is now more frequently being considered in previously untreated patients as an alternative to R-CHOP.

Treatment Options for MCL Patients Unfit for Intensive Therapy

Carlo Visco

(San Bortolo Hospital, Vicenza, Italy)

In an attempt to improve outcomes, the German Low Grade Lymphoma Study Group (GLSG) initiated a randomised trial comparing CHOP and R-CHOP as first-line therapy for advanced-stage MCL.[44] R-CHOP was significantly superior to CHOP in terms of ORR (94% versus 75%; p=0.0054), CR (34% versus 7%; p=0.00024) and time to treatment failure (median, 21 versus 14 months; p=0.0131). No differences were observed for PFS. These results were confirmed in a retrospective, observational study of elderly patients with MCL, and a benefit in terms of OS by the addition of rituximab was reported.[45] In this study, the mean age at diagnosis was 75 years, 75% had stage III/IV disease, 67% had extranodal involvement. When R-CHOP was compared to rituximab, fludarabine and cyclophosphamide (R-FC) in elderly patients with MCL, CR was similar for R-FC and R-CHOP (40% and 34%, respectively; p=0.10), progressive disease was more frequent with R-FC (14%, versus 5% with R-CHOP), OS was significantly shorter with R-FC versus R-CHOP (4-year survival rate, 47% versus 62%; p=0.005) and more patients in the R-FC group died during the first remission (10% versus 4%). Importantly, this study demonstrated a significant OS advantage by maintenance rituximab following R-CHOP, but not after R-FC. In the Rummel 2013 study described earlier, R-CHOP versus BR was also assessed in elderly patients with MCL and BR was found to have a significant effect on PFS.[15] A multicentre, prospective, randomised Phase III trial has been initiated to investigate the impact of adding rituximab maintenance following BR first-line induction for patients with Waldenström's macroglobulinemia, marginal zone, small lymphocytic and MCL (MAINTAIN).[47] There are also a number of other ongoing studies in elderly patients with MCL investigating a number of bendamustine combinations due to be completed between 2014 and 2018.

The combination of rituximab, bendamustine and cytarabine (R-BAC) has been investigated in MCL. Preclinical studies have demonstrated a striking synergy of the drugs when administered consecutively.[48] A Phase II study in patients aged ≥65 years who were previously untreated or who were relapsed/refractory after one prior immunochemotherapy treatment, reported an ORR of 100% (95% CR) for previously untreated and 80% (70% CR) for relapsed/refractory patients.[49] The 2-year PFS rate was 95% for untreated and 70% for relapsed/refractory patients. R-BAC is also being investigated as an induction regimen in an ongoing Phase II study with cytarabine dose reduction to 500 mg/m^2 (R-BAC500).

A number of active single agents are also being investigated. These include bortezomib, temsirolimus, everolimus, lenalidomide and ibrutinib.

Bortezomib as a monotherapy has been investigated in patients with relapsed/refractory MCL in the multicentre Phase II PINNACLE study and was found to be associated with a median OS of 23.5 months and time to progression of 6.7 months, suggesting substantial clinical benefit.[50] It has also been investigated in combination with R-CHOP.[51] For MCL patients, the ORR was 81% with 64% CR/CRu, 2-year PFS was 44% and 2-year OS was 86%.

Treatment with the m-TOR inhibitors temsirolimus and everolimus have also shown promising results. PFS of 1.9-9.7 months and ORR of 2-41% have been reported across several studies.[52-56] In addition, the immunomodulatory drug, lenalidomide, has been associated with PFS of 4-12 months and ORR of 28-53%.[57-60] In a small Phase II study, lenalidomide in combination with rituximab was associated with PFS of 11.1 months and OS of 24.3 months in relapsed/refractory patients with MCL.[61] Similar results have been found with ibrutinib, with PFS of 13.6 months.[62] An international Phase II trial of ibrutinib in 111 patients with relapsed or refractory MCL reported a response rate of 68% (75 patients), with a CR rate of 21% and a PR rate of 47% independent of prior treatment with bortezomib.[63] The estimated median PFS was 13.9 months (95% CI, 7.0 to not reached), and the median OS was not reached. The estimated rate of OS was 58% at 18 months.

There are a number of completed and ongoing clinical trials investigating new treatment options in patients with MCL who are unfit for intensive therapy. R-CHOP plus rituximab maintenance is the standard therapy for many patients. However, BR is now also considered a good first-line option. Pending the outcome of trials investigating several B-cell receptor downstream inhibitors, new treatment options may be available for these difficult to treat patients.

Current and New Approaches for the Younger MCL Patient

Steven Le Gouill

(Université de Nantes, Nantes, France)

There is no single standard initial therapy for MCL. Standard lymphoma therapies yield high response rates, but responses are generally not durable. Even younger patients, receiving high-intensity regimens, will eventually relapse.

Efficacy	Bortezomib-naïve (n=63)	Bortezomib-exposed (n=46)	Total (n=109)
ORR	65%	67%	66%
CR	19%	20%	19%
PR	46%	48%	47%

Table 3. Ibrutinib in relapsed/refractory MCL patients.

To improve clinical outcomes, the European MCL Network initiated a randomised trial in 2005 comparing consolidation with myeloablative radiochemotherapy followed by ASCT to interferon-α (IFNα) maintenance in first remission.[64] Patients aged ≤65 years with advanced-stage MCL were assigned to ASCT or IFNα after achievement of complete or partial remission with CHOP-like induction therapy. Patients in the ASCT arm experienced a significantly longer PFS versus IFNα (median 39 months versus 17 months, p=0.0108). The 3-year OS was 83% after ASCT versus 77% in the IFNα group (p=0.18). Recent Phase II studies suggest that the addition of rituximab and/or high dose cytarabine may significantly improve outcomes. Long-term follow up results from the MCL randomised trial comparing 6 courses of R-CHOP followed by myeloablative radiochemotherapy and ASCT (control arm A) versus alternating courses of 3x CHOP and 3x DHAP plus rituximab followed by a high dose cytarabine containing myeloablative regimen and ASCT (experimental arm B) have shown that, after induction, ORR was similar in both arms (90% versus 95%; p=0.19) but CR and CR/CRu rates were significantly higher in arm B (25% versus 36%; p=0.012 and 40% versus 54%; p=0.0003).[65] The number of patients transplanted was similar in both arms (72% versus 73%). After transplantation, ORR and CR rates were comparable in both arms (98% versus 97% and 63% versus 61%). Although CR rate after ASCT was similar in both arms, remission duration after ASCT was superior in arm B (49 months versus 84 months, p=0.0001). At the time of final analysis, OS was superior in arm B (p=0.045).

The LyMa trial of induction with 4 cycles of R-DHAP alone, without R-CHOP, reported interim data of 76% CR/CRu, prior to HDT/ASCT.[66] Results of the Groupe d'Etude des Lymphomes de l'Adulte Phase II trial using cytarabine and rituximab as the induction regimen before ASCT in patients <66 years of age with stage 3/4 MCL showed an ORR of 93% after R-CHOP and 95% after R-DHAP.[67] With a median follow-up of 67 months, median event-free survival was 83 months and median OS was not reached. Five-year OS is 75%. The role of cytarabine as part of the induction therapy for younger patients with MCL may soon be clarified by the large European MCL Network Phase III trial comparing R-CHOP with R-CHOP alternating with R-DHAP.

Research is ongoing to assess the incorporation of several novel targeted agents into combination regimens for frontline therapy in younger patients, such as temsirolimus, bortezomib and ibrutinib, as well as the rediscovery of bendamustine.

Bendamustine plus rituximab has been found to improve PFS versus R-CHOP (69.5 months versus 31.2 months, p<0.0001) in patients aged ≥18 years with a WHO performance status of ≤2 with newly diagnosed stage III or IV indolent or MCL.[15] Other studies assessing bendamustine are currently ongoing.

Two dose regimens of temsirolimus versus investigator's choice single-agent therapy has also been assessed in relapsed/refractory MCL.[48] Median PFS was 4.8, 3.4 and 1.9 months for the temsirolimus 175/75 mg, 175/25 mg and investigator's choice groups, respectively. Patients treated with temsirolimus 175/75 mg had significantly longer PFS than those treated with investigator's choice therapy (p=0.0009). However, median OS was not significantly different between the treatment groups.

A number of studies have investigated bortezomib in MCL. ORR ranged from 29-46%, CR/Cru ranged from 4-21%.[43, 68-71]

Single-agent ibrutinib has also been investigated in relapsed/refractory MCL patients, including those <65 years of age.[47] The patients were either bortezomib-naïve or bortezomib-exposed (prior treatment with at least two cycles of bortezomib). Response rates were similar between the two groups (Table 3).

Conclusions

In recent years, the introduction of rituximab and the rediscovery of bendamustine have provided significant benefits for patients with iNHL, including FL. However, many new and hopefully more effective drugs are being developed for progressive disease, including agents that target either cell surface antigens, intracellular pathways or the microenvironment. These are the treatments of the future. Many challenges exist in determining the optimal use of these novel agents, such as how to combine them with other therapies, management of toxicities and identifying which patient populations will benefit the most from these therapies.

For MCL, increases in treatment intensity and duration have yielded significant benefit for many patients. However, MCL continues to be characterised by a pattern of continuous relapse. Novel approaches, in particular with bortezomib, temsirolimus, everolimus, lenalidomide and ibrutinib, have shown promise in these settings, with many agents currently poised to make significant additional impact.

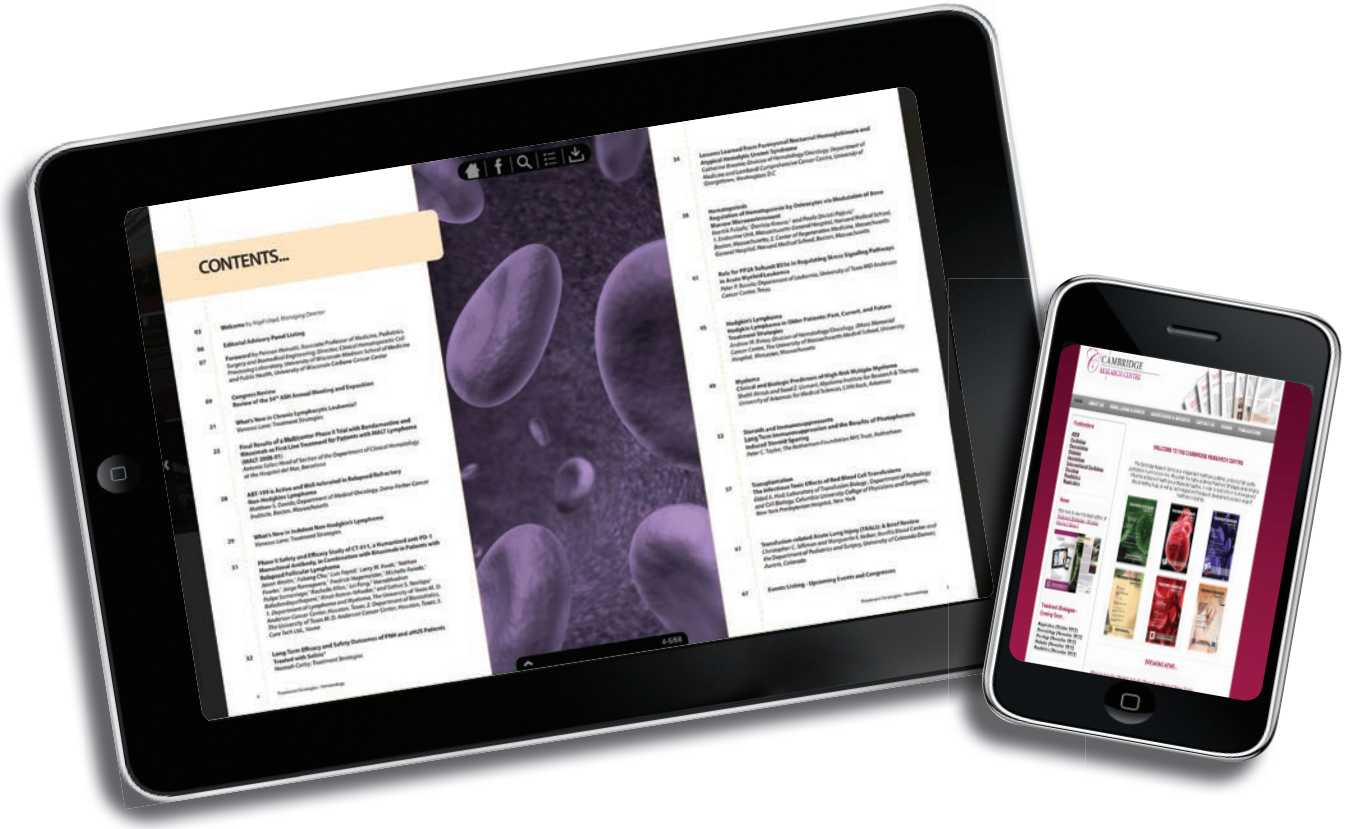
References

1. Armitage JO, Weisenburger DD. New approach to classifying non-Hodgkin's lymphomas: Clinical features of the major histologic subtypes. Non-Hodgkin's Lymphoma Classification Project. J Clin Oncol 1998;16:2780-2795.
2. Jemal A, Siegel R, Ward E *et al*. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71-96.
3. The Non-Hodgkin's Lymphoma Classification Project. A clinical evaluation of the International. Lymphoma Study Group classification of non-Hodgkin's lymphoma. Blood 1997;89:3909-3918.
4. Fisher RI, LeBlanc M, Press OW *et al*. New treatment options have changed the survival of patients with follicular lymphoma. J Clin Oncol 2005;23:8447-8452.
5. Herold M. Addition of rituximab to first-line MCP (mitoxantrone, chlorambucil, prednisolone) chemotherapy prolongs survival in advanced follicular lymphoma - 4 year follow-up results of a Phase III trial of the East German Study Group Hematology and Oncology (OSHO#39). Blood 2006;108:147:Abstract 483.
6. Ghielmini M, Vitolo U, Kimby E *et al*. ESMO Guidelines consensus conference on malignant lymphoma 2011. Part 1: diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) and chronic lymphocytic leukemia (CLL). Ann Oncol 2013;24:561-576.
7. Fisher RI *et al*. Lymphoma Annual Update 2005;29-41.
8. Gascoyne R. Lymphoma Annual Update 2005;5-20.
9. Humala K, Younes A. Current and emerging new treatment strategies for mantle cell lymphoma. Leuk Lymphoma 2013;54:912-921.
10. Dreyling M, Thieblemont C, Gallamini A *et al*. ESMO Consensus conferences: guidelines on malignant lymphoma. Part 2: Marginal zone lymphoma, mantle cell lymphoma, peripheral T-cell lymphoma. Ann Oncol 2013;24:857-877.
11. Brice P, Bastion Y, Lepage E *et al*. Comparison in low-tumor-burden follicular lymphomas between an initial no-treatment policy, prednimustine, or interferon alfa: A randomized study from the Groupe d'Etude des Lymphomes Folliculaires. J Clin Oncol 1997;15:1110-1117.
12. Ardeshna KM, Smith P, Norton A, *et al*. Long-term effect of a watch and wait policy versus immediate systemic treatment for asymptomatic advanced-stage non-Hodgkin lymphoma: a randomised controlled trial. Lancet 2003;362:516-522.
13. Rummel JM, Niederle N, Maschmeyer G, *et al*. Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. Lancet 2013; 381: 1203-10.
14. Federico M, Bellei M, Marcheselli L *et al*. Follicular lymphoma international prognostic index 2: A new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. J Clin Oncol 2009;27:4555-4562.
15. Salles G, Seymour JF, Offner F *et al*. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): A phase 3, randomised controlled trial. Lancet 2011;377:42-51.
16. Federico M, Luminari S, Dondi A *et al*. R-CVP versus R-CHOP versus R-FM for the initial treatment of patients with advanced-stage follicular lymphoma: results of the FOLL05 trial conducted by the Fondazione Italiana Linfomi. J Clin Oncol 2013;31:1506-1513.
17. Robinson KS, Williams ME, van der Jagt RH *et al*. Phase II multicenter study of bendamustine plus rituximab in patients with relapsed indolent B-cell and mantle cell non-Hodgkin's lymphoma. J Clin Oncol 2008;26:4473-4479.
18. Rummel MJ, Al-Batran SE, Kim SZ *et al*. Bendamustine plus rituximab is effective and has a favorable toxicity profile in the treatment of mantle cell and low-grade non-Hodgkin's lymphoma. J Clin Oncol 2005;23:3383-3389.
19. Rummel MJ, Niederle N, Maschmeyer G *et al*. Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: An open-label, multicentre, randomised, phase 3 non-inferiority trial. Lancet 2013;381:1203-1210.
20. http://clinicaltrials.gov/show/NCT00877214
21. Dana BW, Dahlberg S, Nathwani BN *et al*. Long-term follow-up of patients with low-grade malignant lymphomas treated with doxorubicin-based chemotherapy or chemoimmunotherapy. J Clin Oncol 1993;11:644-651.
22. Ezdinli EZ, Harrington DP, Kucuk O *et al*. The effect of intensive intermittent maintenance therapy in advanced low-grade non-Hodgkin's lymphoma. Cancer 1987;60:156-160.
23. Peterson BA, Petroni GR, Frizzera G, *et al*. Prolonged single-agent versus combination chemotherapy in indolent follicular lymphomas: a study of the cancer and leukemia group B. J Clin Oncol 2003;21:5-15.
24. Steward WP, Crowther D, McWilliam LJ *et al*. Maintenance chlorambucil after CVP in the management of advanced stage, low-grade histologic type non-Hodgkin's lymphoma. A randomized prospective study with an assessment of prognostic factors. Cancer 1988;61:441-447.
25. van Oers MH, Klasa R, Marcus RE *et al*. Rituximab maintenance improves clinical outcome of relapsed/resistant follicular non-Hodgkin lymphoma in patients both with and without rituximab during induction: Results of a prospective randomized phase 3 intergroup trial. Blood 2006;108:3295-301.
26. van Oers MH, Van Glabbeke M, Giurgea L *et al*. Rituximab maintenance treatment of relapsed/resistant follicular non-Hodgkin's lymphoma: Long-term outcome of the EORTC 20981 phase III randomized intergroup study. J Clin Oncol 2010;28:2853-2858.
27. Forstpointner R, Dreyling M, Repp R *et al*. The addition of rituximab to a combination of fludarabine, cyclophosphamide, mitoxantrone (FCM) significantly increases the response rate and prolongs survival as compared with FCM alone in patients with relapsed and refractory follicular and mantle cell lymphomas: Results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood 2004;104:3064-3071.
28. Czuczman MS, Grillo-Lo´pez AJ, White CA *et al*. Treatment of patients with low-grade B-Cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. J Clin Oncol 1999;17:268-276.
29. Witzig TE, Gordon LI, Cabanillas F *et al*. Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. J Clin Oncol 2002;20:2453-2463.
30. Hagenbeek A, Gadeberg O, Johnson P *et al*. First clinical use of ofatumumab, a novel fully human anti-CD20 monoclonal antibody in relapsed or refractory follicular lymphoma: Results of a phase 1/2 trial. Blood 2008;111:5486-5495.
31. Hiddemann W, Kneba M, Dreyling M *et al*. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: Results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood 2005;106:3725-3732.
32. Boland A, Bagust A, Hockenhull J *et al*. Rituximab for the treatment of relapsed or refractory stage III or IV follicular non-Hodgkin's lymphoma. Health Technol Assess 2009;13 Suppl 2:41-48.
33. Rummel MJ, Kaiser I, Balser C *et al*. Bendamustine plus rituximab versus fludarabine plus rituximab in patients with relapsed follicular, indolent and mantle cell lymphomas – final results of the randomized Phase III study NHL 2-2003 on behalf of the StiL (Study Group Indolent Lymphomas, Germany). ASH 2010:Abstract 856.
34. Czuczman MS, Fayad L, Delwail V *et al*. Ofatumumab monotherapy in rituximab-refractory follicular lymphoma: Results from a multicenter study. Blood 2012;119:3698-3704.
35. Reichert JM. Antibodies to watch in 2013: Mid-year update. MAbs 2013;5:513-517.
36. Leonard J, Coleman M, Ketas J *et al*. Phase I/II trial of epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma. J Clin Oncol 2003;21:3051-3059.
37. Leonard J, Schuster S, Emmanouilides C *et al*. Durable complete responses from therapy with combined epratuzumab and rituximab: Final results from an international multicenter, phase 2 study in recurrent, indolent, non-Hodgkin lymphoma. Cancer 2008;113:2714-2723.
38. Dang N, Smith M, Offner F *et al*. Anti-CD22 immunoconjugate inotuzumab ozogamicin (CMC-544) + rituximab: Clinical activity including survival in patients with recurrent/refractory follicular or 'aggressive' lymphoma. ASH 2009;114:584.
39. Fowler NH, Neelapu SS, Hagemeister FB *et al*. Lenalidomide and rituximab for untreated indolent lymphoma: Final results of a Phase II study. ASH 2012:Abstract 901.
40. Witzig T, Wiernik P, Moore T *et al*. Lenalidomide oral monotherapy produces durable responses in relapsed or refractory indolent non-Hodgkin's Lymphoma. J Clin Oncol 2009;27:5404-5409.
41. Coiffier B, Osmanov E, Hong X *et al*. Bortezomib plus rituximab versus rituximab alone in patients with relapsed, rituximab-naive or rituximab-sensitive, follicular lymphoma: A randomised phase 3 trial. Lancet Oncol 2011;12:773-784.
42. Fowler N, Kahl B, Lee P *et al*. Bortezomib, bendamustine, and rituximab in patients with relapsed or refractory follicular lymphoma: The phase II VERTICAL study. J Clin Oncol 2011;29:3389-3395.
43. Merli M, Ferrario A, Basilico C *et al*. Novel agents in indolent lymphomas. Ther Adv Hematol 2013;4:133-148.
44. Lenz G, Dreyling M, Hoster E *et al*. Immunochemotherapy with rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone significantly improves response and time to treatment failure, but not long-term outcome in patients with previously untreated mantle cell lymphoma: Results of a prospective randomized trial of the German Low Grade Lymphoma Study Group (GLSG). J Clin Oncol 2005;23:1984-1992.
45. Griffiths R, Mikhael J, Gleeson M *et al*. Addition of rituximab to chemotherapy alone as first-line therapy improves overall survival in elderly patients with mantle cell lymphoma. Blood 2011;118:4808-4816.
46. Kluin-Nelemans HC, Hoster E, Hermine O *et al*. Treatment of older patients with mantle cell lymphoma. N Engl J Med 2012;367:520-531.
47. Rummel MJ, Lerchenmüller C, Greil R, *et al*. Bendamustine-rituximab induction followed by observation or rituximab maintenance for newly diagnosed patients with Waldenström's macroglobulinemia: Results from a prospective, randomized, multicenter study (StiL NHL 7-2008 – MAINTAIN. ASH 2012:2739.
48. Visco C, Castegnaro S, Chieregato K *et al*. The cytotoxic effects of bendamustine in combination with cytarabine in mantle cell lymphoma cell lines. Blood Cells Mol Dis 2012;48:68-75.
49. Visco C, Finotto S, Zambello R *et al*. Combination of rituximab, bendamustine, and cytarabine for patients with mantle-cell non-Hodgkin lymphoma ineligible for intensive regimens or autologous transplantation. J Clin Oncol 2013;31:1442-1449.

50. Goy A, Bernstein SH, Kahl BS *et al*. Bortezomib in patients with relapsed or refractory mantle cell lymphoma: Updated time-to-event analyses of the multicenter phase 2 PINNACLE study. Ann Oncol 2009;20:520-525.
51. Ruan J, Martin P, Furman RR *et al*. Bortezomib plus CHOP-rituximab for previously untreated diffuse large B-cell lymphoma and mantle cell lymphoma. J Clin Oncol 2011;29:690-697.
52. Witzig TE, Geyer SM, Ghobrial I *et al*. Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. J Clin Oncol 2005;23:5347-5356.
53. Ansell SM, Inwards DJ, Rowland KM Jr *et al*. Low-dose, single-agent temsirolimus for relapsed mantle cell lymphoma: a phase 2 trial in the North Central Cancer Treatment Group. Cancer 2008;113:508-514.
54. Ansell SM. Beyond targeting malignant B cells. Lancet Oncol 2012;13:657-658.
55. Hess G, Herbrecht R, Romaguera J *et al*. Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. J Clin Oncol 2009;27:3822-3829.
56. Renner C, Zinzani PL, Gressin R *et al*. A multicenter phase II trial (SAKK 36/06) of single-agent everolimus (RAD001) in patients with relapsed or refractory mantle cell lymphoma. Haematologica 2012;97:1085-1091.
57. Habermann TM, Lossos IS, Justice G *et al*. Lenalidomide oral monotherapy produces a high response rate in patients with relapsed or refractory mantle cell lymphoma. Br J Haematol 2009;145:344-349.
58. Witzig TE, Vose JM, Zinzani PL *et al*. An international phase II trial of single-agent lenalidomide for relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma. Ann Oncol 2011;22:1622-167.
59. Goy A, Sinha R, Vose J *et al*. Single-agent lenalidomide in patients with relapsed/refractory mantle cell lymphoma following bortezomib: Efficacy, safety and pharmacokinetics from the multicenter Phase II MCL-001 "EMERGE" trial. EHA 2013: Abstract 3819.
60. Zaja F, De Luca S, Vitolo U *et al*. Salvage treatment with lenalidomide and dexamethasone in relapsed/refractory mantle cell lymphoma: clinical results and effects on microenvironment and neo-angiogenic biomarkers. Haematologica 2012;97:416-422.
61. Wang M, Fayad L, Wagner-Bartak N *et al*. Lenalidomide in combination with rituximab for patients with relapsed or refractory mantle-cell lymphoma: A phase 1/2 clinical trial. Lancet Oncol 2012;13:716-723.
62. Advani RH, Buggy JJ, Sharman JP *et al*. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. J Clin Oncol 2013;31:88-94.
63. Wang ML, Rule S, Martin P *et al*. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. N Engl J Med. 2013 Jun 19. [Epub ahead of print]
64. Dreyling M, Lenz G, Hoster E *et al*. Early consolidation by myeloablative radiochemotherapy followed by autologous stem cell transplantation in first remission significantly prolongs progression-free survival in mantle-cell lymphoma: Results of a prospective randomized trial of the European MCL Network. Blood 2005;105:2677-2684
65. Hermine O, Hoster E, Walewski J *et al*. Alternating courses of 3x CHOP and 3x DHAP plus rituximab followed by a high dose ARA-C containing myeloablative regimen and autologous stem cell transplantation (ASCT) increases overall survival when compared to 6 courses of CHOP plus rituximab followed by myeloablative radiochemotherapy and ASCT in mantle cell lymphoma: Final analysis of the MCL younger trial of the European mantle cell lymphoma network (MCL net). ASH 2012:Abstract 151.
66. Le Gouill S, Callanan M, Macintyre E *et al*. Clinical, metabolic and molecular responses after 4 courses of R-DHAP and after autologous stem cell transplantation for untreated mantle cell lymphoma patients included in the LyMa trial, a Lysa study. ASH 2012:Abstract 152.
67. Delarue R, Haioun C, Ribrag V *et al*. CHOP and DHAP plus rituximab followed by autologous stem cell transplantation in mantle cell lymphoma: A phase 2 study from the Groupe d'Etude des Lymphomes de l'Adulte. Blood 2013;121:48-53.
68. O'Connor OA, Wright J, Moskowitz C *et al*. Phase II clinical experience with the novel proteasome inhibitor bortezomib in patients with indolent non-Hodgkin's lymphoma and mantle cell lymphoma. J Clin Oncol 2005;23:676-684.
69. Goy A, Younes A, McLaughlin P *et al*. Phase II study of proteasome inhibitor bortezomib in relapsed or refractory B-cell non-Hodgkin's lymphoma. J Clin Oncol 2005;23:667-675.
70. Strauss SJ, Maharaj L, Hoare S *et al*. Bortezomib therapy in patients with relapsed or refractory lymphoma: potential correlation of in vitro sensitivity and tumor necrosis factor alpha response with clinical activity. J Clin Oncol 2006;24:2105-2112.
71. Belch A, Kouroukis CT, Crump M *et al*. A phase II study of bortezomib in mantle cell lymphoma: the National Cancer Institute of Canada Clinical Trials Group trial IND.150. Ann Oncol 2007;18:116-121.

CAMBRIDGE
RESEARCH CENTRE

Visit the Treatment Strategies Series online to view our eBooks, subscribe to the series and email the papers in PDF format.



Our eBooks are: -

- Free-to-view online
- Provide an exciting interactive experience
- Offer a wide range of dynamic features
- Easily accessible and user friendly

View our online publications at www.cambridgeresearchcentre.co.uk

Hodgkin Variant of Richter Syndrome in Chronic Lymphocytic Leukaemia

Krzysztof Jamroziak,[1] and **Anna Stępień**[2]
1. Department of Hematology, Medical University of Lodz, Lodz; 2. Laboratory of Clinical and Transplant Immunology and Genetics, Copernicus Memorial Hospital in Lodz, Lodz

The development of a secondary high-grade lymphoma during the course of chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) is referred as Richter syndrome (RS) or Richter's transformation.[1] RS defines an entity that is significantly heterogeneous in regard to genetic, molecular, immunological and clinical characteristics.[2, 3] As suggested by the term transformation, the majority of RS (circa 80% cases) have direct clonal relation to the pre-existing CLL/SLL.[4, 5] However, in the remaining cases the cell of origin is independent from the CLL/SLL clone.[6] Various histological subtypes of RS have been reported, of which the most common is secondary diffuse large B-cell lymphoma (DLBCL-RS). DLBCL-RS constitutes roughly 70% of all cases of RS and its molecular pathogenesis has recently been relatively well characterised.[7]

In contrast, less is known on other histological subtypes of RS transformation that occur with significantly lower frequency.[8-11] The second most common subtype of RS is secondary Hodgkin lymphoma, also known as Hodgkin variant of Richter syndrome (HvRS).[12, 13] The most distinct and important clinical feature of HvRS as compared to primary Hodgkin lymphoma is its refractoriness to therapy and poor prognosis. Here, we briefly review epidemiology, ethiopathophysiology, and clinical presentation as well as current and potential therapeutic strategies in HvRS based on our clinical experience and observations published by others.[8, 10, 12-17]

Epidemiology

RS is commonly regarded as a rare diagnosis, though the incidence of this severe CLL/SLL complication may be underestimated. According to retrospective analyses and limited prospective data from clinical trials, histologically confirmed RS develops in 2-10% of patients with pre-existing CLL/SLL.[8, 10, 12, 18] Of note, CLL/SLL transformation can be distinguished from rapid CLL progression only by a biopsy of the involved organ that should demonstrate histological shift to aggressive lymphoma.[19] However, lymph node biopsy is not a routine procedure in every case of dynamic CLL/SLL progression. Furthermore, histological features of transformation may be present only in selected lymph nodes, especially when RS and CLL/SLL progression occur synonymously. Therefore, the real incidence of RS is likely to be higher than that which is reported.

It has been found that the cumulative prevalence of RS development steadily increases at a rate of 1% per year.[7] This can be related to current strongly immunosuppressive therapy of CLL/SLL that potentially promotes clonal selection and transformation. On the other hand, prolongation of CLL patient survival that has been achieved with rituximab or alemtuzumab-based immunochemotherapy also extended the time interval for secondary tumour development.[20]

HvRS is diagnosed significantly less often than DLBCL-RS. The incidence of this rare entity is estimated at around 10% of all cases of RS and circa 1% (from 0.4% to 2.3%) of all CLL/SLL patients.[8, 10, 12, 21, 22] The largest reported series of HvRS included 18 cases that is 0.4% of 4121 CLL/SLL patients who were screened through electronic database search in the University of Texas M. D. Anderson Cancer Center.[12] Recently, we have performed a retrospective analysis of 786 patients with CLL/SLL admitted to our centre during years 2000-2011 in order to evaluate the incidence and clinical course of RS.[10] For all patients in whom RS diagnosis was confirmed by histology or at least cytology and immunophenotyping, hospital records and ambulatory charts were reviewed to determine clinical presentation, laboratory results, treatment and outcome. We have identified 40 patients with confirmed diagnosis of RS that constituted 5.1% of all analysed CLL/SLL cases. The majority of these patients had DLBCL-RS histology, but in 6 patients HvRS was diagnosed, and one subject had plasmablastic lymphoma.

Krzysztof Jamroziak is an Assistant Professor in the Department of Haematology of the Medical University of Lodz, Poland. His primary research interest is in genetic epidemiology, pharmacogenetics and new therapeutic modalities in chronic lymphocytic leukaemia and multiple myeloma. He is also actively involved in clinical trials within the Polish Adult Leukaemia Study Group (PALG) and Polish Myeloma Group.

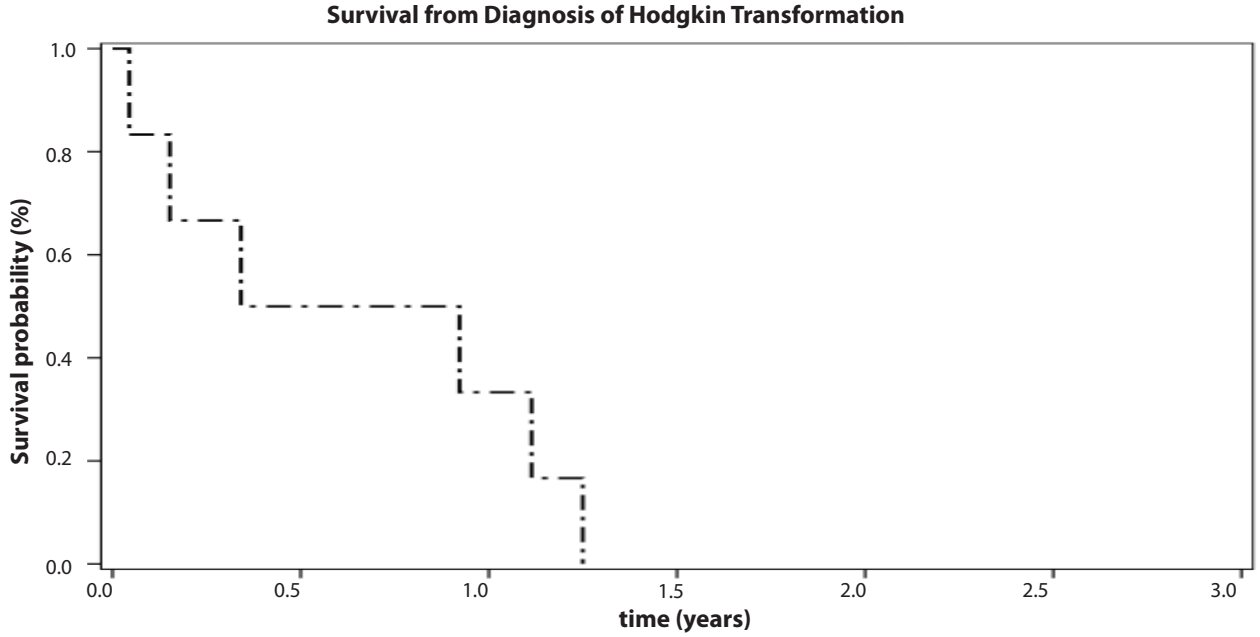


Figure 1. Overall survival from the time of transformation in six patients with Hodgkin variant of Richter syndrome identified by the retrospective analysis of 786 patients with CLL/SLL admitted to the Department of Hematology of Medical University of Lodz between 2000 and 2011. Median survival in this series was 7.6 months (range 0.5 – 15 months). Data were updated in regard to original report.[10]

Thus, in our series HvRS diagnosis accounted for 15% of RS cases and 0.8% of CLL/SLL patients.[10]

In a recent excellent comprehensive analysis of all HvRS cases and case series reported in the medical literature between 1975 and 2011, Bockorny *et al*.[8] identified 86 cases with diagnosis of HvRS. Based on an analysis of those cases they concluded that this entity predominantly affects older men.[8] The median age at Richter's transformation was 65.7 years (range 34-85), and HvRS developed at the median time interval of 4.3 years from diagnosis of CLL/SLL. The most common histological Hodgkin lymphoma subtype was mixed cellularity.[8] Results of analysis of our small series are in line with those epidemiological findings.[10] We identified 5 (83%) males and one female (17%) with median age at transformation of 70.6 years (range 55-76). HvRS was diagnosed after a median of 2.6 years (range 1.2-4.0) from CLL/SLL diagnosis. Histological subtypes of Hodgkin lymphoma were mixed cellularity in four patients, nodular sclerosis in one patient and not available for one patient.[10]

Etiopathogenesis

The etiology of HvRS is not well understood. Among factors that are suspected as events contributing to transformation are considered treatment of CLL/SLL with purine analogs (fludarabine, cladribine or pentostatin)-based chemotherapy and infection with Epstein-Barr virus.[23-27] The immunosuppression caused by treatment with purine analogs is a potential factor that drives Epstein-Barr virus reactivation and transformation of CLL/SLL cells.[28] The relation of previous treatment with fludarabine and Ebstein-Barr immunological status in HvRS was tested in the retrospective analysis of all reported HvRS cases, but the results were inconclusive, possibly due to limited number of cases.[8] In our series all patients received cladribine-based regimens before transformation, and a trend suggesting more common preceding treatment with purine analog as compared to DLBCL was noted (p=0.063).[10] Therefore, the role of purine analog therapy dependent immunosuppression and Epstein-Barr virus infection in the etiopathogenesis of this rare event remains to be clarified.[29-31]

The pathogenesis of RS has been studied in more detail regarding DLBCL-RS subtype.[7] Similarly to HvRS, this entity includes two different conditions: DLBCL-RS that is clonally derived from CLL or less common DLBCL-HS unrelated to the CLL clone. During the transition from CLL to clonally related DLBCL-RS novel genetic alterations are acquired.[7] This may be the explanation for the high level of chemotherapy resistance of this type of RS. Interesting data were also reported regarding predictive factors of RS in CLL. Conventional predictors of DLBCL-RS include expression of CD38, absence of del13q14, and a lymph node size greater than 3 cm.[4] Among novel factors that correlate with elevated risk of transformation to DLBCL-RS there were identified the host genotype of the CD38 locus and of other genes; telomere length of CLL/SLL cells, stereotypy B-cell receptors and usage of specific immunoglobulin variable genes (IGHV4-39).[32] It is not known, however, whether the same factors are also predictive of HvRS development.

Clinical Presentation and Diagnosis

According to our clinical experience and reports of cases and case series published by other researchers, HvRS presents typically as a rapidly growing peripheral or/and visceral lymphadenopathy or splenomegaly with concomitant general symptoms including fatigue, fever, night sweats and loss of weight.[10, 12] However, atypical non-lymphoid sites of involvement were also reported.[33, 34] Due to rapid progression the disease is typically diagnosed at an advance stage. In our series of six patients in whom HvRS developed after cladribine chemotherapy, 100% of patients presented with extensive disease involvement including

three patients with Ann Arbor Hodgkin lymphoma classification stage IIIB and three patients with stage IVB.[10] Moreover, in two patients concomitant progression of CLL/SLL was documented. Accordingly, in the large analysis of all published HvRS cases 29/35 patients (83%) were classified as Ann Arbor stage III or IV.[8]

Diagnosis of RS requires a histological assessment of the material obtained from the suspected organ, most commonly by biopsy of an enlarged lymph node or trephine bone marrow biopsy.[19] Although not supported by guidelines, in selected situations and in the experienced hands, cytology and immunophenotyping of a specimen obtained through fine-needle aspiration may also be considered.[35] Histological examination may reveal two types of possible transformation including presence of Reed-Stenberg cells scattered on the background of CLL/SLL clone or picture of Reed-Stenberg cells on the background of inflammatory infiltration as seen in primary Hodgkin lymphoma.[36, 37]

Treatment and Prognosis

Due to extremely rare occurrence of HvRS, no randomised trials comparing different treatment approaches could be performed. According to our experience and published reports, the most used first-line treatment strategies for HvRS are based on Hodgkin lymphoma-like protocols. Unfortunately, such treatment seems to have low efficacy in majority of cases.[8, 10]

In our single-centre series 5 out of 6 patients with HvRS received regimens typical for Hodkin lymphoma, most often ABVD (adriamycin, bleomycin, vinblastine, and dacarbazine) combination that was given in 50% of cases. Such chemotherapy resulted in only two partial remissions, while four patients did not respond.[10] Regarding long-term outcomes, at the time of our previous report one patient remained alive and on treatment, while five patients died at 0.5, 2, 4, 11 and 13 months from Richter's transformation.[10] With the present update, the last patient died at 15 months due to active HvRS complicated by pneumonia and massive fungal mucositis. Thus, in this series median overall survival duration from the time of transformation reached only 7.6 months (range 0.5 – 15 months). The updated survival probability curve for this small series of HvRS cases is illustrated in Figure 1.

In the retrospective analysis by Bockborny *et al*.,[8] the most commonly used treatment schedules were also Hodgkin lymphoma type regimens including ABVD administered to 31.3% of patients and MOPP (mechloretamine, oncovin, procarbazine and prednisone) received by 16.4% individuals.[8] Importantly, almost half of patients (47.3%) did not respond to such therapy. The overall survival reached 1.7 years (range (0-14). Interestingly, the patients in whom CLL/SLL had been treated with fludarabine had significantly shorter survival after transformation compared to the ones not treated with fludarabine.[8] The authors hypothesised that shorter survival may reflect a more aggressive course of HvRS in patients who had been treated with fludarabine. In agreement with this hypothesis, we have found that in our series in which all patients had received cladribine, the outcome was poor with overall survival even significantly shorter as compared to patients with DLBCL-RS (p=0.019).[10] Of note, despite several earlier case reports that indicated relatively satisfactory prognosis in HvRS, the median survival time in the largest series of HvRS, which included 85% fludarabine-treated patients, was only 0.8 years.[12-14] Treatment with purine analogs results with profound and prolonged T-cell depletion that could contribute both to selection of resistant CLL/SLL clones and subsequent transformation to HvRS.[8, 10]

Another possible reason of poor prognosis in HvRS is the treatment with non-intensive Hodgkin lymphoma therapy that is only based on histological similarity of two conditions. As can be concluded from our and others' reports, such low-dose alkylating agents-based combinations seem suboptimal, especially if CLL/SLL had been treated with purine analogs before transformation.[8, 10] Moreover, in a significant proportion of cases CLL/SLL progression is simultaneous with HvRS development, and purine analog-exposed CLL/SLL tumour cells are likely to be resistant to alkylating drugs-based therapy.

Interestingly, our retrospective analysis revealed no survival difference in patients with DLBCL-RS who received or not previous purine analog-containing therapy.[10] This may suggest that more intensive treatment, similar to immunochemotherapy or high-dose therapy used in DLBCL-RS may overcome negative prognostic influence of pretreatment with purine analogs.[18, 38]

On the other hand, it needs to be taken into account that HvRS is diagnosed predominantly in patients in their 7th decade, often with poor performance status and other concomitant diseases.[8, 10, 12] Administration of high-dose protocols or/and stem cell transplantation in such population is very risky or not feasible.[38] In this light, the more promising option would be immunochemotherapy in which monoclonal antibodies typically add little toxicity to the regimen. Interestingly, half of Hodgkin-RT express CD20, thus rituximab-based treatment can be effective in large proportion of patients.[39, 40] Another option is alemtuzumab, though this antibody is very immunosupressive and less effective against bulky lymph nodes. Finally, an interesting novel drug to be tested in this setting is anti-CD30 monoclonal antibody brentuximab vedotin.[41] Brentuximab vedotin was highly effective in resistant Hodkin lymphoma and anaplastic large cell lymphoma, and can be used as monotherapy that would allow treatment of elderly frail patients.[41] However, as far as we know brentuximab vedotin has not yet been tested in a patient with HvRS, thus its potential clinical utility in HvRS remains to be proven.

Conclusions

HvRS is a very rare but severe complication that significantly shortens

CLL/SLL patients' survival. This type of transformation typically affects elderly men, and presents as an progressive advanced stage lymphadenopathy with B-symptoms. Although chemotherapy regimens originally developed for primary Hodgkin lymphoma are most commonly used in HvRS, such treatment results in low response rates and poor long-term outcomes. Novel treatment strategies including immunochemotherapy need to be introduced to improve dismal prognosis of patients with HvRS.

References

1. Richter, MN. "Generalized reticular cell sarcomaof lymph nodes associated with lymphatic leukemia", Am. J. Pathol. (1928), 4, 285-292.
2. Rossi D, Gaidano G. "Richter syndrome: molecular insights and clinical perspectives", Hematol Oncol, (2009), 27, 1–10.
3. Tsimberidou, AM, Keating MJ. "Richter syndrome: biology, incidence, and therapeutic strategies", Cancer (2005), 103, 216–228.
4. Rossi D, Cerri M, Capello *et al*. "Biological and clinical risk factors of chronic lymphocytic leukaemia transformation to Richter syndrome", Br. J. Haematol (2008), 142, 202–215.
5. van den Berg A, Maggio E, Rust, *et al*. "Clonal relation in a case of CLL, ALCL, and Hodgkin composite lymphoma", Blood (2002), 100, 1425–1429.
6. de Leval L, Vivario M, De Prijck B *et al*. "Distinct clonal origin in two cases of Hodgkin's lymphoma variant of Richter's syndrome associated with EBV infection". Am. J. Sur. Pathol (2004), 28, 679–686.
7. Fangazio M, De Paoli L, Rossi D *et al*. "Predictive markers and driving factors behind Richter syndrome development", Expert Rev Anticancer Ther. (2011) Mar;11(3):433-42.
8. Bockorny B, Codreanu I, Dasanu CA. „Hodgkin lymphoma as Richter transformation in chronic lymphocytic leukaemia: a retrospective analysis of world literature", Br. J. Haematol. (2012), 156, 50-66
9. Duchayne E, Delsol G, Kuhlein E *et al*."Hairy cell transformation of a B-cellchronic lymphocytic leukemia: a morphological,cytochemical, phenotypic and molecular study", Leukemia (1991), 5, 150–155.
10. Jamroziak K, Grzybowska-Izydorczyk O, Jesionek-Kupnicka D *et al*. „Poor prognosis of Hodgkin variant of Richter transformation in chronic lymphocytic leukemia treated with cladribine", Br. J. Haematol. 2012 Jul;158(2):286-8;
11. Lee A, Skelly ME, Kingma DW *et al*. "B-cell chronic lymphocytic leukemia followed by high grade T-cell lymphoma.An unusual variant of Richter's syndrome", Am. J. Clin. Pathol. (1995), 103, 348–352.
12. Tsimberidou AM, O'Brien S, Kantarjian HM *et al*. "Hodgkin transformation of chronic lymphocytic leukemia: the M. D. Anderson Cancer Center experience", Cancer (2006), 107, 1294-1302.
13. Brecher M, Banks, PM "Hodgkin's disease variant of Richter's syndrome.Report of eight cases. Am. J. Clin. Pathol. (1990); 93, 333–339.
14. Serratrice De Roux C, Coso D, Bouabdallah R *et al*. "Chronic lymphocytic leukemia and Hodgkin's disease. Clinicopathologic study of three cases with good prognosis", Haematologica (2000), 85, 878-879
15. Choi H, Keller RH. "Coexistence of chronic lymphocytic leukemia and Hodgkin's disease", Cancer (1981), 48, 48–57
16. Ekstrand BC, Lucas JB, Horwitz SM *et al*. "Rituximab in lymphocyte- predominant Hodgkin disease: results of a phase 2 trial", Blood (2003), 101, 4285–4289.
17. Dujardin F, Lefrancq T, Blechet C *et al*. "Hodgkin's disease variant of Richter's syndrome. two cases and literature review", Ann. Pathol. (2008), 28, 311–316.
18. Molica SA. "Systematic review on Richter syndrome: what is the published evidence?" Leukemia&Lymphoma (2010), 51, 415-421.
19. Hallek M, Cheson BD, Catovsky D *et al*. "Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines", Blood (2008), 111, 5446–5456.
20. Cheson, BD, Vena, DA, Barrett, J *et al*. "Second malignancies as a consequence o nucleoside analog therapy for chronic lymphoid leukemias", J. Clin. Oncol. (1999)., 17, 2454– 2460.
21. Juneja S, Carney D, Ellis D *et al*. "Hodgkin's disease type Richter's syndrome in chronic lymphocytic leukemia", Leukemia (1999), 13, 826–827.
22. Weisenberg E, Anastasi J, Adeyanju M *et al*. "Hodgkin's disease associated with chronic lymphocytic leukemia. Eight additional cases, including two of the nodular lymphocyte predominant type", Am. J. Clin. Pathol. (1995), 103,479–484.
23. Abruzzo LV, Rosales CM, Medeiros LJ *et al*. "Epstein–Barr virus-positive B-cell lymphoproliferative disorders arising in immunodeficient patients previously treated with fludarabine for low-grade B-cell neoplasms", Am. J. Surg. Pathol (2002), 26, 630–636.
24. Ansell SM, Li CY, Lloyd RV *et al*. "Epstein–Barr virus infection in Richter's transformation", Am. J. Hematol. (1999), 60, 99–104
25. Dolcetti R, Carbone A. "Epstein–Barr virus infection and chronic lymphocytic leukemia:a possible progression factor?", Infect. Agents Cancer (2010), 5, 22.
26. Fong D, Kaiser A, Spizzo G *et al*. "Hodgkin's disease variant of Richter's syndrome in chronic lymphocytic leukaemia patients previously treated with fludarabine", Br. J. Haematol. (2005), 129, 199–205.
27. Nemets A, Ben Dor D, Barry T *et al*. "Variant Richter's syndrome: a rare case of classical Hodgkin's lymphoma developing in a patient with chronic lymphocytic leukemia treated with fludarabine", Leukemia &Lymphoma (2003), 44, 2151–2154.
28. Lazzarino M, Orlandi E, Baldanti F *et al*. "The immunosuppression and potential for EBV reactivation of fludarabine combined with cyclophosphamide and dexamethasone in patients with lymphoproliferative disorders", Br. J. Haematol.(1999), 107, 877–882.
29. Momose H, Jaffe ES, Shin SS *et al*. "Chronic lymphocytic leukemia/ small lymphocytic lymphoma with Reed– Sternberg-like cells and possible transformation to Hodgkin's disease. Mediation by Epstein–Barr virus", Am. J. Surg. Pathol. (1992), 16, 859–867.
30. Petrella T, Yaziji N, Collin F *et al*. "Implication of the Epstein–Barr virus in theprogression of chronic lymphocytic leukaemia/small lymphocytic lymphoma to Hodgkin-like lymphomas", Anticancer Research (1997), 17, 3907–3913.
31. Thornton PD, Bellas C, Santon A *et al*. "Richter's transformation of chronic lymphocytic leukemia. The possible role of fludarabine and the Epstein–Barr virus in its pathogenesis", Leukemia Research (2005), 29, 389–395.
32. Aydin S, Rossi D, Bergui L *et al*. "CD38 gene polymorphism and chronic lymphocytic leukemia: a role in transformation to Richter syndrome?", Blood (2008), 111, 5646–5653.
33. Almhanna K, Wongchaowart N, Sweetenham J. "Intracerebral Hodgkin's lymphoma in a patient with chronic lymphocytic leukemia/small lymphocytic lymphoma: a case report and literature review", Cancer Investigation (2009), 27, 215–220.
34. Reddy N, Thompson-Arildsen MA. "Hodgkin's lymphoma: Richter's transformation of chronic lymphocytic leukemia involving the liver", J. Clin. Oncol. (2010) 28, 543–544.
35. Simpson JL, Moriarty AT, Earls J *et al*. "Hodgkin's disease variant of Richter's syndrome. Report of a case with diagnosis by fine needle biopsy", Acta Cytologica (1997), 41, 823–829.
36. Pescarmona E, Pignoloni P, Mauro FR *et al*. "Hodgkin/ Reed–Sternberg cells and Hodgkin's disease in patients with B-cell chronic lymphocytic leukaemia: an immunohistological, molecular and clinical study of four cases suggesting a heterogeneous pathogenetic background", Virchows Archiv (2000), 437, 129–132.
37. Kanzler H, Kuppers R, Helmes S *et al*. "Hodgkin and Reed–Sternberg-like cells in B-cell chronic lymphocytic leukemia represent the outgrowth of single germinal-center B-cellderived clones: potential precursors of Hodgkin and Reed–Sternberg cells in Hodgkin's disease", Blood (2000), 95, 1023–1031.
38. Tsimberidou AM, O'Brien S, Khouri I *et al*. "Clinical outcomes and prognostic factors in patients with Richter's syndrome treated with chemotherapy or chemoimmunotherapy with or without stem-cell transplantation", J. Clin. Oncol. (2006), 24, 2343–2351.
39. Al-Ali HK, Wittekind C, Niederwieser D. "Complete remission of relapsed mixed cellularity Hodgkin's disease treated with rituximab", Deutsche medizinische Wochenschrift (2007), 132, 1688–1691.
40. Keilholz U, Szelenyi H, Siehl J *et al*. „Rapid regression of chemotherapy refractory lymphocyte predominant Hodgkin's disease after administration of rituximab (anti CD20 monoclonal antibody) and interleukin-2", Leukemia&Lymphoma (1999), 35, 641– 642.
41. Younes A, Bartlett NL, Leonard JP *et al*. (2010) "Brentuximabvedotin (SGN-35) for relapsed CD30-positive lymphomas", N. Eng. J. Med., 363, 1812-1821.

Management of Comorbid/Elderly Patients with Chronic Lymphocytic Leukaemia: The Role of Dose-reduced Fludarabine Combination Regimens

Lukáš Smolej
4th Department of Medicine - Hematology, Charles University Hospital and Faculty of Medicine, Hradec Králové

Introduction

Chronic lymphocytic leukaemia (CLL), the most predominant adult leukaemia in the Western hemisphere,[1-3] is still considered incurable in spite of extraordinary progress seen in the last two decades. Allogeneic stem cell transplantation, the only therapeutic method with curative potential, is feasible in highly selected, young and fit patients only. The combination of monoclonal antibodies with chemotherapy represents a major breakthrough in the management of CLL. Recently, two large randomised studies proved that the addition of monoclonal anti-CD20 antibody rituximab to fludarabine and cyclophosphamide (FCR) was associated with a significantly higher overall response rate (ORR), complete responses (CR) and longer progression-free survival (PFS);[4, 5] moreover, patients treated with FCR in first-line achieved significantly longer overall survival – the first clinical study in the history of CLL treatment to achieve this endpoint. Therefore, FCR protocol is currently accepted as the gold standard in the treatment of physically fit CLL patients. However, the results of these studies are not applicable to the general CLL population as young and fit patients treated within these trials represent less than 50% of all CLL patients (Table 1),[1-11] as the median age at diagnosis of CLL lies between 65 and 72 years.[1-3, 12] Age has also been reported as one of predictive factors for overall survival.[13-14] Interestingly, a retrospective analysis of patients treated at Hospital Clinic in Barcelona showed that overall survival in patients ≥ 70 years has not significantly improve in the last decade in comparison to younger patients.[15] Therefore, it is not clear whether older and/or comorbid patients profit from novel treatments such as purine analog- based chemoimmunotherapy.[16]

Lukáš Smolej is Associate Professor of Internal Medicine at the Charles University of Prague, Faculty of Medicine, Hradec Králové, Czech Republic, and Head of the Hematology Laboratory at 4th Department of Medicine – Hematology, University Hospital, Hradec Králové, Czech Republic. His research interests include novel prognostic factors in chronic lymphocytic leukaemia, modern chemoimmunotherapy and use of high-dose steroids in CLL and treatment of elderly/comorbid CLL patients. Dr. Smolej currently serves as the Chairman of Czech Study Group for Chronic Lymphocytic Leukaemia (CCLLSG) and is a member of multiple national and international societies including European Hematology Association (EHA) and European Research Initiative on CLL (ERIC). He has authored or co-authored more than 50 articles in peer-reviewed journals including *Blood*, *Cancer*, *Haematologica*, *Annals of Oncology*, *Leukemia Research*, and *European Journal of Haematology*.

Specific Issues in Elderly/Comorbid Patients

In elderly patients, organ function decreases with advancing age. This is particularly true for kidneys. Creatinine clearance decreases at the rate of approximately 1% yearly.[17] Muscle mass and urinary creatinine excretion decrease at a similar rate with advancing age, while mean serum creatinine concentrations may stay almost constant for a long time in spite of decreasing renal function. Therefore, the measurement of serum creatinine is not reliable in elderly patients.[17] Such information has important implications because most cytotoxic agents are excreted via the kidneys.[18] Renal function can be conveniently calculated using Cockroft-Gault formula.[19] Cancer and Leukemia Group B (CALGB) study 9011 showed that creatinine clearance ≤ 80ml/min. but not age was predictive of higher toxicity of treatment in untreated patients who received fludarabine monotherapy.[20] Comorbidities in general have an unfavourable effect on the survival of cancer patients.[21] A population-based study specifically focused on the role of comorbidities in patients with malignant lymphomas reported shorter overall survival in patients with severe comorbidities.[22] In contrast, a single-centre analysis by Mayo Clinic identified age rather than comorbidities as a significant factor for overall survival.[13] The study was, however, influenced by the fact that comorbid diseases were assessed at the time of CLL diagnosis and not at the time of treatment (many patients require treatment for CLL only years after diagnosis and comorbidities may occur/worsen meanwhile). In an analysis of patients enrolled in two phase III trials by a German CLL Study Group, there was a trend towards shorter survival in significantly comorbid patients (with multiple comorbidities or with severe comorbid diseases); independent of age.[23]

The intensity of treatment should be tailored to the individual patient´s overall condition/fitness/comorbidities, and therapeutic goals should

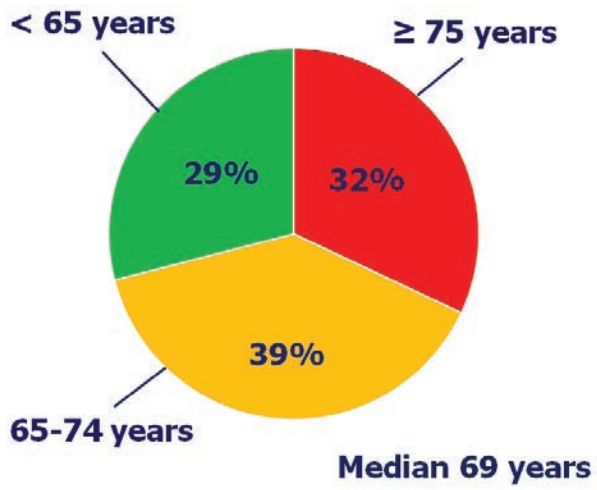


Figure 1. Age distribution of unselected CLL population; data from 4th Department of Medicin - Hematology, University Hospital, Hradec Kralove, Czech Republic.

be selected accordingly. Eastern Cooperative Oncology Group (ECOG) performance status is a valuable tool; however, it does not provide information about the number and severity of comorbid conditions. Ideally, elderly patients should be evaluated for functional capacity and comorbidities using Comprehensive Geriatric Assessment (CGA).[24] One of the possibilities is to use the Cumulative Illness Rating Scale (CIRS).[25] This system scores the number and severity of comorbidities in each of the organ system, and its predictive power has been demonstrated in patients with solid tumours.[26] A threshold of 6 points is used in German CLL Study Group trials to distinguish between fit and significantly comorbid patients. Patients aged 65-70 (possibly up to 75) in a good general condition, performance status 0-1, CIRS score ≤ 6 creatinine clearance ≥ 70ml/min. and no severe comorbidities (e.g., advanced cardiac failure or ischemic heart disease) represent the best candidates for a more intensive approach such as full-dose FCR. Health-related quality of life (HR-QoL) is an extremely important endpoint in elderly/comorbid CLL patients given the incurability of the disease and potential side effects of treatment. Unfortunately, patient-related outcomes (PRO) have not been included among standard assessments within clinical trials in CLL until recently. Advanced CLL is associated with a significant disturbance of HR-QoL and the achievement of therapeutic response resulted in improvement of HR-QoL.[27,28]

Treatment Options for Elderly/Comorbid CLL Patients

First-line Treatment

Chlorambucil can still be considered to be the treatment of choice in frail patients because no other regimen has proved to be superior. Several schedules of chlorambucil have been used: a) low dose, 0.1 mg/kg continuously, b) pulse schedule, 0.4-0.8 mg/kg every 2 weeks (used by German CLL Study Group),[29] c) 10 mg/m^2 day 1-7 repeated every 28 days for up to 12 cycles - used in British studies,[6] d) 40 mg/m^2 once monthly - used by CALGB.[10] Overall response rate and progression-free survival ranged from 37 to 72% and 9 to 20 months, respectively, depending on the patient population; complete responses were infrequent. A higher dose of chlorambucil per cycle seems to be more effective in historical comparison, but there has never been randomised trials comparing different chlorambucil schedules. The only trial which specifically focused on the elderly CLL population so far was the German CLL Study Group CLL-5 study. Fludarabine monotherapy (25 mg/m^2 iv day 1-5, maximum 6 cycles) was compared to chlorambucil (0.4 mg/kg every 2 weeks, possible dose escalation by 0.1 mg/kg increments up to 0.8 mg/kg in subsequent cycles, maximum 24 cycles) in 193 untreated elderly patients (median age, 70 years). Dludarabine induced significantly more overall responses (ORR) and complete remissions (CR) than chlorambucil (ORR, 72 vs. 51%, p=0.003; CR, 7 vs 0%, p=0.011); however, this did not translate into longer progression-free survival (median 19 vs 18 months, p=0.7) and non-significantly shorter overall survival in fludarabine-treated patients (median 46 months vs 64 months, p=0.15). These results could be explained by the fact that significantly less patients in the fludarabine group were retreated on relapse/progression (50 vs 77%; p=0.006).[29] German CLL Study Group therefore considers chlorambucil as the standard treatment for elderly CLL patients. Results of CLL-5 study underscore the need for specifically targeted clinical trials, as it is not possible to simply extrapolate data from trials involving different patient population.

Contrary to German CLL-5 trial, a subanalysis of British CLL4 trial (chlorambucil vs. fludarabine vs. FC in untreated CLL) showed that FC combination was better than chlorambucil in terms of ORR, CR and PFS in all subgroups including patients ≥ 70 years (approximately 30% of

First Author	Year	Study Design	Median Age	Reference
Rai	2001	F vs CLB vs F+CLB	64/62/63	10
Eichhorst	2006	FC vs F	58/59	7
Catovsky	2007	FC vs F vs. CLB	64/65/65	6
Flinn	2007	FC vs F	61/61	8
Knauf	2009	B vs CLB	64/64	9
Hallek	2009	FCR vs FC	61/61	4
Robak	2010	FCR vs FC	62/63	5
Robak	2010	FC vs CC	59/58	11

Table 1. Median age in randomised studies in CLL is markedly lower than in general CLL population. Median age in each randomisation group.
F = fludarabine; CLB = chlorambucil; FC = fludarabine and cyclophosphamide; B = bendamustine; FCR = fludarabine, cyclophosphamide, and rituximab; CC = cladribine and cyclophosphamide.

First Author	Year	n	Median Age	Line of Treatment	Schedule	ORR/CR (%)	Myelotoxicity	Other Toxicity
Bocchia	1999	30*	68	Rel/Ref, UT	F 15mg/m^2 i.v. D1-4, C 250mg/m^2 i.v. D1-4, E 60mg/m^2 i.v. D1 q28d	81/36	ANC gr.IV 43%	FUO 26 %
Marotta	2000	20	75	Rel/Ref	F 15mg/m^2 i.v. D1-4, C 200mg/m2 i.v. D1-4 q28d	85/15	ANC gr.IV 20%	1x infection
Fabbri	2004	28**	74	Rel/Ref	F 25mg/m^2 p.o. D1-4, C 150mg/m^2 p.o. D1-4 q28d	100/0	NS	gr.IV, 1x TLS
Fabbri	2007	25***	74	UT	F 25mg/m^2 p.o. D1-4, C 150mg/m^2 p.o. D1-4 q28d	88/50	ANC gr. III/IV 16%	1x fatal sepsis 3xFUO, 1x pneumonia
Forconi	2008	14	71	UT	25mg/m^2 p.o. D1-4, C 120mg/m^2 p.o. D1-4 q28d	100/62	ANC gr. III/IV 21%	-
Forconi	2008	12	71	Rel/Ref	F 25mg/m^2 p.o. D1-4, C 120mg/m^2 p.o. D1-4 q28d	84/25	ANC gr. III/IV 25%	2x pneumonia

Table 2. Results of studies evaluating low-dose fludarabine-based regimens in the treatment of CLL and small lymphocytic lymphoma (SLL). UT = untreated; Rel/Ref = relapsed/refractory; F = fludarabine; C = cyclophosphamide; E = epirubicin; D = day; ANC = neutropenia; NS = not stated; FUO = fever of unknown origin; Ref. = reference. *patients with indolent lymphoma including 11 cases of SLL; **patients with indolent lymphoma including 4 cases of SLL; ***patients with indolent lymphoma including 8 cases of SLL.

study population). Overall survival was not significantly different. FC-treated patients had more myelotoxicity but there was no significant difference in infections. It is necessary to emphasise that CLL4 trial was not specifically designed for elderly patients.[6]

Fludarabine Combination Regimens in Attanuated Doses

The most dreaded complications in elderly/comorbid population treated with intensive fludarabine-based chemo(immuno)therapy are myelotoxicity and infections. Ferrajoli *et al*. treated 125 patients older than 70 years by FC or FCR: grade III/IV myelotoxicity occured in 60% patients treated by FC and 82% patients treated by FCR; severe infections were recorded in 42% and 22%; median number of cycles was 2 for FC and 3 for FCR.[30] The Israeli Group on CLL reported unacceptable toxicity in 82 relapsed/refractory CLL patients treated with fludarabine monotherapy or combinations (median age, 70). ORR/CR was lower in older patients (59/0% vs 80/20%) and severe bacterial infections were seen in 44% and neutropenic fever in 25% patients, leading to at least one hospitalisation in 63% of patients. Only 31% of patients completed the planned treatment.[31]

Dose-reduced fludarabine protocols represent a logical option with the aim of milder toxicity but preserved efficacy. Several pilot single-centre studies reported promising efficacy and acceptable toxicity of low-dose fludarabine-based combinations,[32-36] (Table 2).

The next step was the addition of monoclonal antibodies to chemotherapy. The "FCR-Lite" protocol developed used low-dose FC (fludarabine 20 mg/m^2 and cyclophosphamide 150 mg/m^2 i.v. day 1-3) and double dose of rituximab (500 mg/m^2 day 1 and 14 of the cycle, repeated every 28 days) with maintenance treatment with rituximab 500 mg/m^2 every three months for 2 years.[44] Results in a highly selected group of 49 untreated patients were impressive: ORR/CR was achieved in 100/85%; 7/8 patients in CR achieved MRD-negativity. Grade III/IV neutropenia occured in 12% of cycles only. Unfortunately, the patients were neither elderly nor significantly comorbid: median age was 58 years, patients were mostly in intermediate Rai stages and had excellent performance status; thus, results of this study are not relevant for the elderly/comorbid CLL patient population. The largest study using dose-reduced FCR in elderly/comorbid CLL patients is currently protocol "Q-lite" of the Czech CLL Study Group. Preliminary results are available on 182 patients (CLL, n=172, SLL, n=10) with median age of 70 years. Dose reduction of chemotherapy in comparison to full-dose FCR was: 50% of fludarabine dose (12 mg/m^2 i.v. or 20 mg/m^2 orally on day 1-3) and 60% of cyclophosphamide dose (150 mg/m^2 i.v./p.o. on days 1-3). Rituximab was administered in the standard schedule (375 mg/m^2 i.v. day 1 in 1st cycle, 500 mg/m^2 i.v. day 1 from 2nd cycle). Treatment was repeated every 4 weeks. Antimicrobial prophylaxis with

sulfamethoxazol/trimethoprim and aciclovir or equivalents was routinely used. ORR/CR (including clinical CR [without bone marrow biopsy] and CRi [with incomplete marrow recovery]) was 80/38% in first-line (n=92) and 69/32% in relapsed/refractory setting (n=90). Serious (CTCAE grade III/IV) neutropenia was frequent but serious infections occurred in 14% of patients.[38]

The Australasian Research CLL Consortium is currently running a CLL5 trial which compares standard FCR, fludarabine+rituximab and dose-reduced FCR (fludarabine 24 mg/m^2 and cyclophosphamide 150 mg/m^2 orally on day 1-3, rituximab in standard CLL dose) in previously untreated CLL patients ≥ 65 years. Preliminary results on 94 patients (median age, 73 years, 60% males, Binet C stage in 36%) suggest very good efficacy (ORR 89.8% in pooled patient population) and acceptable toxicity. All 6 cycles of treatment were completed by 44% of patients.[39] A phase II trial testing the low-dose FC is also currently running in Israel.[40] A combination of pentostatin (2 mg/m^2), cyclophosphamide (600 mg/m^2), and rituximab (375 mg/m^2 was tested in 64 untreated patients with CLL.[41] Eighteen patients were ≥ 70 years and 25 had creatinine clearance ≤ 70 ml/min. These subgroups did not have significantly worse ORR/CR or more frequent cytopenia; older patients still required more treatment delays and individuals with impaired renal function needed more dose reductions; in addition, the study was not primarily designed for elderly patients. In summary, further data are needed to address the question whether pentostatin could be more suitable than fludarabine in this scenario.

Relapsed/Refractory CLL

In the case of relapsed CLL with PFS ≥ 12 months, it is usually recommended to repeat the previous treatment line as the sensitivity to treatment is maintained in majority of patients. Management of refractory CLL, however, is a truly challenging mission.[42] Moreover, results specific for elderly/comorbid patients are very rare. Monoclonal anti-CD52 antibody alemtuzumab is approved for fludarabine-refractory CLL. ORR is 30-40% but responses often last less than 12 months.[43] Patients refractory to fludarabine and alemtuzumab or fludarabine-refractory with bulky lymphadenopathy have a highly unfavourable prognosis.[44] One of the most promising treatment possibilities for these patients is a combination of high-dose glucocorticoids with monoclonal antibodies. A combination of high-dose methylprednisolone (HDMP, of 1 g/m^2 i.v. day 1-5) with rituximab (375 mg/m^2 i.v. day 1 repeated every 4 weeks) was explored in 14 heavily pretreated patients, six of them ≥65 years.[45] ORR was 93% with 2 CRs. Responses were short (median PFS, 7 months) and serious infections developed in spite of combined prophylaxis in 6 patients. Authors from Mayo Clinic treated 37 relapsed CLL patients with HDMP plus weekly rituximab. ORR was 78% and median PFS 12 months. Five patients died of infections during the first month of treatment.[46] Castro *et al*. treated 14 fludarabine-refractory patients by 3 cycles of R-HDMP. ORR/CR were 93/36%; median PFS 15 months and median time to next treatment 22 months. Serious adverse events were relatively rare.[47] Two studies aimed to decrease the toxicity of glucocorticoids by attenuation of the dose. Dexamethasone (40 mg on day 1-4 repeated every 28 days) plus rituximab was used by Quinn *et al*. in six patients with similar results to those treated by HDMP but at a lower toxicity.[48] A two-centre retrospective study recently reported efficacy and safety of the same regimen using two different schedules of rituximab: I, 500 mg/m^2 day 1, 8, 15, 22 (375 mg/m^2 in 1st dose) every 4 weeks (n=29); II, 500 mg/m^2 day 1 (375 mg/m^2 in 1st cycle) repeated every 3 weeks (n=25). Dexamethasone was given 40 mg orally on day 1-4 and 10-13. ORR/CR was 62/24% and 69/4%. Serious infections developed in 32% patients. Median progression-free survival was 6 and 6.9 months.[49]

Ofatumumab, the first fully human anti-CD20 antibody with enhanced complement-dependent cytotoxicity and better *in vitro* killing of neoplastic lymphocytes, is approved in United States and Europe for the treatment of CLL patients refractory to fludarabine and alemtuzumab on the basis of a large international phase II trial.[50] A total of 138 patients (double refractory, n=59; bulky fludarabine refractory, n=79) were enrolled with no age limit. The median age was 64 and 62 years. The ofatumumab regimen consisted of 8 weekly intravenous infusions (1x 300 mg + 7x2000 mg) followed by four monthly doses of 2000 mg. ORR was 51% in double-refractory patients and 44% in bulky fludarabine-refractory group. Median PFS was 6 months and median OS was 14 and 15 months, respectively. Toxicity was acceptable: Infusion-related side effects (fever, rash etc.) were frequent (61-64 %) but predominantly mild. Infections developed in 67% of patients but only 26% were serious.

Novel Agents

The combination of chlorambucil and anti-CD20 monoclonal antibodies offers a new possibility for improvement of efficacy without significant increase in toxicity. British CLL208 trial treated 100 patients (median age, 70 years) with chlorambucil 10 mg/m^2 orally day 1-7 repeated every 28 days) and rituximab (500 mg/m^2, i.v. day 1; 375 mg/m^2 in 1st cycle) for 6 cycles. ORR was better with the combination in historical matched pair comparison to patients from CLL4 trial treated with chlorambucil alone (ORR, 82 vs. vs 66%); CR was 9% and PFS 23.5 months. Grade III/IV neutropenia was relatively frequent (39%); serious adverse events occured in 37% of patients; neutropenic sepsis/febrile neutropenia accounted for 5 and 4%.[51] Italian ML21445 study presented data on 54 patients (median age, 71 years) treated with the same combination (chlorambucil 8 mg/m2 p.o. days 1-7 + rituximab 375 mg/m^2 in cycle 3 and 500 mg/m^2 in cycles 4-8, repeated every 28 days, maximum 8 cycles). ORR/CR were 81/17%. Grade III-IV neutropenia occured in 17% of patients with no grade III-IV infections.[52] Neither of these studies evaluated comorbidities; therefore, it is not known how many patients could have been treated with more intensive regimens. Other eagerly expected studies include CLL-11, a phase III trial by German CLL Study Group comparing chlorambucil monotherapy, chlorambucil + rituximab and chlorambucil + obinutuzumab (GA-101), a novel class II anti-CD20 monoclonal antibody.[53] This study is specifically designed for

significantly comorbid CLL patients with CIRS score of >6 or significantly impaired renal function (creatinine clearance <70ml//min).[40] The combination of chlorambucil with ofatumumab vs chlorambucil monotherapy is tested in another phase III trial.[40]

Bendamustine is a unique molecule combining the properties of an alkylating agent and a purine analog. Bendamustine is approved in USA and Europe for the first-line treatment of CLL on the basis of better ORR/CR and PFS in comparison to chlorambucil monotherapy in a large phase III randomised trial.[9] The combination of bendamustine with rituximab was recently reported in 78 patients (median age, 67 years) with relapsed/refractory CLL. The regimen achieved ORR/CR in 59/9%. Median event-free survival was 15 months. Severe infections were seen in 13% of patients; grade III/IV neutropenia and thrombocytopenia occured in 23 and 28%.[53]

Lenalidomide, an orally available immunomodulatory drug targeting the bone marrow and lymph node microenvironment, was tested in 60 untreated elderly CLL patients (median age, 72 years). The drug was given continuously until disease progression, starting with 5 mg and possible dose escalation to 25 mg. ORR/CR was 65/10% and estimated 2-year PFS 60%. Grade III/IV neutropenia developed in 34% of treatment cycles; serious infections/febrile neutropenia occurred in 12% of patients. Tumour flare grade I/II developed in 52% of patients, but there were no severe cases of tumour flare or tumour lysis syndrome.[54] A large phase III randomised trial compares chlorambucil monotherapy to lenalidomide in older untreated CLL patients .[40]

Conclusions

The lack of data from randomised trials make the treatment decisions in elderly/comorbid CLL patients difficult. Chlorambucil monotherapy can still be considered standard in this patient population until randomised trials evaluating chlorambucil ± anti-CD20 antibodies, monotherapy with lenalidomide and other agents prove otherwise. Comprehensive geriatric assessment should help us identify comorbid patients with higher risk of complications. Creatinine clearance is highly important for patients treated with fludarabine-based protocols. Low-dose fludarabine combinations achieved promising results in pilot studies; therefore, confirmation in large randomised trials is necessary. Treatment options in refractory CLL include alemtuzumab, ofatumumab and high-dose glucocorticoids ± rituximab. Novel agents and treatment combinations will hopefully lead to prolongation of overall survival and improvement in quality of life of elderly/comorbid CLL patients in the near future.

Acknowledgements and Conflict of Interest Statement

The author has received honoraria and/or travel grants from Roche, Bayer-Schering Pharma, Sanofi and GlaxoSmithKline. This work was supported by grant IGA NT/13412-4 from Ministry of Health, Czech Republic.

References

1. Dores GM, Anderson WF, Curtis RE, *et al*. Chronic lymphocytic leukaemia and small lymphocytic lymphoma: overview of the descriptive epidemiology. Br J Haematol. 2007;139(5):809-19.
2. Linet MS, Schubauer-Berigan MK, Weisenburger DD, *et al*. Chronic lymphocytic leukaemia: an overview of aetiology in light of recent developments in classification and pathogenesis. Br J Haematol. 2007;139(5):672-86.
3. National Cancer Institute: SEER Cancer Statistics Review. Available at http://seer. cancer.gov. Accessed on 10.2.2010
4. Hallek M., Fischer K, Fingerle-Rowson G, *et al*. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet 2010; 376: 1164-74.
5. Robak T, Dmoszynska A, Solal-Céligny P, *et al*. Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. J Clin Oncol. 2010;28(10):1756-65
6. Catovsky D, Richards S, Matutes E, *et al*. Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. Lancet. 2007;370(9583):230-9.
7. Eichhorst BF, Busch R, Hopfinger G, *et al*. Fludarabine plus cyclophosphamide versus fludarabine alone in first-line therapy of younger patients with chronic lymphocytic leukemia. Blood. 2006;107(3):885-91.
8. Flinn IW, Neuberg DS, Grever MR, *et al*. Phase III trial of fludarabine plus cyclophosphamide compared with fludarabine for patients with previously untreated chronic lymphocytic leukemia: US Intergroup Trial E2997. J Clin Oncol. 2007;25(7):793-8.
9. Knauf WU, Lissichkov T, Aldaoud A, *et al*. Phase III randomized study of bendamustine compared with chlorambucil in previously untreated patients with chronic lymphocytic leukemia. J Clin Oncol. 2009;27(26):4378-84.
10. Rai KR, Peterson BL, Appelbaum FR, *et al*. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med. 2000;343(24):1750-7.
11. Robak T, Jamroziak K, Gora-Tybor J, *et al*. Comparison of Cladribine Plus Cyclophosphamide With Fludarabine Plus Cyclophosphamide As First-Line Therapy for Chronic Lymphocytic Leukemia: A Phase III Randomized Study by the Polish Adult Leukemia Group (PALG-CLL3 Study). J Clin Oncol. 2010;28(11):1863-9.
12. Diehl LF, Karnell LH, Menck HR. The American College of Surgeons Commission on Cancer and the American Cancer Society. The National Cancer Data Base report on age, gender, treatment, and outcomes of patients with chronic lymphocytic leukemia. Cancer. 1999;86(12):2684-92.
13. Thurmes P, Call T, Slager S, *et al*. Comorbid conditions and survival in unselected, newly diagnosed patients with chronic lymphocytic leukemia. Leuk Lymphoma. 2008;49(1):49-56.
14. Wierda WG, O'Brien S, Wang X, *et al*. Characteristics associated with important clinical end points in patients with chronic lymphocytic leukemia at initial treatment. J Clin Oncol. 2009;27(10):1637-43.
15. Abrisqueta P, Pereira A, Rozman C, *et al*. Improving survival in patients with chronic lymphocytic leukemia (1980-2008): the Hospital Clinic of Barcelona experience. Blood. 2009;114(10):2044-50
16. Eichhorst B, Goede V, Hallek M. Treatment of elderly patients with chronic lymphocytic leukemia. Leuk Lymphoma. 2009;50(2):171-8.
17. Lindeman RD. Changes in renal function with aging. Implications for treatment. Drugs Aging. 1992;2(5):423-31.
18. Plunkett W, Gandhi V, Huang P, *et al*. Fludarabine: pharmacokinetics, mechanisms of action, and rationales for combination therapies. Semin Oncol. 1993;20(5 Suppl 7):2-12.
19. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31-41.
20. Martell RE, Peterson BL, Cohen HJ, *et al*. Analysis of age, estimated creatinine clearance and pretreatment hematologic parameters as predictors of fludarabine toxicity in patients treated for chronic lymphocytic leukemia: a CALGB (9011) coordinated intergroup study. Cancer Chemother Pharmacol. 2002;50(1):37-45.
21. Piccirillo JF, Tierney RM, Costas I, *et al*. Prognostic importance of comorbidity in a hospital-based cancer registry. JAMA 2004;291:2441–2447.
22. Janssen-Heijnen ML, van Spronsen DJ, Lemmens VE, *et al*. A population-based study of severity of comorbidity among patients with non-Hodgkin's lymphoma: prognostic impact independent of

International prognostic index. Br J Haematol 2005;129: 597–606.
23. Cramer P, Goede V, Jenke P, *et al*. Impact of different chemotherapy regimen in comorbid patients with advanced chronic lymphocytic leukemia: metaanalysis of two phase-III-trials of the German CLL study group. Blood 2006;108 (abstract 2840)
24. Extermann M, Hurria A. Comprehensive geriatric assessment for older patients with cancer. J Clin Oncol. 2007;25(14):1824-31.
25. Linn BS, Linn MW, Gurel L. Cumulative illness rating scale. J Am Geriatr Soc. 1968;16(5):622-6
26. Extermann M, Overcash J, Lyman GH, *et al*. Comorbidity and functional status are independent in older cancer patients. J Clin Oncol 1998;16:1582–1587.
27. Else M, Smith AG, Cocks K, *et al*. Patients' experience of chronic lymphocytic leukaemia: baseline health-related quality of life results from the LRF CLL4 trial. Br J Haematol. 2008;143:690-697.
28. Eichhorst BF, Busch R, Obwandner T, *et al*. , Health-related quality of life in younger patients with chronic lymphocytic leukemia treated with fludarabine plus cyclophosphamide or fludarabine alone for first-line therapy: a study by the German CLL Study Group. J Clin Oncol 2007;25:1722-1731.
29. Eichhorst BF, Busch R, Stilgenbauer S, *et al*. First-line therapy with fludarabine compared with chlorambucil does not result in a major benefit for elderly patients with advanced chronic lymphocytic leukemia. Blood. 2009;114(16):3382-91.
30. Ferrajoli A, O'Brien S, Wierda W, *et al*. Treatment of patients with CLL 70 years old and older: a single center experience of 142 patients. Leuk Lymphoma 2005;46:(Suppl 1):S86 (abstract P95).
31. Shvidel L, Shtalrid M, Bairey O, *et al*; Israeli Study Group on CLL. Conventional dose fludarabine-based regimens are effective but have excessive toxicity in elderly patients with refractory chronic lymphocytic leukemia. Leuk Lymphoma. 2003;44(11):1947-50.
32. Bocchia M, Bigazzi C, Marconcini S, *et al*. Favorable impact of low-dose fludarabine plus epirubicin and cyclophosphamide regimen (FLEC) as treatment for low-grade non-Hodgkin's lymphomas. Haematologica. 1999;84(8):716-20.
33. Fabbri A, Lenoci M, Gozzetti A, *et al*. Low-dose oral fludarabine plus cyclophosphamide in elderly patients with chronic lymphoproliferative disorders. Hematol J. 2004;5(6):472-4.
34. Fabbri A, Lenoci M, Gozzetti A, *et al*. Low-dose oral fludarabine plus cyclophosphamide as first-line treatment in elderly patients with indolent non-Hodgkin lymphoma. Br J Haematol. 2007;139(1):90-3.
35. Forconi F, Fabbri A, Lenoci M, *et al*. Low-dose oral fludarabine plus cyclophosphamide in elderly patients with untreated and relapsed or refractory chronic lymphocytic Leukaemia. Hematol Oncol. 2008;26(4):247-51.
36. Marotta G, Bigazzi C, Lenoci M, *et al*. Low-dose fludarabine and cyclophosphamide in elderly patients with B-cell chronic lymphocytic leukemia refractory to conventional therapy. Haematologica. 2000;85(12):1268-70
37. Foon KA, Boyiadzis M, Land SR, *et al*. Chemoimmunotherapy with low-dose fludarabine and cyclophosphamide and high dose rituximab in previously untreated patients with chronic lymphocytic leukemia. J Clin Oncol. 2009;27(4):498-503.
38. Smolej L, Brychtová Y, Špaček M, *et al*. Low-dose FCR in the treatment of elderly/comorbid patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL): updated results of project Q-lite by Czech CLL Study Group. 17th Congress of Eueopean Hematology Association. Haematologica 2012;97 (Suppl 1): abstract 0151
39. Mulligan SP, Gill D, Turner P, *et al*. Safety and Tolerability of Oral Fludarabine, with or without Oral Cyclophosphamide and Intravenous Rituximab Therapy, in Previously Untreated Patients with Chronic Lymphocytic Leukaemia Aged 65 Years or Older: Second Interim Analysis from the Australasian Leukaemia and Lymphoma Group and CLL Australian Research Consortium CLL5 Study. International Workshop in CLL. Clin Lymphoma Myeloma Leukemia 2011;11 (Suppl 2): S259-60 (abstract 5.25)
40. National Cancer Institute: Registry of clinical trials. Cited on 20/09/2011. Available at http:// http://www.cancer.gov/clinicaltrials/search.
41. Shanafelt TD, Lin T, Geyer SM, *et al*. Pentostatin, cyclophosphamide, and rituximab regimen in older patients with chronic lymphocytic leukemia. Cancer 2007;109:2291–2298.
42. Smolej L. Modern concepts in the treatment of chronic lymphocytic leukemia. Hematology 2009; 14: 249-54.
43. Gribben JG, Hallek M. Rediscovering alemtuzumab: current and emerging therapeutic roles. Br J Haematol. 2009;144(6):818-31.
44. Tam CS, O'Brien S, Lerner S, *et al*. The natural history of fludarabine-refractory chronic lymphocytic leukemia patients who fail alemtuzumab or have bulky lymphadenopathy. Leuk Lymphoma. 2007;48(10):1931-9.
45. Dungarwalla M, Evans SO, Riley U, *et al*. High dose methylprednisolone and rituximab is an effective therapy in advanced refractory chronic lymphocytic leukemia resistant to fludarabine therapy. Haematologica. 2008;93(3):475-6
46. Bowen DA, Call TG, Jenkins GD, *et al*. Methylprednisolone-rituximab is an effective salvage therapy for patients with relapsed chronic lymphocytic leukemia including those with unfavorable cytogenetic features. Leuk Lymphoma. 2007;48(12):2412-7
47. Castro JE, Sandoval-Sus JD, Bole J, *et al*. Rituximab in combination with high-dose methylprednisolone for the treatment of fludarabine refractory high-risk chronic lymphocytic leukemia. Leukemia. 2008;22(11):2048-53.
48. Quinn J, Sajir M, Chipperfield K, *et al*. Efficacy of rituximab in combination with steroids in refractory chronic lymphocytic leukaemia. Leuk Lymphoma 2008;49:1995–1998
49. Smolej L, Doubek M, Panovská A *et al*. Rituximab in combination with high-dose dexamethasone for the treatment of relapsed/refractory chronic lymphocytic leukemia. Leuk Res. 2012;36(10):1278-82.
50. Wierda WG, Kipps TJ, Mayer J, *et al*. Ofatumumab As Single-Agent CD20 Immunotherapy in Fludarabine-Refractory Chronic Lymphocytic Leukemia. J Clin Oncol 2010; 28: 1749-55.
51. Hillmen P, Gribben JG, Follows GA, *et al*. Rituximab Plus Chlorambucil In Patients with CD20-Positive B-Cell Chronic Lymphocytic Leukemia (CLL): Final Response Analysis of An Open- Label Phase II Study. Blood (ASH Annual Meeting Abstracts), 2010;116: abstract 697.
52. Foà R, Ciolli S, Di Raimondo F, *et al*. A Phase II Study of Chlorambucil Plus Rituximab Followed by Maintenance Versus Observation In Elderly Patients with Previously Untreated Chronic Lymphocytic Leukemia: Results of the First Interim Analysis. Blood (ASH Annual Meeting Abstracts) 2010;116: abstract 2462
53. Bologna L, Gotti E, Manganini M *et al*. Mechanism of action of type II, glycoengineered, anti-CD20 monoclonal antibody GA101 in B-chronic lymphocytic leukemia whole blood assays in comparison with rituximab and alemtuzumab. J Immunol. 2011;186(6):3762-9
54. Fischer K, Cramer P, Busch R, *et al*. Bendamustine Combined With Rituximab in Patients With Relapsed and/or Refractory Chronic Lymphocytic Leukemia: A Multicenter Phase II Trial of the German Chronic Lymphocytic Leukemia Study Group. J Clin Oncol. 2011;29(26):3559-66
55. Badoux XC, Keating MJ, Wen S, *et al*. Lenalidomide as initial therapy of elderly patients with chronic lymphocytic leukemia. Blood. 2011;118(13):3489-981.

Which is the Objective of CML Treatment in Elderly Patients in the TKI Era? To "Cure" or to "Take Care"?

Michele Malagola and **Domenico Russo**
Chair of Hematology, Unit of Blood Diseases and Stem Cell Transplantation, University of Brescia, AO Spedali Civili di Brescia, Brescia

Introduction

Chronic Myeloid Leukaemia (CML) is a myeloprolipherative disease characterised by a biphasic clinical course: the chronic phase (CP), consisting of an abnormal expansion of the clonal haematopoiesis with an apparent normal differentiation, and the accelerated-blastic phase (A-BP), leading to the development of a secondary acute leukaemia, which fatally closes the course of the disease.[1] Philadelphia (Ph+) chromosome, a balanced translocation between chromosomes 9 and 22, t(9;22)(q34;q11.2), translating into a BCR-ABL p210 protein with increased tyrosine kinase (TK) activity, is responsible for the pathogenesis and progression of CML;[2-5] thus, the suppression of Ph+ leukemic clone is mandatory and remains the main goal of CML therapy.

Over the last two decades, CML therapy has evolved from the use of non-specific cytotoxic agents (i.e. Hydroxurea, Busulfan)[6, 7] to Interferon-α (IFN-α)[8-12] or allogeneic stem cell transplantation (allo-SCT)[13-19] and more recently to Tyrosine Kinase Inhibitors (TKIs), namely imatinib (Glivec),[20-22] that is now recognised as the first-line treatment of Ph+ CML. The introduction of imatinib and the more recent use of 2nd generation TKIs, dramatically changed the prognosis of CML, from a fatal disease to a "curable" disease.[22]

As part of this radical change of prognosis, two issues particularly deserve to be considered and discussed. First, if there is a viable treatment strategy based on the use of TKIs that can lead to the "cure" of the disease, and whether it should be applied equally to all patients, regardless of age, disease risk or other factors. Second, if a therapeutic strategy and its objectives in the elderly may be different from those of a younger age.

In this review we will address and discuss: the efficacy and safety of imatinib and second- generation TKIs, with particular reference to the population of older patients; the objectives that should be considered as a policy guide of treatment in this latter group of patients and the possible therapeutic strategies that could be adopted.

Imatinib for Elderly CML Patients

Imatinib (IM) is a dihydropirimidine derivative that binds ABL moiety of p210 in its inactive conformation, preventing p210 from assuming the active conformation that is required for TK activity.[20] Turning off p210 TK activity, imatinib induces the apoptosis of Ph+ cells and a progressive debulky of Ph+ leukemic clone.[20, 21] The most solid results on efficacy and toxicity of imatinib, were firstly generated by the IRIS study, the only prospective-randomised trial comparing imatinib at 400 mg daily with IFNα plus low dose ara-C (LDAC).[22] The IRIS study led to the registration of imatinib as first-line treatment in patients with CML in chronic phase (CP) and the results can be briefly summarised as follows: more than 90% of the patients can achieve a complete haematological response (CHR); 80%, 40% and less than 10% can achieve a complete cytogenetic response (CCgR= 0% of Ph+ bone marrow metaphases), a major molecular response (MMR=BCR-ABL/ABL[IS] 0.1%) and a complete molecular response (CMR= a 4.5 log reduction in BCR-ABL transcript level (<0.01%[IS]), with a sensitivity of at least 10,000 ABL copies), respectively;[22] then, after 6 years of follow-up, patients in CCgR and MMR may have a freedom from progression to A/BP and an overall survival (OS) of 83% and 88%, respectively.[23] These results on the efficacy of imatinib were confirmed by other phase II studies, many including large cohorts of patients,[24-31] and altogether they contributed to produce the ELN[32] and the ESMO[33] guidelines through which optimal responders can be distinguished from non-optimal responders or non-responders, allowing us to modulate the better treatment. However, both the IRIS study and the other controlled clinical trials included CML cases with a median age of approximately 55 years, almost 10 years younger than the median age of CML patients outside

Domenico Russo achieved his *Summa cum Laude* at the University of Bologna School of Medicine and has been a Full Professor of Hematology, Department of Medical and Surgical Sciences, University of Brescia Medical School since 2011. Since 2004, Dr. Russo has acted as Head of Unit of Blood Diseases and Cell Therapies, I Division of General Medicine, Spedali Civili of Brescia, and Director of Medical School of Specialization in Hematology, University of Brescia. He is also a member of the Research Council of Brescia University. Dr. Russo's research interests are the Prognosis and therapy of Chronic Myeloid Leukaemia, therapy of acute leukaemias, lymphomas and myeloma and Multidrug resistance (MDR) stem cell transplantation.

clinical trials.[22, 24-31] Therefore, these data could not be considered to be particularly helpful in informing us of the effects of imatinib in elderly patients. Thus, the first question to address is whether elderly patients respond to and tolerate imatinib as well as younger patients.

The data published in the literature prove that imatinib is effective in elderly patients as well as in younger patients and that it is likely able to abrogate the negative impact of age on outcome,[34] particularly when elderly patients are treated in early chronic phase (early-CP).[35-40] Being more detailed, in this subset of patients, CCgRs and MMRs were achievable in more than 80% of the patients, with a PFS and an OS ranging from 75 to more than 90% and from 78 to 82%, respectively.[35, 38, 40] In elderly patients treated in late-CP, imatinib was somehow less potent, leading a CCgR in 36 to 70% of the patients according to the different studies.[35, 37, 38] Nevertheless, the long-term outcome in terms of PFS and OS was extremely good, with more than 90% of patients being alive and disease-free at 48 months.[37]

From a clinical point of view, these data suggest that all the patients with CML, irrespective of their age, should be treated up-front with imatinib. This is true for elderly patients treated in controlled clinical trials and also for those treated outside. This latter group of elderly patients may also benefit from a treatment with imaninib as shown by observational studies. These studies are not considered to be less important than controlled clinical trials because they provide complementary data of "real life" clinical practice, although they are not the best approach to test the effects of a clinical intervention.[41] In this regard, the results of a large population-based study on 3173 CML patients diagnosed in Sweden from 1973 to 2008 showed that patients older than 80 years were those with the poorer outcome because of the limited use of imatinib, while a major improvement in outcome was observed in CML patients up to 79 years of age diagnosed from 2001 to 2008, because of an increasing use of imatinib. To confirm this, we can also cite a recent study by the Czech Republic Group, where 152 consecutive adult CP-CML patients were treated with imatinib outside a controlled clinical trial.[42] The estimated 4-year cumulative incidences of CHR, CCgR, MMR, and CMR were 95.3%, 80.6%, 65.4%, and 39.2%, respectively, and the 4-year probability of overall and progression-free survival (PFS) was 91.5% and 78.1%, respectively. Furthermore, the 4-year EFS, which also counted failure events according to valid recommendations (e.g. ELN recommendations) or imatinib discontinuation due to intolerance, was only 60.7%. This latter point is very important and underlines that a relatively high percentage of patients discontinue imatinib not only because of the loss of optimal response but also because of intolerance or difficulties to adhere chronic treatment.

Concerning imatinib toxicity, the published data show that haematological and non-haematological adverse events in elderly patients were not different from the ones observed in the youngers, but that elderly population suffered from a higher incidence of severe (grade III-IV WHO) adverse events and that these events clearly influence therapy interruption and discontinuation. The incidence of permanent discontinuation varied from 4% to 33% and also the incidence of haematological and non-haematological toxicities leading to discontinuation varied from 12% to 66%.[34-40] This wide range may be partially explained by different patient populations included in the study (e.g. early versus late CP), but this testifies the fact that tolerance to chronic therapy is a crucial problem, and that this problem is even more important in the elderly.

We have so far examined the efficacy and tolerability of imatinib in elderly patients. Now we will see if there are advantages and possibly what types of advantages from 2nd TKIs, namely nilotinb or dasatinib, that have become available for those patients who develop imatinib intolerance or resistance and that, more recently, have been registered for first-line therapy of CML.

Second Generation TKIS in Elderly CML Patients

Data concerning second-line therapy with nilotinib or dasatinib in elderly CML patients generally come from sub-analysis of multi-centre trials testing second generation TKIs at different doses in patients resistant or intolerant to imatinib. They show that CCgR is achievable in 31 to 85% of the cases, that a molecular response may be achieved in 31 to 72% of cases and that this response can be major (MMR) in 15 to 70% of the cases.[43-45] These results translated into an OS at 1 year of 91% with nilotinib.[43] With dasatinib, the OS and EFS at 2 and 4 years was 93.1%, 60.8% and 84.2%, 49.5%, respectively.[45]

When second generation TKIs were tested for first-line therapy of elderly patients, the results were extremely good. In particular, as reported in the DASISION trial, with dasatinib the cumulative incidence of CCgR in the elderly (> 65 years) was 85%, comparable to the 78% and 88% observed in patients between 46-65 and <46 years, respectively. The results were similar when considering MMR, which was 50% in the elderly, very close to the 47% and 45% observed in patients between 46-65 and < 46 years, respectively.[46] Concerning nilotinib, as reported in the ENESTn trial, the cumulative incidence of CCgR and MMR was 83% and 72% with nilotinib 300 mg BID and 68% and 61% with nilotinib 400 mg BID. The advantage of the lower dose schedule was probably related to the lower incidence of therapy discontinuation due to toxicity. These results compare very favourably with the 87% of CCgR and the 72-61% of MMR observed in patients younger than 65 years treated with the same nilotinib doses.[47] Overall, dasatinib and nilotinib appeared to be more potent than imatinib when used for first-line treatment of CML patients and were demonstrated to be able to induce faster and deeper CCgR and MMR.

Nevertheless, although the first data on the first-line treatment of CP-CML with the more potent second generation TKIs appear to be in line with what is believed and expected, the proportion of responders who may become free of treatment would hardly exceed 20%, and, like in the case of imatinib, the majority of responders are destined to a policy of chronic treatment at the same standard schedule and dose.[48] This point opens important questions regarding treatment toxicity of a chronic therapy,

particularly in the elderly. In this view, haematological and non-haematological toxic profiles observed in elderly patients treated with nilotinib or dasatinib reproduce the one observed in the younger patients. The incidence of grade III-IV WHO episodes of neutropenia and/or thrombocytopenia and/or anemia with nilotinib or dasatinib was not higher than 25% when these drugs were used for first-line therapy of elderly CML patients[46, 47] and ranged between 10 and 50% of the cases in patients treated for second-line.[43-45] Interestingly, with dasatinib a severe haematological toxicity was more frequent with the dose of 140 mg/day.[43- 45] Concerning extra-haematological toxicity, nilotinib showed some peculiar side effects, that consisted on: clinically asymptomatic laboratory abnormalities (elevation of lipase and bilirubin levels),[43] QTcF elongation (> 500 msec)[43] and hyperglycemia.[47] The incidence of these events ranged between 1% and 23%. Overall, according to Le Coutre and Colleagues the incidence of drug-related grade III-IV adverse events was 56% in patients ≥ 60 years and 53% in patients ≥ 70 years and the incidence of therapy discontinuation was 46% in patients ≥ 60 years and 41% in patients ≥ 70 years.[44] On the other hand, dasatinib extra-haematological peculiar toxicities consisted on: fluid retention episodes (up to 35% of patients aged > 65 years) and pleuro- pericardial effusions (less than 10% of the cases).[45, 46] In general, the number of patients who required a dose reduction was higher when dasatinib was administered at a dose of 140 mg/day and the incidence of permanent dasatinib discontinuation in the whole population was 15%, mainly due to toxicity.[46]

CML in Elderly: To "Cure" or to "Take Care"?

The goal of the treatment of CML have become more ambitious: not only a delay of progression and prolongation of survival, but, possibly, a real "cure" of the disease with a normalisation of survival and treatment discontinuation without molecular relapse.[41, 49-53]

The current therapeutic strategy for CP-CML patients is to use a TKI (e.g. imatinib, nilotinib, dasatinib) at the "best standard dose" to achieve and maintain a reduction of the disease burden able to induce a "normalisation" of survival. With imatinib at the standard dose of 400 mg daily, 80-90% of patients achieving CCgR and MMR are alive at 8 years, while only a few patients can achieve a molecular response (CMR) allowing a discontinuation of therapy without a disease recurrence.[53]

Although elderly patients have cytogenetic and molecular responses comparable to youngers, they tolerate imatinib worse and this causes higher rate of therapy discontinuation and less adherence to chronic treatment. Improve cost-effectiveness in this setting of patients is not irrelevant. It has to be considered that, the median age of CML patients at diagnosis is around 60 years, and that the patients who are aged 65 or older represent a very high proportion of all the patients who are currently cared for Ph+ CML.[41, 51] Therefore, the issue is if there are other potential or possible policies to optimise the TKI chronic therapy in the elderly.

Results could be improved by the use of second generation TKIs, but their toxicity remains an issue and even if they were very successful, the proportion of patients who would become free of treatment and remain free of leukaemia would hardly be greater than 20%.[54-69]

Another therapeutic strategy that have been proposed is based on the identification of minimal effective dose or schedule of imatinib to maintain the optimal response without increasing the toxicity. This alternative approach was explored by a phase II multicentric exploratory trial that evaluated the effects of a policy of intermittent imatinib (INTERIM) treatment (one month on/one month off) on cytogenetic and molecular responses in a selected population of patients ≥ 65 years treated with imatinib for > 2 years and in stable CCgR.[60] With a minimum follow-up of four years, out of 76 patients enrolled in this study, 13 patients (17%) lost CCgR and MMR, and 14 (18 %) lost MMR only. All these patients resumed imatinib continuously, and all of them regained CCgR and MMR, while no patients progressed to accelerated or blastic phase, or developed clonal chromosomal abnormalities in Ph+ cells, or BCR-ABL mutations. This data show that in elderly CML Ph+ patients highly selected for a stable CCgR (long lasting > 2 years), a policy of intermittent imatinib treatment affects the markers of residual disease, but not the clinical outcomes (overall and progression-free survival). In elderly patients, who have a lower tolerance, have other co-morbidities, and take many other drugs, this innovative strategy could lead to improve the tolerance and to reduce the costs without affecting the long-term outcome. For example, it could be employed in those patients who have difficulties to adhere to chronic treatment and do not achieve the criteria necessary for treatment discontinuation.

Although the concept of "cure" remains more attractive, this strategy may actually be carried out on a minority of CML patients. However, even if a policy of therapy based on the minimum effective dose to achieve an "operationally cure" could be considered less attractive, this strategy could be considered a practice and good therapeutic alternative for a consistent proportion of elderly patients with CML.

References

1. Sawyers CL. Chronic myeloid leukemia. N Engl J Med (1999), 340: pp. 1330-1340.
2. Melo JV. The biology of CML and targets for molecular therapy. American Society of Hematology Education Program – 45th Congress of the American Society of Hematology (2003):pp. 132-152.
3. Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. Blood (2000), 96: pp. 3343–3356.
4. Holyoake TL. Recent advances in the molecular and cellular biology of chronic myeloid leukemia: lessons to be learned from the laboratory. Br J Haematol (2001), 113: pp. 11-23
5. Goldman J. Management of chronic myeloid leukemia. Semin Hematol (2003), 40: pp. 1-103.
6. Tura S, Baccarani M, Gugliotta L, *et al*. A clinical trial of early splenectomy, Hydroxyurea, and cyclic Arabinosyl Cytosine, Vincristine and Prednisone in chronic myeloid leukemia. Series Haematologica (1975), 8: pp. 121-142.
7. Silver RT, Woolf SH, Hehlmann R, *et al*. An evidence-based analysis of the effect of busulfan,

hydroxyurea, interferon, and allogeneic bone marrow transplantation in treating the chronic phase of chronic myeloid leukemia: developed for the American Society of Hematology. Blood (1999), 94: pp. 1517-36.
8. The Italian Cooperative Study Group on Chronic Myeloid Leukemia (Writing committee: S. Tura, M. Baccarani, E. Zuffa, D. Russo, R. Fanin, A. Zaccaria, M. Fiacchini). Interferon alfa-2a as compared with conventional chemotherapy for the treatment of chronic myeloid leukemia. N Engl J Med (1994), 330: pp. 820-825.
9. Bonifazi F, de Vivo A, Rosti G, *et al*. Chronic myeloid leukemia and interferon-alpha: a study of complete cytogenetic responders. Blood (2001), 98: pp. 3074-81.
10. Hehlmann R, Berger U, Pfirrmann M, *et al*; German CML-Study Group. Randomized comparison of interferon alpha and hydroxyurea with hydroxyurea monotherapy in chronic myeloid leukemia (CML-study II): prolongation of survival by the combination of interferon alpha and hydroxyurea. Leukemia (2003), 17: pp. 1529-37.
11. Baccarani M, Russo D, Rosti G, *et al*. Interferon-alfa for chronic myeloid leukemia. Semin Hematol (2003), 40: pp. 22-33.
12. Baccarani M, Rosti G, de Vivo A, *et al*; Italian Cooperative Study Group on Myeloid Leukemia. A randomized study of interferon-alpha versus interferon-alpha and low-dose arabinosyl cytosine in chronic myeloid leukemia. Blood (2002), 99: pp. 1527-35.
13. Italian Cooperative Study Group on Chronic Myeloid Leukemia and Italian Group for Bone Marrow Transplantation. Monitoring treatment and survival in chronic myeloid leukemia. J Clin Oncol (1999), 17: pp. 1858-68.
14. Simonsson B, Oberg G, Bjoreman M, *et al*. Intensive treatment and stem cell transplantation in chronic myelogenous leukemia: long-term follow-up. Acta Haematol (2005), 113: pp. 155-62.
15. Gratwohl A, Brand R, Apperley J, *et al*; Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation (CLWP-EBMT). Graft-versus-host disease and outcome in HLA-identical sibling transplantations for chronic myeloid leukemia. Blood (2002), 100: pp. 3877-86.
16. Robin M, Guardiola P, Devergie A, *et al*. A 10-year median follow-up study after allogeneic stem cell transplantation for chronic myeloid leukemia in chronic phase from HLA-identical sibling donors. Leukemia (2005), 19: pp. 1613-20.
17. Gratwohl A, Brand R, Apperley J, *et al*. Allogeneic hematopoietic stem cell transplantation for chronic myeloid leukemia in Europe 2006: transplant activity, long term data and current results: an analysis by the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Haematologica (2006), 91: pp. 513-521
18. Gratwohl A, Hermans J, Goldman JM, *et al*. Risk assessment for patients with chronic myeloid leukemia before allogeneic blood or marrow transplantation. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Lancet. (1998), 352: pp. 1087-92.
19. Passweg JR, Walker I, Sobocinski KA, *et al*; Chronic Leukemia Study Writing Committee of the International Bone Marrow Transplant Registry. Validation and extension of the EBMT Risk Score for patients with chronic myeloid leukemia (CML) receiving allogeneic haematopoietic stem cell transplants. Br J Haematol (2004), 125: pp. 613-20.
20. Druker BJ, Tamura S, Buchdunger E, *et al*. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med (1996), 2: pp. 561-566
21. Druker BJ, Sawyers CL, Kantarjian H, *et al*. Activity of a specific inhibitor of the BCR- ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med (2001), 344: pp. 1038-1042
22. O'Brien SG, Guilhot F, Larson RA, *et al*. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med (2003), 348: pp. 994-1004.
23. Hochhaus A, O'Brien SG, Guilhot F, *et al*; IRIS Investigators Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. Leukemia (2009), 23: pp. 1054-61.
24. Cortes JE, Kantarjian HM, Goldberg SL, *et al*; Rationale and Insight for Gleevec High-Dose Therapy (RIGHT) Trial Study Group. High-dose imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: high rates of rapid cytogenetic and molecular responses. J Clin Oncol (2009), 27: pp. 4754-9.
25. Castagnetti F, Palandri F, Amabile M, *et al*; GIMEMA CML Working Party Results of high- dose imatinib mesylate in intermediate Sokal risk chronic myeloid leukemia patients in early chronic phase: a phase 2 trial of the GIMEMA CML Working Party. Blood (2009), 113: pp. 3428-34.
26. Palandri F, Iacobucci I, Castagnetti F, *et al*; GIMEMA Working Party on CML Front-line treatment of Philadelphia positive chronic myeloid leukemia with imatinib and interferon- alpha: 5-year outcome. Haematologica (2008), 93: pp. 770-4.
27. Morishima Y, Ogura M, Nishimura M, *et al*. Efficacy and safety of imatinib mesylate for patients in the first chronic phase of chronic myeloid leukemia: results of a Japanese phase II clinical study. Int J Hematol (2004), 80: pp. 261-6.
28. Gardembas M, Rousselot P, Tulliez M, *et al*; CML French Group. Results of a prospective phase 2 study combining imatinib mesylate and cytarabine for the treatment of Philadelphia- positive patients with chronic myelogenous leukemia in chronic phase. Blood (2003), 102: pp. 4298-305.
29. Cortes JE, Baccarani M, Guilhot F, *et al*. Phase III, randomized, open-label study of daily imatinib mesylate 400 mg versus 800 mg in patients with newly diagnosed, previously untreated chronic myeloid leukemia in chronic phase using molecular end points: tyrosine kinase inhibitor optimization and selectivity study. J Clin Oncol (2010), 28: pp. 424-30.
30. Baccarani M, Rosti G, Castagnetti F, *et al*. Comparison of imatinib 400 mg and 800 mg daily in the front-line treatment of high-risk, Philadelphia-positive chronic myeloid leukemia: a European LeukemiaNet Study. Blood (2009), 113: pp. 4497-504.
31. Castagnetti F, Palandri F, Amabile M, *et al*; GIMEMA CML Working Party. Results of high-dose imatinib mesylate in intermediate Sokal risk chronic myeloid leukemia patients in early chronic phase: a phase 2 trial of the GIMEMA CML Working Party. Blood (2009), 113: pp. 3428-34.
32. Baccarani M, Cortes J, Pane F, *et al*; European LeukemiaNet. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. J Clin Oncol (2009), 27: pp. 6041-51.
33. Baccarani M, Dreyling M; ESMO Guidelines Working Group Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol (2010), 21 Suppl 5:v165-7.
34. Breccia M, Tiribelli M, Alimena G. Tyrosine kinase inhibitors for elderly chronic myeloid leukemia patients: A systematic review of efficacy and safety data. Crit Rev Oncol Hematol 2012, 84: pp. 93-100.
35. Cortes J, Talpaz M, O'Brian S, *et al*. Effects of Age on Prognosis with Imatinib Mesylate Therapy for Patients with Philadelphia Chromosome-Positive Chronic Myeloid Leikemia. Cancer (2003), 98: pp. 1105-13.
36. Latagliata R, Breccia M, Carmosino I, *et al*. Elderly patients with Ph+ chronic myelogenous leukemia (CML): results of imatinib mesylate treatment. Leuk Res (2005), 29: pp. 287-91.
37. Rosti G, Iacobucci I, Bassi S, *et al*. Impact of age on the outcome of patients with chronic myeloid leukemia in late chronic phase: results of a phase II study of the GIMEMA CML Working Party. Haematologica (2007), 92: pp. 101-5.
38. Latagliata R, Breccia M, Carmosino I, *et al*. "Real.life" results of front-line treatment with Imatinib inolder patients (≥ 65 years): with newly diagnosed chronic myelogenous leukemia. Leuk Res (2010), 34: pp. 1472-75.
39. Sanchez-Guijo FM, Duran S, Galende J, *et al*. Evaluation of tolerability and efficacy of imatinib mesylate in elderly patients with chronic phase CML: ELDERGLI study. Leuk Res (2011), 35: pp. 1184-87.
40. Gugliotta G, Castagnetti F, Palandri F, *et al* on behalf of the Gruppo Italiano Malattiem Ematologiche dell'Adulto CML Working Party. Frontline imatinib treatment of chronic myeloid leukemia: no impact of age on outcome, a survey by the GIMEMA CML Working Party. Blood (2011), 117: pp. 5591-99.
41. Björkholm M, Ohm L, Eloranta S, *et al*. Success story of targeted therapy in chronic myeloid leukemia: a population-based study of patients diagnosed in Sweden from 1973 to 2008. J Clin Oncol (2011), 29: pp. 2514-20.
42. Zackova D, Klamova H, Dusek L, *et al*. Imatinib as the first-line treatment of patients with chronic myeloid leukemia diagnosed in the chronic phase: can we compare real life data to the results from clinical trials? Am J Hematol (2011), 86: pp. 318-21.
43. Lipton JH, le Coutre P, Wang J, *et al*. Nilotinib in elderly chronic myeloid leukemia patients in chronic phase (CML-CP) with Imatinib resistance or intolerance: efficacy and safety analysis. Proceedings of the 50th Meeting of the American Society of Hematology, San Francisco, USA, December 6 - 9 (2008), 112: p. 3233.
44. Le Coutre PD, Turkina A, Kim DW, *et al*. Efficacy and safety of Nilotinib in elderly patients with Imatinib-resistant or intolerant chronic myeloid leukemia (CML) in chronic phase (CP): a sub-analysis of the ENACT (expanding Nilotinib access in clinical trials) study. Proceedings of the 51st Meeting of the American Society of Hematology, New Orleans, USA, December 5-8 (2009), 114: p. 3286.
45. Latagliata R, Breccia M, Castagnetti F, *et al*. Dasatinib is safe and effective in unselected chronic myeloid leukemia elderly patients resistant/intolerant to imatinib. Leuk Res (2011), 35: pp. 1164-69.
46. Khoury JH, Cortes J, Kantarjian H, *et al*. Safety and efficacy of Dasatinib (DAS) vs Imatinib (IM) by baseline comorbidity in patients with chronic myeloid leukemia in chronic phase (CML-CP): analysis of the DASISION trial. Proceedings of the 52nd Meeting of the American Society of Hematology, Orlando, USA, December 4-7 (2010), 116: p. 3421.
47. Larson R, Bunworasqte U, Turkina AG, *et al*. Nilotinib shows safety and efficacy in older patients (≥ 65 years) with newly diagnosed chronic myeloid leukemia in chronic phase comparable with that in younger patients with chronic myeloid leukemia in chronic phase; results from the ENESTn. Proceedings of the 53rd Meeting of the American Society of Hematology, San Diego, USA, December 10-13 (2011), 118: p. 3768.
48. Rea D, Rousselot P, Nocolini F, *et al*. Discontinuation of Dasatinib or Nilotinib in CML patients with stable undetectable BCR-ABL transcripts: results from the French CML Group (FILMC). Proceedings of the 53rd Meeting of the American Society of Hematology, San Diego, USA, December 10-13 (2011), 118: p. 632
49. Goldman JM. How I treat chronic myeloid leukemia in the imatinib era. Blood (2007), 110: pp. 2828-37.
50. Druker BJ. Translation of the Philadelphia chromosome into therapy for CML. Blood. 2008;112(13):4808-4815.
51. Hehlmann R, Hochhaus A, Baccarani M. Chronic myeloid leukaemia. Lancet (2007), 370: pp. 342-50.
52. Kantarjian H, O'Brien S, Jabbour E *et al*. Improved survival in chronic myeloid leukemia since the introduction of imatinib therapy: a single institution experience. Blood (2012), 119: pp. 1981-1987.
53. Mahon FX, Réa D, Guilhot J *et al*. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission

for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol (2010), 11: pp. 1029- 35.
54. Jabbour E, Cortes JE, Giles FJ, *et al*. Current and emerging treatment options in chronic myeloid leukemia. Cancer (2007), 109: pp. 2171-81.
55. Rosti G, Palandri F, Castagnetti F, *et al*. Nilotinib for the frontline treatment of Ph(+) chronic myeloid leukemia. Blood (2009), 114: pp. 4933-8.
56. Saglio G, Kim DW, Issaragrisil S, *et al*. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med (2010), 362: pp. 2251-9.
57. Kantarjian H, Shah NP, Hochhaus A, *et al*. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med (2010), 362: pp. 2260-70.
58. Kantarjian HM, Shah NP, Cortes JE *et al*. Dasatinib or imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: 2-year follow-up from a randomized phase 3 trial (DASISION). Blood (2012), 119: pp. 1123-1129.
59. Larson RA, Hochhaus A, Hughes TP, *et al*. Nilotinib vs imatinib in patients with newly diagnosed Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase: ENESTnd 3-year follow-up. Leukemia (2012), 26: pp. 2197-203.
60. Domenico Russo, Giovanni Martinelli, Michele Malagola *et al*. Effects and outcome of a policy of intermittent imatinib treatment in elderly patients with chronic myeloid leukemia. Blood (2013), in press.

Further Reading

Please see the abstracts below for further reading on chronic lymphocytic leukaemia. These articles plus others are available in previous editions of *Treatment Strategies - Hematology*. Please see our website for more articles on chronic lymphocytic leukaemia and a range of other topics within haematology.

Clinical Development of B-Cell Receptor Pathway Inhibitors in Chronic Lymphocytic Leukaemia

Matthew S. Davids and **Jennifer R. Brown**
CLL Center, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts

The B-cell receptor (BCR) pathway has been increasingly recognised as a key mediator of chronic lymphocytic leukaemia (CLL) cell survival and proliferation. Recently, inhibitors of several of the key mediators of this pathway, including spleen tyrosine kinse (Syk), phosphoinositide 3'-kinase (PI3K), mammalian target of rapamycin (mTOR), and Bruton's tyrosine kinase (Btk), have been developed and found in pre-clinical studies to induce CLL cell apoptosis both through direct killing and through modulation of the microenvironment. These oral agents have the potential to spare patients many of the toxicities of cytotoxic chemotherapy, and also to be effective in patients with high-risk disease such as del(17p). Here, we focus on the clinical development of inhibitors of the BCR pathway, reviewing recently presented data on the safety, efficacy, and unique pattern of response of these agents in early phase clinical trials, and describing several of the key ongoing studies. We also highlight some of the critical questions that arise regarding how best to integrate these exciting new therapies into the treatment approach for patients with CLL.

Chronic Lymphocytic Leukemia (CLL): From Biology to Targeted Therapy

Jan A. Burger
Department of Leukemia, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Over the past few years, it became increasingly clear that external signals from the microenvironment are critical factors for the disease progression in CLL and other B-cell malignancies. Many of these signals and activation pathways are similar to those activated in normal B-cells during the induction of specific immune responses. B-cell receptor (BCR) signaling is the quintessential example for such conserved mechanism, given that it promotes the expansion of normal and malignant B-cells, including CLL cells. CLL cells proliferate within the secondary lymphatic tissues in areas called "proliferation centres", where BCR signaling pathways are turned on, regulating leukaemia cell survival and proliferation and modulating CLL cell migration and tissue homing. Updated response data and more detailed insight into the mechanism of action of different small molecule inhibitors of BCR signaling were presented at the 2011 ASH meeting. Specifically, the pre-clinical and clinical activities of the Bruton's tyrosine kinase (Btk) inhibitor PCI-32765 and the phosphoinositide 3'-kinase (PI3K) isoform p110δ (PI3Kδ) inhibitor GS-1101 (previously called CAL-101) were highlighted in oral and poster presentations. Interestingly, these two agents both induce rapid resolution of lymphadenopathy and organomegaly in CLL patients, which is accompanied by a transient surge in lymphocyte counts due to the release of tissue-resident CLL cells into the peripheral blood. Then, often after months of continuous therapy, a major proportion of patients achieve remissions. This paper reviews the development of this exciting new class of kinase inhibitors and other agents that target the CLL microenvironment.

Progress in Langerhans Cell Histiocytosis: Back to Histiocytosis X

Stephen J. Simko, Kenneth L. McClain and **Carl E. Allen**
Baylor College of Medicine and Texas Children's Cancer Center, Houston, Texas

Overview

Langerhans cell histiocytosis (LCH), the most common histiocytic disorder in humans, occurs in approximately 5 per 1,000,000 children under the age of 15 per year.[1-3] The clinical presentation is highly variable, ranging from a self-resolving, single-site disease to a potentially lethal multi-system disease. Because the symptoms of LCH may mimic features of many more common conditions, diagnosis is often challenging. However, once LCH is considered, diagnosis is relatively straightforward with biopsy, where histopathology demonstrates pathologic dendritic cells expressing CD1a and CD207, along with a background inflammatory infiltrate (Figure 1 A-B). Here we review the pathogenesis, clinical presentation, current therapies, and future therapeutic directions for this disorder.

Pathogenesis

Around the turn of the 19th Century, physicians began to report series of patients with a repeated pattern of unusual findings such as multiple lytic bone lesions along with pituitary abnormalities (Hand-Schüller-Christian disease); or skin rash, hepatosplenomegaly, cytopenias, and bone lesions (Letterer-Siwe disease); or solitary eosinophilic granulomas (reviewed in [4]) (Figure 1 C-F). In 1953, Dr. Lichtenstein observed a common histology of histiocytes (or tissue macrophages/dendritic cells) along with inflammatory infiltrate in biopsies from the range of clinical manifestations and proposed that they represent a common condition he named "Histiocytosis X".[5] The "X" indicated the incomplete knowledge of the identity of the pathologic histiocytes. With the advent of electron microscopy in the 1970s, the discovery of pentalaminar cytoplasmic inclusions known as Birbeck granules histiocytes in Histiocytosis X, that had previously only been observed in epidermal Langerhans cells (LCs), led Dr. Nezelof and colleagues to hypothesise that this disease arose from pathologic epidermal LCs. The disease was then re-branded Langerhans Cell Histiocytosis.[6]

While LCH has, for decades, been hypothesised to arise from epidermal LCs, the mechanism of pathogenesis remains undefined and the debates over immune dysregulation versus neoplastic transformation continue. LCH clearly has an inflammatory component, as lesions contain a variety of inflammatory cells in addition to the pathologic CD207+ dendritic cells (DCs), and the LCH lesion microenvironment has been described as a "cytokine storm" with expression of inflammatory cytokines and T-cell costimulatory molecules.[7-9] LCH also has features suggestive of malignancy, including clonality of the pathologic DCs[10] and potentially aggressive tissue invasion by the LCH lesions. However, these lesions are not histopathologically dysplastic, they are cytogenetically normal, and they typically lack Ki-67 expression or mitotic figures.[11] LCH DCs do not grow *in vitro* without growth factor support, and they have not to date been propagated in xenografts.[12] Many investigations for recurrent mutations in LCH reported only negative data (reviewed in [13]). However, a more recent study with next-generation mutation screening and sequencing identified activating BRAF-V600E point mutations in most LCH lesions.[14] BRAF is a kinase involved in the RAS/RAF/MEK pathway that promotes cell growth and proliferation. Though BRAF mutations are also found in benign neoplasms such as nevi, they also are implicated in pathogenesis, tumour maintenance and metastasis in many malignancies (reviewed in [15]).

Advances in experimental technologies and our understanding of immunology once again question assumptions about the origin of the pathologic histiocytes in LCH. Comparison of gene expression profiles of purified CD207+ cells from LCH lesions was distinct from those of epidermal Langerhans cells. Furthermore, the LCH CD207+ cells had relatively increased expression of cell-surface markers associated with immature myeloid DCs.[8] In both mouse and man, expression of langerin, the gene that encodes CD207 protein, is more promiscuous than initially anticipated. CD207+ DC populations have recently been described in many DC lineages in many anatomic locations.[16-20] LCH, therefore, has the potential to arise from cells other than epidermal LCs. Clonality, recurrent BRAF mutations, and re-characterisation of the cell of origin as an immature myeloid DC could be consistent with updated branding of LCH as a myeloid neoplasia.

Clinical Presentation

Clinical manifestations of LCH are protean. Most cases present in

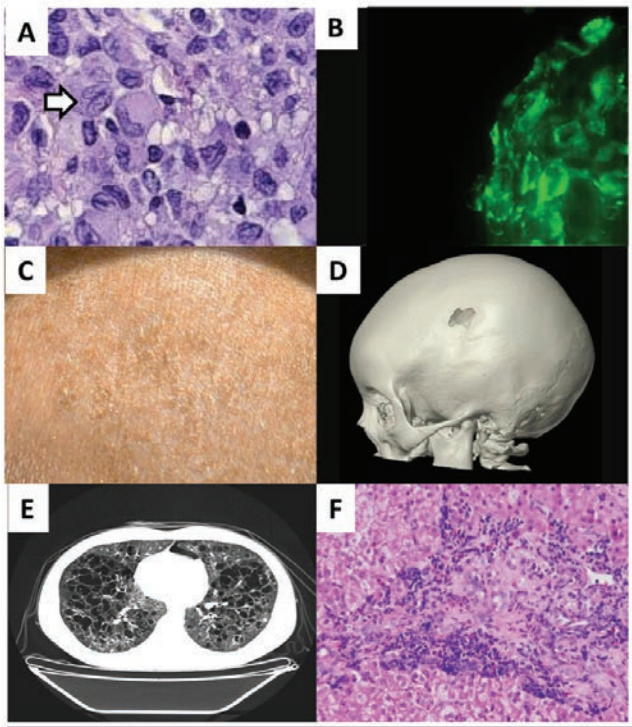


Figure 1. A. H&E slide of a typical LCH lesion with "histiocytes" in an inflammatory background. The white arrow points to a classic LCH DC with reniform nucleus. (Image courtesy of Dr. John Hicks); B. Immune fluorescence of CD207 antibody demonstrates surface langerin/CD207 expression in pathologic DCs in LCH lesions.; C. This photograph of a LCH skin rash represents a relatively mild manifestation of LCH that could easily be confused with many more common paediatric rashes.; D. This CT scan is an example of an isolated LCH bone lesion; E. This lung CT scan is from a patient with severe lung LCH. Note the multiple cysts that result from destruction of normal lung tissue; F. H&E slide of liver from a patient with high-risk LCH, including infiltration of portal tracts with classic LCH DCs and inflammatory cells. (Image courtesy of Dr. John Hicks).

children, although the LCH does occur *de novo* in adults.[21, 22] The disease is restricted to a single organ system in approximately 65% of cases, and to a single site in approximately 50% of cases. Typical sites of involvement include skin, bone, lymph node, lung, lymph node, and central nervous system, with isolated bone disease being the most common presentation.[23] Presentation varies by age: Infants and toddlers commonly present with skin-limited disease or multisystem disease, including risk organ involvement; isolated bone disease tends to be a presentation in older patients. Patients with "risk organ" involvement (defined as liver, spleen, and/or bone marrow) have worse overall survival than those with non-risk organ involvement. Although relapse rates in the two groups are similar, patients with risk organ involvement typically require more intensive salvage therapy to achieve disease resolution.[24-27] Late effects, including endocrine abnormalities, growth delay, and neurocognitive problems, remain challenges in the management of patients with LCH.[28]

Current Therapies

Diagnostic Evaluation

All patients should undergo comprehensive diagnostic evaluation (outlined in [29]) and should never be presumed to have single-site disease. For example, in infants, skin LCH could represent isolated, spontaneously resolving disease, or could be part of the constellation of symptoms in a patient with potentially fatal high-risk multisystem disease.

Response to therapy is classified according to both disease state and response, based on established guidelines. Disease state categories include:

- NAD: no active disease.
- AD: active disease.

Response categories include:[29]

- Better:
→ Complete resolution.
→ Regression.
- Intermediate:
→ Mixed: some lesions better, some lesions worse.
→ Stable disease.
- Worse:
→ Disease progression.

Single System

The treatment of single-system LCH is variable and depends primarily on the disease site. Lesion location is the critical determinant of whether systemic therapy should be initiated. Involvement of lung (in children) or central nervous system always requires chemotherapeutic intervention, as do certain lesions considered "special sites" because of their anatomic risk (see "Special Sites" below). In adult smokers, isolated lung disease has been reported to resolve with smoking cessation alone in some cases.[30] "CNS-risk" bone lesions (see bone section below) also require systemic therapy due to the risks of developing CNS complications.

Multiorgan Disease

Standard front-line therapy for children with multiorgan disease, irrespective of risk organ involvement, utilises vinblastine and prednisone. This combination of drugs has been developed over the course of multiple international randomised controlled trials.[24, 27, 31-33] Patients undergo a six-week induction phase receiving:

- Vinblastine 6 mg/m^2 IV weekly.
- Prednisone 40 mg/m^2/day PO for 4 weeks, with 2 week taper.

The Histiocyte Society LCH-II trial demonstrated no additional benefit of etoposide when added to this backbone;[24] the LCH-III international trial demonstrated no added benefit of co-administration of methotrexate to patients with risk organ disease in the induction phase of therapy.[27]

Patients who have no active disease after six weeks of induction therapy proceed to continuation therapy. Patients with AD/better or AD/intermediate response receive a second six-week course of induction therapy, and patients with worse disease after six weeks receive alternate

salvage therapy. After second induction, AD/better patients after twelve weeks proceed to continuation therapy; patients classified as AD/intermediate or worse after two courses of induction also switch to salvage therapy.

Continuation therapy consists of vinblastine 6 mg/m^2 IV, plus prednisone 40 mg/m^2/day PO daily for five days, every three weeks. Patients with risk organ disease also receive oral 6-mercaptopurine daily. The LCH-III trial demonstrated a decrease in relapse rates with twelve months of therapy compared to six months of therapy.[27] Standard of care for children could therefore be considered 12 months of therapy according to the LCH-III protocol. Adults are frequently intolerant of the side effects of prednisone/vinblastine. In a case series, adult patients with LCH bone lesions had less toxicity and superior outcomes with cytarabine therapy.[34] We recommend that imaging response assessments be conducted at six and twelve months of continuation in addition to studies at the end of the induction period.

Central Nervous System and CNS-risk Lesions

Due to low numbers of patients and poor response to existing therapies, studies of therapy options for central nervous system involvement of LCH are limited to case reports and series. Patients with primary central nervous system tumours, which typically infiltrate the hypothalamic-pituitary axis, infrequently respond to systemic chemotherapy or radiation. Even if radiographic improvement or resolution is seen, resultant endocrinopathies such as diabetes insipidus rarely resolve, but some improvement may be seen (reviewed in [35]). The primary goal of therapy is to prevent progression of the disease and worsening of the pituitary hormone deficiencies. Cladribine (2-CdA), a purine analog, has yielded promising results in one case series of patients with LCH CNS tumours.[36]

A poorly understood "neurodegenerative" syndrome in LCH (LCH-ND) exists in which patients develop characteristic white matter changes on MRI, ataxia, learning difficulties, dysarthria, and dysmetria.[35] Symptoms typically manifest over 5 years after initial diagnosis of LCH. LCH-ND may be driven by a T-cell mediated autoimmune process.[37] Case series reporting the use of all-trans retinoic acid, IVIG, and cytarabine have been published, with improvement reported in the majority of patients treated with cytarabine and stabilisation reported with all modalities.[38-40] Given these limited findings, we currently utilise cytarabine as an initial therapy for patients with neurodegeneration.

Patients with orbital, mastoid, temporal, or sphenoid bone involvement are considered to have "CNS-risk" lesions, as these patients are predisposed to the development of CNS lesions, diabetes insipidus, and neurodegeneration.[41] These patients are treated similarly to patients with non-risk organ multisystem disease, with systemic vinblastine/prednisone for one year as described above.

Bone

Unifocal bone lesions comprise over 40% of LCH lesions.[23] Patients with single non-CNS risk lesions can be treated with surgical curettage where clear margins are generally not necessary for a complete response. Intralesional glucocorticoid injection at time of curettage may assist with pain control and improved range of motion.[42] For large lesions in which aggressive surgical resection may be highly morbid, systemic chemotherapy with vinblastine/prednisone may be considered, as patients restore normal bone architecture after systemic therapy for LCH. Most long-term complications in this population involve orthopaedic complications (i.e. vertebra plana or limb-length discrepancy), and neurologic progression is rare.[23] Radiation therapy is usually avoided except in special site lesions (see below), due to concerns of long-term effect of bone growth.[43]

Though most single bone lesions can be treated surgically or with minimal intervention, the involvement of "CNS risk" sites (orbit, mastoid, temporal bone, or sphenoid bone) predisposes patients to developing central nervous system LCH, including pituitary disease and resultant endocrinopathies. CNS risk bone lesions require systemic therapy to reduce the risk of subsequent CNS involvement.

Skin

Many infants with skin LCH at birth have spontaneous regression of disease. Provided that diagnostic evaluation shows no other disease sites, children with mild to moderate skin involvement can be observed without medical intervention. For patients with severe or progressive disease, no consistent treatment strategy for this patient population exists. Suggested therapeutic modalities include topical steroids, nitrogen mustard or imiquimod; surgical resection of isolated lesions; phototherapy or photochemotherapy; or systemic interferon α2b, methotrexate, 6-mercaptopurine, vincristine, vinblastine, thalidomide, steroids, cladribine, and/or cytarabine.[44-52] No regimen, however, has been evaluated in a randomised-controlled trial, and long-term follow-up data are lacking. In our institution, we typically observe most patients with skin-only disease but initiate therapy with oral methotrexate for moderate disease burden, then escalate to vinblastine/prednisone for highly morbid or refractory cases. We emphasise that careful evaluation for multisystem LCH is required at diagnosis and periodically during therapy, as skin-only LCH is a diagnosis of exclusion.

Pulmonary

LCH involvement of the lung, though previously considered a "risk organ" site, is now no longer thought to be of prognostic significance.[53] Isolated lung LCH typically occurs in adult smokers.[30] Treatment with vinblastine/prednisone is typical for children. Patients with irreversible disease often require lung transplantation due to the destructive cystic change that occurs.

Special Sites

Patients with "special site" involvement, in which disease places vital structures at risk, require therapy. In particular, involvement of the odontoid process, vertebrae with soft tissue extension or with risk or

collapse, or femoral neck requires intervention. In addition to systemic therapy, radiation therapy for special site lesions is often administered to maximise local disease control.[43, 54, 55] No specific interventions are needed once vertebra plana has already occurred.

Salvage Therapy

Many strategies are utilised to treat recurrent or refractory LCH, although few have been tested in clinical trials. While low-risk patients who recur more than 12 months from the end of therapy may be successfully retreated with vinblastine/prednisone,[25] risk-organ and early-relapse disease should be treated with alternate therapy. Commonly employed strategies include the use of cytarabine (+/- vincristine), cladribine, and combination cladribine/cytarabine. The LCH-S-98 trial evaluated the effectiveness of cladribine monotherapy in recurrent disease.[56] Patients without risk organ disease had favourable responses (only 11% had progressive disease), but patients with risk organ disease had a 22% rate of "good response." Mortality was high (73%) in patients who did not respond to cladribine monotherapy. A subsequent multicentre pilot study of combined cladribine and cytarabine[26] demonstrated good response in six of seven patients who completed two cycles of therapy, but septic events (including death) and delayed immune reconstitution are common with this intensive approach.[26, 57]

Solid organ transplantation is utilised in patients with lung and liver disease who fail salvage therapies and have irreversible end-organ damage. Bone marrow transplantation appears to be an effective strategy for treating refractory disease in multiple case series, but with a risk of substantial treatment related mortality.[58-60] A clinical trial of transplantation in LCH with reduced intensity conditioning is ongoing.

Future Directions for Research and Therapy

One of the major challenges in treatment of LCH is optimising salvage strategies. An international Histiocyte Society trial, LCH-S-2005, evaluated the effectiveness of combined cytarabine/cladribine, with results pending publication. Recent case series have shown promising results with the use of clofarabine for salvage therapy.[61, 62] Clofarabine is a second-generation purine nucleoside analog, similar to cladribine but with biochemical modifications to promote resistance to degradation and deamination.[63] Our experience with this drug has been consistent with their findings, and in our view a multicentre trial of this drug is warranted.

Until recently, rational therapeutic strategies to LCH were not possible. The discovery of BRAF-V600E mutation in the majority of LCH lesions raises the question of whether constitutive BRAF activation (or activation of downstream pathways involving MEK and ERK) is an essential pathway in pathogenesis of all cases of LCH, and whether inhibition of these pathways can cure LCH. Multiple BRAF inhibitors (vemurafenib, dabrafenib, trametinib) have been approved by the United States Food and Drug Administration for use in unresectable metastatic melanoma. A preliminary report of two adults with LCH lesions in the context of Erdheim-Chester Disease, a related histiocytic disorder of adults, describes initial response to vemurafenib.[64] The widely available tyrosine kinase inhibitor sorafenib has known activity against BRAF, CRAF, and other tyrosine kinases,[65] but it is unknown whether it would have activity against LCH. BRAF-specific inhibitors have not been reported for phase I trials in children, though some studies are in development. Given the well-characterised association between BRAF inhibition and the development of squamous cell carcinoma[65, 66] as well as unknown effects on growth and development, we would not recommend use of BRAF inhibitors for this disorder for children outside of a carefully monitored clinical trial.

The Next 100 Years

Over the past 100-plus years, this enigmatic disease has morphed from Hand-Schüller-Christian/Letterer-Siwe disease to Histiocytosis X to Langerhans Cell Histiocytosis, and current data suggest Histiocytosis X may in fact be the most appropriate label. The identity crisis of LCH as an immune dysregulation versus malignancy has made it difficult for the disease to find a home among both physicians and scientists. While incidence is similar to Hodgkin lymphoma or acute myelogenous leukemia, the number of LCH studies funded by NIH or the number of LCH talks presented at ASH meetings does not compare. Emerging data suggest LCH may be a bona fide myeloid neoplasia. Future studies that further refine identity of the LCH DCs and pathogenesis of the inflammatory lesions will unveil opportunities for improved care of patients with LCH, including novel diagnostic tools, risk-stratification strategies, optimisation of chemotherapy dose and duration, and targeted therapies.

Acknowledgements

The Texas Children's Cancer Center Histiocytosis Program receives support from HistioCure Foundation. Grant support includes NIH R01 (CA154489) (CEA, KLM), NIH SPORE in Lymphoma (P50CA126752) (CEA), NIH K12 (CA090433) (SJS). The authors have no conflicting financial interests.

References

1. Guyot-Goubin A, Donadieu J, Barkaoui M, *et al.*, "Descriptive epidemiology of childhood Langerhans cell histiocytosis in France, 2000-2004", Pediatr Blood Cancer (2008), 51(1): pp. 71-5.
2. Salotti J A, Nanduri V, Pearce M S, *et al.*, "Incidence and clinical features of Langerhans cell histiocytosis in the UK and Ireland", Arch Dis Child (2009), 94(5): pp. 376-80.
3. Stalemark H, Laurencikas E, Karis J, *et al.*, "Incidence of Langerhans cell histiocytosis in children: a population-based study", Pediatr Blood Cancer (2008), 51(1): pp. 76-81.
4. Arceci R J, "The histiocytoses: the fall of the Tower of Babel", Eur J Cancer (1999), 35(5): pp. 747-67; discussion 767-9.
5. Lichtenstein L, "Histiocytosis X; integration of eosinophilic granuloma of bone, Letterer-Siwe disease, and Schuller-Christian disease as related manifestations of a single nosologic entity", AMA Arch Pathol (1953), 56(1): pp. 84-102.
6. Nezelof C, Basset F, and Rousseau M F, "Histiocytosis X histogenetic arguments for a Langerhans cell origin", Biomedicine (1973), 18(5): pp. 365-71.
7. Geissmann F, Lepelletier Y, Fraitag S, *et al.*, "Differentiation of Langerhans cells in Langerhans cell histiocytosis", Blood (2001), 97(5): pp. 1241-8.
8. Allen C E, Li L, Peters T L, *et al.*, "Cell-specific gene expression in Langerhans cell histiocytosis lesions reveals a distinct profile compared with epidermal Langerhans cells", J Immunol (2010), 184(8): pp. 4557-67.
9. Laman J D, Leenen P J, Annels N E, *et al.*, "Langerhans-

cell histiocytosis 'insight into DC biology'", Trends Immunol (2003), 24(4): pp. 190-6.
10. Willman C L, Busque L, Griffith B B, *et al.*, "Langerhans'-cell histiocytosis (histiocytosis X)--a clonal proliferative disease", N Engl J Med (1994), 331(3): pp. 154-60.
11. Senechal B, Elain G, Jeziorski E, *et al.*, "Expansion of regulatory T cells in patients with Langerhans cell histiocytosis", PLoS Med (2007), 4(8): pp. e253.
12. Nezelof C and Basset F, "An hypothesis Langerhans cell histiocytosis: the failure of the immune system to switch from an innate to an adaptive mode", Pediatr Blood Cancer (2004), 42(5): pp. 398-400.
13. da Costa C E, Szuhai K, van Eijk R, *et al.*, "No genomic aberrations in Langerhans cell histiocytosis as assessed by diverse molecular technologies", Genes Chromosomes Cancer (2009), 48(3): pp. 239-49.
14. Badalian-Very G, Vergilio J A, Degar B A, *et al.*, "Recurrent BRAF mutations in Langerhans cell histiocytosis", Blood (2010), 116(11): pp. 1919-23.
15. Davies H, Bignell G R, Cox C, et al., "Mutations of the BRAF gene in human cancer", Nature (2002), 417(6892): pp. 949-54.
16. Ginhoux F, Collin M P, Bogunovic M, *et al.*, "Blood-derived dermal langerin+ dendritic cells survey the skin in the steady state", J Exp Med (2007), 204(13): pp. 3133-46.
17. Chikwava K and Jaffe R, "Langerin (CD207) staining in normal pediatric tissues, reactive lymph nodes, and childhood histiocytic disorders", Pediatr Dev Pathol (2004), 7(6): pp. 607-14.
18. Helft J, Ginhoux F, Bogunovic M, *et al.*, "Origin and functional heterogeneity of non-lymphoid tissue dendritic cells in mice", Immunol Rev (2010), 234(1): pp. 55-75.
19. Poulin L F, Henri S, de Bovis B, *et al.*, "The dermis contains langerin+ dendritic cells that develop and function independently of epidermal Langerhans cells", J Exp Med (2007), 204(13): pp. 3119-31.
20. Segerer S, Heller F, Lindenmeyer M T, *et al.*, "Compartment specific expression of dendritic cell markers in human glomerulonephritis", Kidney Int (2008), 74(1): pp. 37-46.
21. Baumgartner I, von Hochstetter A, Baumert B, *et al.*, "Langerhans'-cell histiocytosis in adults", Med Pediatr Oncol (1997), 28(1): pp. 9-14.
22. Arico M, Girschikofsky M, Genereau T, *et al.*, "Langerhans cell histiocytosis in adults. Report from the International Registry of the Histiocyte Society", Eur J Cancer (2003), 39(16): pp. 2341-8.
23. Titgemeyer C, Grois N, Minkov M, *et al.*, "Pattern and course of single-system disease in Langerhans cell histiocytosis data from the DAL-HX 83- and 90-study", Med Pediatr Oncol (2001), 37(2): pp. 108-14.
24. Gadner H, Grois N, Potschger U, *et al.*, "Improved outcome in multisystem Langerhans cell histiocytosis is associated with therapy intensification", Blood (2008), 111(5): pp. 2556-62.
25. Minkov M, Steiner M, Potschger U, et al., "Reactivations in multisystem Langerhans cell histiocytosis: data of the international LCH registry", J Pediatr (2008), 153(5): pp. 700-5, 705 e1-2.
26. Bernard F, Thomas C, Bertrand Y, et al., "Multi-centre pilot study of 2-chlorodeoxyadenosine and cytosine arabinoside combined chemotherapy in refractory Langerhans cell histiocytosis with haematological dysfunction", Eur J Cancer (2005), 41(17): pp. 2682-9.
27. Gadner H, Minkov M, Grois N, *et al.*, "Therapy prolongation improves outcome in multisystem Langerhans cell histiocytosis", Blood (2013), 121(25): pp. 5006-14.
28. Haupt R, Nanduri V, Calevo M G, *et al.*, "Permanent consequences in Langerhans cell histiocytosis patients: a pilot study from the Histiocyte Society-Late Effects Study Group", Pediatr Blood Cancer (2004), 42(5): pp. 438-44.
29. Minkov M, Grois N, McClain K, *et al.* Histiocyte Society Evaluation and Treatment Guidelines, April 2009. 2009; Available from: www.histiocytesociety.org/document.doc?id=290.
30. Vassallo R and Ryu J H, "Pulmonary Langerhans' cell histiocytosis", Clin Chest Med (2004), 25(3): pp. 561-71, vii.
31. Gadner H, Heitger A, Grois N, *et al.*, "Treatment strategy for disseminated Langerhans cell histiocytosis. DAL HX-83 Study Group", Med Pediatr Oncol (1994), 23(2): pp. 72-80.
32. Gadner H, Grois N, Arico M, *et al.*, "A randomized trial of treatment for multisystem Langerhans' cell histiocytosis", J Pediatr (2001), 138(5): pp. 728-34.
33. Minkov M, Grois N, Heitger A, *et al.*, "Treatment of multisystem Langerhans cell histiocytosis. Results of the DAL-HX 83 and DAL-HX 90 studies. DAL-HX Study Group", Klin Padiatr (2000), 212(4): pp. 139-44.
34. Cantu M A, Lupo P J, Bilgi M, *et al.*, "Optimal therapy for adults with Langerhans cell histiocytosis bone lesions", PLoS One (2012), 7(8): pp. e43257.
35. Grois N, Fahrner B, Arceci R J, *et al.*, "Central nervous system disease in Langerhans cell histiocytosis", J Pediatr (2010), 156(6): pp. 873-81, 881 e1.
36. Dhall G, Finlay J L, Dunkel I J, *et al.*, "Analysis of outcome for patients with mass lesions of the central nervous system due to Langerhans cell histiocytosis treated with 2-chlorodeoxyadenosine", Pediatr Blood Cancer (2008), 50(1): pp. 72-9.
37. Barthez M A, Araujo E, and Donadieu J, "Langerhans cell histiocytosis and the central nervous system in childhood: evolution and prognostic factors. Results of a collaborative study", J Child Neurol (2000), 15(3): pp. 150-6.
38. Allen C E, Flores R, Rauch R, *et al.*, "Neurodegenerative central nervous system Langerhans cell histiocytosis and coincident hydrocephalus treated with vincristine/cytosine arabinoside", Pediatr Blood Cancer (2010), 54(3): pp. 416-23.
39. Imashuku S, Okazaki N, Nakayama M, *et al.*, "Treatment of neurodegenerative CNS disease in Langerhans cell histiocytosis with a combination of intravenous immunoglobulin and chemotherapy", Pediatr Blood Cancer (2008), 50(2): pp. 308-11.
40. Idbaih A, Donadieu J, Barthez M A, *et al.*, "Retinoic acid therapy in "degenerative-like" neuro-langerhans cell histiocytosis: a prospective pilot study", Pediatr Blood Cancer (2004), 43(1): pp. 55-8.
41. Grois N, Potschger U, Prosch H, *et al.*, "Risk factors for diabetes insipidus in langerhans cell histiocytosis", Pediatr Blood Cancer (2006), 46(2): pp. 228-33.
42. Yasko A W, Fanning C V, Ayala A G, *et al.*, "Percutaneous techniques for the diagnosis and treatment of localized Langerhans-cell histiocytosis (eosinophilic granuloma of bone)", J Bone Joint Surg Am (1998), 80(2): pp. 219-28.
43. Kotecha R, Venkatramani R, Jubran R F, *et al.*, "Clinical Outcomes of Radiation Therapy in the Management of Langerhans Cell Histiocytosis", Am J Clin Oncol (2013). Epub 8 March 2013.
44. Sakai H, Ibe M, Takahashi H, *et al.*, "Satisfactory remission achieved by PUVA therapy in Langerhans cell hisiocytosis in an elderly patient", J Dermatol (1996), 23(1): pp. 42-6.
45. Kwon O S, Cho K H, and Song K Y, "Primary cutaneous Langerhans cell histiocytosis treated with photochemotherapy", J Dermatol (1997), 24(1): pp. 54-6.
46. Lindahl L M, Fenger-Gron M, and Iversen L, "Topical nitrogen mustard therapy in patients with Langerhans cell histiocytosis", Br J Dermatol (2012), 166(3): pp. 642-5.
47. Failla V, Wauters O, Caucanas M, *et al.*, "Photodynamic therapy for multi-resistant cutaneous Langerhans cell histiocytosis", Rare Tumors (2010), 2(2): pp. e34.
48. Steen A E, Steen K H, Bauer R, *et al.*, "Successful treatment of cutaneous Langerhans cell histiocytosis with low-dose methotrexate", Br J Dermatol (2001), 145(1): pp. 137-40.
49. McClain K L, "Drug therapy for the treatment of Langerhans cell histiocytosis", Expert Opin Pharmacother (2005), 6(14): pp. 2435-41.
50. Do J E, Lee J Y, and Kim Y C, "Successful treatment of cutaneous Langerhans' cell histiocytosis with targeted narrowband ultraviolet B phototherapy in an infant", Clin Exp Dermatol (2009), 34(7): pp. e280-1.
51. Chang S E, Koh G J, Choi J H, *et al.*, "Widespread skin-limited adult Langerhans cell histiocytosis: long-term follow-up with good response to interferon alpha", Clin Exp Dermatol (2002), 27(2): pp. 135-7.
52. Sander C S, Kaatz M, and Elsner P, "Successful treatment of cutaneous langerhans cell histiocytosis with thalidomide", Dermatology (2004), 208(2): pp. 149-52.
53. Ronceray L, Potschger U, Janka G, *et al.*, "Pulmonary involvement in pediatric-onset multisystem Langerhans cell histiocytosis: effect on course and outcome", J Pediatr (2012), 161(1): pp. 129-33 e1-3.
54. Willis B, Ablin A, Weinberg V, *et al.*, "Disease course and late sequelae of Langerhans' cell histiocytosis: 25-year experience at the University of California, San Francisco", J Clin Oncol (1996), 14(7): pp. 2073-82.
55. Selch M T and Parker R G, "Radiation therapy in the management of Langerhans cell histiocytosis", Med Pediatr Oncol (1990), 18(2): pp. 97-102.
56. Weitzman S, Braier J, Donadieu J, *et al.*, "2'-Chlorodeoxyadenosine (2-CdA) as salvage therapy for Langerhans cell histiocytosis (LCH). results of the LCH-S-98 protocol of the Histiocyte Society", Pediatr Blood Cancer (2009), 53(7): pp. 1271-6.
57. Apollonsky N and Lipton J M, "Treatment of refractory Langerhans cell histiocytosis (LCH) with a combination of 2-chlorodeoxyadenosine and cytosine arabinoside", J Pediatr Hematol Oncol (2009), 31(1): pp. 53-6.
58. Akkari V, Donadieu J, Piguet C, *et al.*, "Hematopoietic stem cell transplantation in patients with severe Langerhans cell histiocytosis and hematological dysfunction: experience of the French Langerhans Cell Study Group", Bone Marrow Transplant (2003), 31(12): pp. 1097-103.
59. Kudo K, Ohga S, Morimoto A, *et al.*, "Improved outcome of refractory Langerhans cell histiocytosis in children with hematopoietic stem cell transplantation in Japan", Bone Marrow Transplant (2010), 45(5): pp. 901-6.
60. Steiner M, Matthes-Martin S, Attarbaschi A, *et al.*, "Improved outcome of treatment-resistant high-risk Langerhans cell histiocytosis after allogeneic stem cell transplantation with reduced-intensity conditioning", Bone Marrow Transplant (2005), 36(3): pp. 215-25.
61. Rodriguez-Galindo C, Jeng M, Khuu P, *et al.*, "Clofarabine in refractory Langerhans cell histiocytosis", Pediatr Blood Cancer (2008), 51(5): pp. 703-6.
62. Abraham A, Alsultan A, Jeng M, *et al.*, "Clofarabine salvage therapy for refractory high-risk langerhans cell histiocytosis", Pediatr Blood Cancer (2013), 60(6): pp. E19-22.
63. Parker W B, Shaddix S C, Chang C H, *et al.*, "Effects of 2-chloro-9-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl) adenine on K562 cellular metabolism and the inhibition of human ribonucleotide reductase and DNA polymerases by its 5'-triphosphate", Cancer Res (1991), 51(9): pp. 2386-94.
64. Haroche J, Cohen-Aubart F, Emile J F, *et al.*, "Dramatic efficacy of vemurafenib in both multisystemic and refractory Erdheim-Chester disease and Langerhans cell histiocytosis harboring the BRAF V600E mutation", Blood (2013), 121(9): pp. 1495-500.
65. Arnault J P, Mateus C, Escudier B, *et al.*, "Skin tumors induced by sorafenib; paradoxic RAS-RAF pathway activation and oncogenic mutations of HRAS, TP53, and TGFBR1", Clin Cancer Res (2012), 18(1): pp. 263-72.
66. Robert C, Arnault J P, and Mateus C, "RAF inhibition and induction of cutaneous squamous cell carcinoma", Curr Opin Oncol (2011), 23(2): pp. 177-82.

TREATMENT STRATEGIES

HEALTHCARE PUBLISHER - REPRINTS

The Cambridge Research Centre publishes a rich and diverse portfolio of fully referenced review articles across numerous healthcare fields. All articles included in Treatment Strategies are available as reprints (minimum order of 500). With tailor-made A4 full-colour booklets, including a bespoke front cover, each publication can be distributed worldwide and produced at the highest quality, on 150gsm (silk) paper.

For further information contact *info@cambridgeresearchcentre.co.uk*.
Separate e-Books are available on request.
Reprints are available both in print and electronically,
in US and European formats and with or without covers.

Prices start from £0.50 per copy
- call 0207 953 8490 for a quotation.

www.cambridgeresearchcentre.co.uk

Acute Promyelocytic Leukaemia in Adult Patients

Felicetto Ferrara
Division of Hematology and Stem Cell Transplantation Unit, Cardarelli Hospital, Naples

Epidemiology and Diagnosis

Acute promyelocytic leukaemia (APL) is a distinct subtype of acute myeloid leukaemia (AML) with peculiar morphologic, cytogenetic, and biomolecular characteristics.[1] Most patients are young, present with leukopenia, and exhibit a life-threatening coagulopathy, which is the most relevant clinical manifestation of the disease at diagnosis.[2] APL accounts for 10%-15% of all AML diagnosed in adults and is more frequent in the Latin Hispanic race than in Caucasians.[3] In most cases, the presumptive diagnosis of APL can easily be made by morphologic examination of peripheral blood or bone marrow aspirate by an experienced haematologist. The peripheral blood smear most frequently shows abnormal promyelocytes, with abundant, often irregular-appearing primary azurophilic granules and bundles of Auer rods (Figure 1, Panel A). At variance with classical APL, APL variant (APLv) displays minimal granulation and relative scarcity of cells with heavy granulation and Auer rods; the nucleus of most cells in the peripheral blood is bilobed, multilobed or reniform (Figure 1, Panel B); however, at least a few cells with all the cytoplasmic features of typical APL are present.[4]

APLv is often associated with a high white blood cell count (WBC > 10 x 10^9/l) and more all-*trans* retinoic acid (ATRA)-related toxicities, particularly pseudotumour cerebri.[5] Appreciation of these details of morphology features is critical, since APL is the one subtype of AML for which immediate treatment with ATRA and intensive blood product support must begin in order to reduce early mortality when the disease is suspected, before confirmation of the diagnosis at immunophenotypic, cytogenetic and molecular level.[6] An immunofluorescence method using an anti-PML monoclonal antibody can rapidly establish the presence of the PML-RARA fusion protein based on the characteristic distribution pattern (Figure 2) of promyelocytic leukaemia gene (PML), that occurs in the presence of the fusion protein but cannot be readily available.[7]

Felicetto Ferrara is Head of the Department of Onco-Hematology of Cardarelli Hospital in Naples, Italy. He has authored many papers in the field of acute leukaemia and autologous stem cell transplantation, and has contributed to the development of guidelines for the treatment of acute myeloid leukaemia for different scientific societies.

Immunophenotypic Features

Immunophenotypic studies have yielded results that included positive staining for CD33, CD13, and CD19 antigens, an absence of HLA-DR expression, and low-frequency occurrence of CD7, CD11b, and CD14 expression. Aberrant surface antigens, including CD2 and CD34, have also been identified.[8,9] Several research groups have demonstrated that the expression of CD2 is associated with APLv morphology and the bcr3 PML-RARα isoform,[10,11] while the presence of the neural cell adhesion molecule CD56 has been shown to be predictive of poor outcome.[12] More recently, data have been reported suggesting that the presence of fms-like tyrosine kinase (FLT3) gene internal tandem duplication (ITD) mutation is closely related to aberrant CD2 expression and high expression levels of FLT3 mRNA.[13]

Genetic and Molecular Characteristics

APL is characterised by a balanced reciprocal translocation between chromosomes 15 and 17, that is, t(15;17)(q22;q21), which results in the fusion between the PML gene and retinoic acid receptor (RARalpha) (Figure 3). Because the efficacy of differentiation treatment based on retinoids and/or arsenic derivatives is strictly dependent on the presence of the PML-RARalpha fusion gene in leukaemic cells, genetic confirmation of this specific lesion is mandatory in all cases.[14-16] In addition, the hybrid gene is extremely important for the monitoring of therapeutic results, APL being the only AML subtype in which molecular remission, defined by the absence of the leukaemic transcript, represents the main indication of therapeutic efficacy.[17,18] Molecular response should be evaluated by real-time polymerase chain reaction (RT-PCR) at the end of consolidation therapy.[19] Re-appearance of the PML-RARAalpha gene, demonstrated in two examinations repeated at an interval of 1 month, defines molecular relapse, which is invariable followed by haematological relapse and should be treated no differently from this. Therefore, standardised RT-PCR evaluation of the PML-RARAalpha gene represents a powerful and reproducible tool for prospective monitoring minimal residual disease.[14-19]

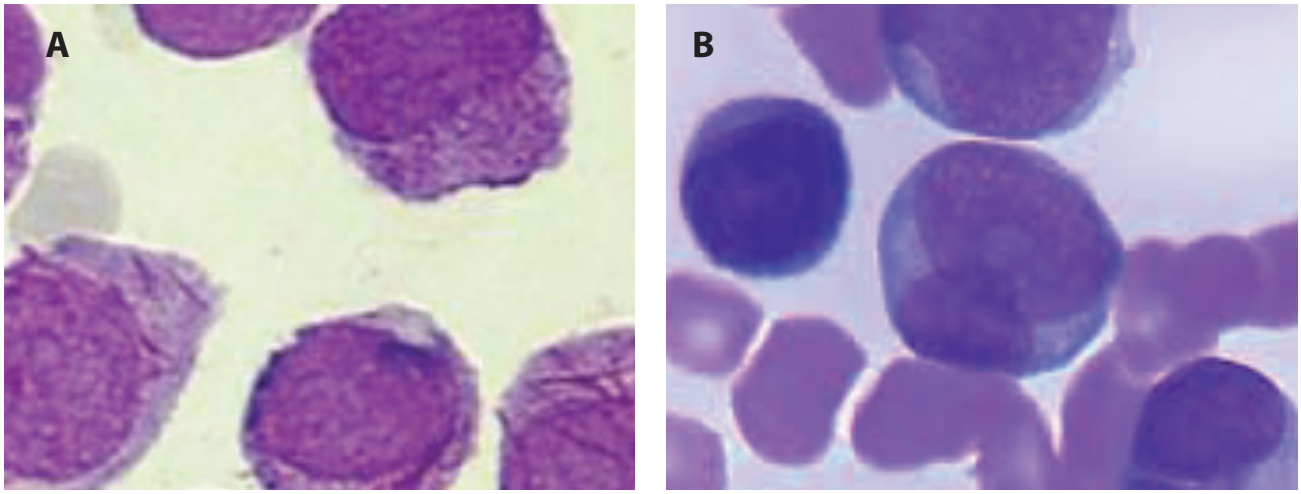


Figure 1. Panel A: morphology of atypical promyelocytes showing abundant, often irregular-appearing primary azurophilic granules and bundles of Auer rods (classical APL). Panel B: Atypical promyelocytes displays cytoplasma with minimal granulation; the nucleus of most cells in the peripheral blood is bilobed, multilobed or reniform.

Prognostic Factors

The most suitable parameters for risk stratification in APL are still under debate. It has been discussed whether patients with APLv might have higher rates of early death because of haemorrhagic complications when compared to patients with the classical APL, however the outcomes of patients with the two subtypes did not differ significantly when adjustment for WBC counts was made. The Sanz score subdivides APL patients according to peripheral blood counts into three risk groups: low (WBC $\leq 10\times10^9$/L and platelet count $>40\times10^9$/L), intermediate (WBC $\leq 10\times10^9$/L and platelet count $\leq 40\times10^9$/L), and high (WBC $>10\times10^9$/L).[20] High-risk APL patients with a WBC count greater than 10×10^9/L were reported to achieve higher complete remission (CR) rates and better survival outcomes when cytarabine (ARA-C) was included in the chemotherapy regimens, whereas for patients with a WBC count less than 10×10^9/L all-*trans* retinoic acid in combination with anthracyclines might be sufficient. The APLv subtype has been associated with higher frequencies of *FLT3*-ITD, which may have a negative prognostic impact.[21, 22]

FLT3-ITD occurrs in 12–38% of all APL patients and tyrosine kinase domain (TKD) mutations in 2–20%. The prognostic impact of *FLT3*-ITD was reported to worsen prognosis in APL in some studies, but others found opposite results,[23-26] so that it remains unclear whether *FLT3*-ITD mutation status should be incorporated into risk-adapted therapeutic algorithms.[26] The Sanz score remains the most used tool for prognostic stratification.

Pathogenesis of APL

The classical APL pathogenetic model describes the PLM-RARalpha fusion product as acting as a dominant negative of RARalpha by forming homodimers, recruiting co-repressors, and inhibiting the expression of target genes necessary for granulocytic differentiation. Furthermore, the PML-RARalpha product might also inhibit the normal function of the PML protein as a tumour suppressor, and therefore act as a dominant negative against both proteins. PML is the organiser of nuclear domains known as

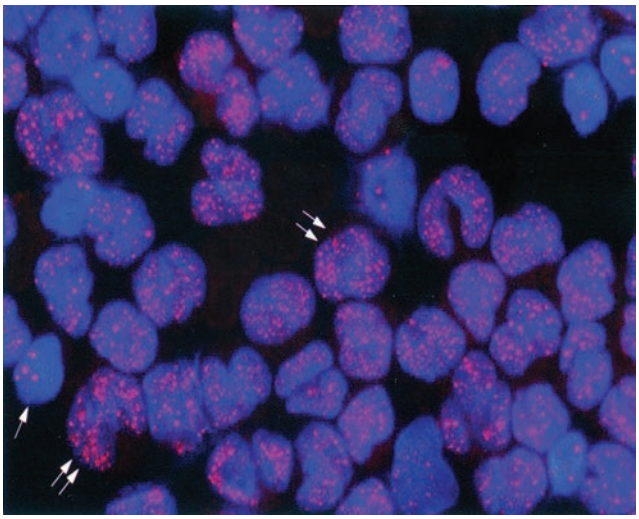


Figure 2. Immundiagnosis of APL: the nuclei of leukaemic promyelocytes (double arrows) display many tiny dots (microgranular pattern). The single arrow points to a residual normal haematopoietic cell showing the speckled positivity of wild-type PML.

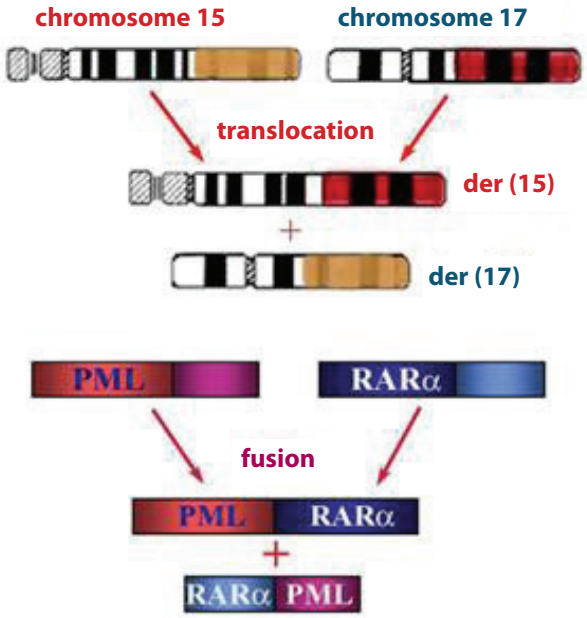


Figure 3. Balanced reciprocal translocation between chromosomes 15 and 17, that is, t(15;17)(q22;q21), which results in the fusion between the promyelocytic leukaemia (PML) gene and retinoic acid receptor (RARalpha).

Induction therapy	
ATRA + antracyclines	Standard of care for any risk
ATRA + ATO	Expected to become the standard of care for low-intermediate risk patients
ATO	Consider in older frail patients and in those previously treated with anthracyclins for other malignancies
Anthracyclines only (daunorubicin)	Consider for pregnant women with APL in the first trimester, when abortion is refused
Consolidation	
Anthracyclines + ATRA	Standard of care for low-intermediate risk patients
Anthracyclines + ATRA + ARA-C	Standard of care for high risk patients
ATO	Consider in older frail patients and in those previously treated with anthracyclins for other malignancies
Gemtuzumab-Ozogamycin	Consider in older frail patients
Maintenance	
ATRA	15 days every three months for 2 years
Chemotherapy	No role
Stem cell transplantation	
Autologous	Consider in patient in late second molecular remission (> 18 months from first CR achievement) with molecularly negative graft
Allogeneic	Consider in patients who are molecularly positive at the end of consolidation and in early relapse (<18 months from first CR achievement)

Table 1. Therapeutic options for APL.

PML nuclear bodies (NB). Pharmacological concentrations of all-*trans* retinoic acid (ATRA) lead to a conformation change of the multifunctional complex around PML-RARalpha. Co-repressors are released, normal regulation of RAR-alpha-responsive genes is restored, and hence terminal differentiation of APL cells is induced.[27] Arsenic trioxide (ATO) also elicits PML/RARA degradation and nuclear bodies reformation, but it acts via the PML rather than the RARA moiety of PML/RARA.[28] Indeed, similar to RA, which degrades RARalpha, ATO degrades normal PML and, thus, PML/RARalpha oncoprotein. This unexpected convergence between two clinically active agents, discovered by chance, supports the idea that PML/RARA degradation, and hence PML NB reformation, contribute together to clinical remissions.[28, 29]

Treatment of APL

Overall, APL treatment involves induction, consolidation, and maintenance phases (Table 1). Unanswered questions still exist and concern the ideal induction therapy, the best initial treatment for older patients, the subset of patients most likely to benefit from maintenance therapy and the most effective relapse regimen.

Induction Therapy

Treatment with ATRA should be initiated the same day that diagnosis is suggested by morphologic examination of blood and/or bone marrow, depending on the favourable risk-benefit ratio associated with this approach;[30] moreover, ATRA is unlikely to have any deleterious effect should genetic assessment fail to confirm the diagnosis of APL.[31] ATRA improves the APL coagulopathy rapidly, decreasing the risk of severe bleeding. Although treatment with single-agent ATRA results in a CR rate > 85 – 90%, there is unanimous consent that induction therapy should be based on the combination of ATRA + chemotherapy. There are two reasons for this: the first relies on the better quality of CR induced by ATRA + chemotherapy (early studies demonstrated that most patients initially treated with ATRA alone did ultimately relapse), the second postulates that the combination is more effective in controlling ATRA-induced leukocytosis, which is often indicative of APL syndrome occurrence (see below). The CR rate achieved with the simultaneous combination is > 90% in large multicentre studies and the most frequent cause of therapeutic failure, which is probably underestimated in the clinical trials, is early death due to fatal cerebral haemorrhage.[32-34] Of note, higher early death rate has been recorded in older APL patient population.[35, 36] Resistance to therapy in APL is virtually absent and would generate the suspicion of incorrect diagnosis. The addition of ATRA is clearly associated with a relevant reduction of mortality due to APL coagulopathy, so that no additional measure is currently indicated for this potentially fatal complication.[37]

One unresolved question regards the type of chemotherapy that should be added to ATRA. Outside of a clinical trial, we use idarubicin (IDA) independently from initial risk, given that in a series of 95 patients with APL we achieved a CR rate of 96%.[38] In the literature, comparable CR rates have been reported using ATRA + daunorubicin and ARA-C or ATRA + IDA alone, with no apparent advantage observed by adding ARA-C.[39] Of interest, a recent randomised clinical trial from United Kingdom did demonstrate that additional chemotherapy, ARA-C in particular, is not required in induction therapy of APL, whatever the risk at diagnosis.[40] As to the type of anthracycline, we prefer IDA but no data have definitively demonstrated an advantage in comparison to daunorubicin. While

simultaneous ATRA and chemotherapy is the current gold standard for newly diagnosed APL resulting in ~80% cure rates, ATO in variable combinations including ± ATRA ± chemotherapy has also been tested as front-line therapy yielding encouraging results in several pilot studies as well as in two phase III studies conducted in China and the US.[41] A recent trial firstly compared ATO + ATRA vs. ATRA + IDA and definitively demonstrated that for patients with newly diagnosed non-high-risk APL, the front-line chemotherapy-free ATO+ATRA combination is at least not inferior for 2 year event free survival (EFS), potentially leading to a new standard of care in non-high risk patients.[42]

Post-induction Therapy

Molecular CR must be the final endpoint of induction/consolidation therapy. The conventional approach is of administering two or three courses of chemotherapy, including anthracyclines with or without ARA-C. Given that any consolidation course induces prolonged pancytopenia, reduction of the intensity of consolidation has been considered according to a 'less is better' approach.[43] The role of ARA-C in consolidation remains somehow controversial, but at least in low- and intermediate-risk patients, risks deriving from more intensive consolidation are not balanced by any clinical benefit; on the contrary, a trend in favour of ARA-C administration was observed for high-risk patients.[44] More recently, a statistically significant reduction in relapse risk was achieved by adding ATRA at standard dose in conjunction with three courses of consolidation chemotherapy,[45] and this approach is currently adopted at our institution.

Recently, aiming at elimination of chemotherapy as well as reinforcement of ATRA efficacy, ATO has emerged as a powerful post-induction treatment of APL. Apart from phase 2 trials on relatively small number of patients,[41] the use of ATO as post-remission treatment has been supported by a large, randomised study by the US Intergroup, in which patients were randomised to receive or not receive two courses of 25 days of ATO (5 days a week for 5 weeks) immediately after CR achievement.[46] The administration of ATO resulted in a significantly better EFS and overall survival (OS), providing further evidence for the use of ATO in consolidation. The question of maintenance therapy for APL patients with molecular negative disease at the end of consolidation is still an unresolved question.[47] Two recent randomised studies showed no benefit for these patients, either when using ATRA, 6-mercaptopurine, and methotrexate (the Italian approach) or when using six courses of intensified therapy (the Japanese approach).[48, 49] Furthermore, tolerance to chemotherapy is poor and more than half of patients discontinue the treatment because of gastrointestinal or hepatic toxicity. Our current approach is to administer ATRA for 15 days at intervals of 3 months for 2 years.

Treatment of Relapse

Relapse occurs in approximately 5 – 30% of patients and is almost exclusively limited to high-risk disease.[50] Approximately 3 – 5% of patients develop extramedullary relapse, in most cases involving the central nervous system, but other sites might also be implicated.[51] Finally, a small group of patients (< 3%) experience isolated molecular relapse. Therapeutic options should take into account different characteristics of patients at relapse, which are independent from initial categorisation. In particular, duration of first CR, the WBC count, the number of previous relapses, and the achievement or not of molecular remission after haematological remission.[52] Patients presenting with none of these factors would be considered as low-risk, all others as high-risk. For patients treated in induction/consolidation with ATRA + chemotherapy, there is no doubt that single-agent ATO represents the treatment of choice.[53] Data from the US multicentre study suggested a CR rate of 85% and a molecular remission rate of 78%, clearly showing that ATO is the most powerful inducer of molecular remission.[54] We suggest two or three additional courses after CR achievement. High-risk patients aged < 45 – 50 years could be considered for allogeneic stem cell transplantation (allo-SCT), whereas patients who do not have a donor and those older than 50 would be candidates for autologous SCT (ASCT), provided that they had a molecularly negative graft (peripheral blood stem cell) and were in molecular remission.[55-57]

Our experience is very favourable in relapsed patients receiving ASCT in the absence of transplant-related mortality and long-term survival exceeding 80%.[58] With regard to allo-SCT, there is unanimous consent that it has no role to play in APL for patients in first molecular CR.[59, 60] The only indication for allo-SCT includes patients who do not achieve molecular CR at the end of consolidation (5% or less) or those who experience early relapse (first CR duration less than 18 – 24 months). Obviously, allo-SCT must also be considered for patients in their second or further relapse.

APL Differentiation Syndrome

The APL differentiation syndrome (DS) is an unpredictable but frequent complication that may develop after administration of ATRA and/or ATO.[61] DS is reported in 2.5 – 30% of APL patients who receive induction therapy with ATRA and/or ATO, while it is absent during consolidation or maintenance therapy with both compounds and during ATRA treatment in non-APL malignancies, implicating that the leukaemic promyelocytes play a crucial role in the development of DS.[62] The wide range of incidence probably depends on the different criteria used for the diagnosis, as well as on differences in induction therapy and supportive measures. Furthermore, as some DS symptoms might be due to concurrent medical problems such as bacteremia, sepsis, pneumonia, pulmonary haemorrhage, or congestive heart failure – DS may be under- or overdiagnosed. On clinical grounds, the syndrome should be suspected in the presence of one of the following symptoms and signs: dyspnea, unexplained fever, weight gain, peripheral edema, unexplained hypotension, acute renal failure or congestive heart failure, and particularly if a chest radiograph demonstrates interstitial pulmonary infiltrates or pleuropericardial effusion. Risk factors for developing DS are not well understood but may include high WBC count, rapidly increasing WBC count, and the expression of cell-surface antigens, mainly adhesion molecules, and chemokine induction.[63, 64] Of interest, in patients treated

with combination of ATRA and Idarubicin, an increased body mass index correlates with incidence of DS.[65] The syndrome is recognised as a distinct complication and a potential life threatening adverse reaction. Therefore, we usually administer specific therapy with dexamethasone at a dose of 10 mg daily by intravenous injection when one symptom is present. In the presence of a high WBC count (>100x10^9/L), our policy is to include the administration of 500 mg ARA-C, even though the risk of myelotoxicity increases substantially. A temporary discontinuation of ATRA and/or ATO is indicated in cases of severe DS, in particular in the presence of acute renal failure or respiratory distress syndrome requiring admission in the intensive care unit.

Indications to Individualised Approach in APL

While inclusion into clinical trials remains the best option for patients with APL, in special situations stringent inclusion criteria of the studies represent reasons of exclusion. Furthermore, in specific situations the optimal approach consisting of the combination of ATRA + anthracyclines may be contra-indicated because of concomitant diseases or poor performance status, unrelated to APL.

APL in Pregnancy

Given the teratogenic potential of chemotherapy, ATRA, and ATO on the fetus, the overall treatment of the pregnant patient with APL should include a discussion about pregnancy termination, especially if APL is diagnosed in the first trimester.[66] In this setting, we favour therapeutic abortion after inducing CR with ATRA plus IDA. After abortion, therapy should be continued on the basis of Sanz risk, as in standard patients. If the pregnancy is to continue due to religious faith or insuppressible patient's desire, then an appropriate chemotherapy regimen needs to be determined, avoiding either ATRA or ATO. In this regard, daunorubicin might be preferred because this agent is known to be effective in APL and there is more published experience of its use in pregnancy.[67] The patient should be informed about the high risk of haemorrage deriving from the lack of ATRA in the therapeutic program. On the contrary, for APL occurring during the second and third trimesters of pregnancy, management should not differ from that used in the daily practice. Notwithstanding, frequent fetal monitoring, along with aggressive management of potential APL-related complications, is necessary to allow for optimal maternal and fetal outcomes.

APL in Older Patients

At variance with other AML subtypes, the haematological, cytogenetic, and molecular features of APL in advanced age are substantially similar to those of young adults and older APL patients seem to be as sensitive to specific APL therapy as younger individuals. However, the prognosis of the disease steadily worsens with increasing age, since induction therapy based on ATRA and anthracyclin can be contraindicated by cardiomyopathy or other severe organ dysfunction.[68, 69] In addition, early death rate is significantly higher; a population-based study from the US reported an early death rate of 24% in APL patients aged 55 years or older.[33] In the Swedish Adult Leukemia Registry, the ED rate of 105 unselected APL patients of all age groups was 29%, while it was 50% in patients over 60 years.[34] Furthermore, consolidation therapy is in turn associated with significant morbidity and mortality.[70] Nonetheless, patients who satisfy inclusion criteria for multicentre clinical trials achieve CR rates and survival similar to young adult APL patients.[71, 72] Accordingly, it appears reasonable to manage fit elderly patients with therapeutic programmes similar to those used in younger patients, slightly attenuated in dose intensity. Those with severe comorbidities and unfit for chemotherapy (especially anthracyclines) are ideal candidates to receive single-agent ATO, since the combination ATRA plus ATO, despite potential better anti-leukaemic activity, could be associated with a considerably higher risk of DS occurrence. The attenuated induction regimen could be followed, after CR achievement, by a molecularly driven post-CR approach aimed at molecular remission achievement. In this setting, Gemtuzumab-Ozogamycin, an anti-CD33 antibody conjugated with the cytotoxic agent calicheamycin, could have a pivotal role.[73]

Treatment-related APL (t-APL)

It is well recognised that treatment-related AML (t-AML), defined as AML occurring after chemo- or radiotherapy for previous cancer, is characterised by poorer prognosis than *de novo* cases.[74] As more patients survive their primary cancers, secondary leukaemias are becoming an increasing healthcare problem. The majority of t-APL cases arise in patients who have undergone treatment for breast cancer, where topoisomerase II inhibitors such as mitoxantrone (MTZ) and epirubicin have been widely used, followed by lymphoma, with a large predominance of non-Hodgkin lymphoma compared with Hodgkin disease.[75] In the past few years, however, an increasing number of reports on t-APL occurring in multiple sclerosis (MS) in patients given MTZ have been published.[76] At variance with t-AML, secondary and *de novo* APL display abnormal promyelocytes with similar morphologic and immunophenotypic features, comparable cytogenetic findings, comparable rates of FLT3 mutations, and similar rates of recurrent disease and death.[75] No prospective studies have specifically addressed the outcome of patients with t-APL, and literature data demonstrate a favourable prognosis not significantly different from *de novo* cases. Accordingly, treatment should not differ with the possible exception of patients given high dose anthracyclines for antecedent malignancies, in whom single agent ATO or combination of ATRA and ATO could be considered.

Conclusions

While APL represents a paradigm of therapeutic success, treatment still needs to be improved. The time has come to approach low-risk patients with ATRA and ATO, either in induction or consolidation, avoiding any chemotherapy. In more detail, the combination ATRA/ATO could be used as induction (with careful attention to the APL syndrome), followed by between four and six courses of ATO as consolidation. For high-risk patients, the combination of ATRA plus anthracyclines remains the induction treatment of choice; two or three courses of ARA-C combined with anthracyclines represents current standard of consolidation therapy, even though recent data suggest to de-escalate treatment by

removing non-anthracycline treatment irrespective of risk group. The benefits may not be in overall survival but reduced myelosuppression and its consequences, mainly in terms of reduced hospitalisation and improved quality of life.[40] ATO represents the ideal therapy for older frail APL patients as well as for those previously treated with anthracyclines because of previous malignancies; in these cases consolidation with GO could offer the best risk:benefit ratio. Finally, any effort should be made to minimise early haemorrhagic mortality, which still accounts for over 10-15 % of all newly diagnosed patients also in developed countries. Physicians caring for patients with APL treated with ATRA or ATO should be aware of early symptoms or signs suggestive of the APL differentiation syndrome. Finally, it is to be considered that current tools for diagnosis and treatment can be not available in many developing countries, including some in Latin America, where the disease may be particularly common. Recently, an International Consortium (IC-APL) was established with the aim of creating a network in developing countries that would exchange experience and data and receive support from well-established cooperative groups from United States and Europe. The establishment of the IC-APL network resulted in a nearly 50% decrease in early mortality and a 30% improvement in survival compared to historical controls, resulting in outcome similar to those reported in developed countries.[77]

References

1. Ferrara F, Schiffer CA. Acute myeloid leukaemia in adults. Lancet 2013;381:484-95
2. Lo-Coco F, Ammatuna E, Montesinos P, *et al*. Acute promyelocytic leukemia: recent advances in diagnosis and management. Semin Oncol. 2008;35:401-9
3. Douer D. The epidemiology of acute promyelocytic leukaemia. Best Pract Res Clin Haematol. 2003;16:357-67
4. Liso V, Bennett J. Morphological and cytochemical characteristics of leukaemic promyelocytes. Best Pract Res Clin Haematol. 2003;16:349-55
5. Ferrara F. Acute promyelocytic leukemia: what are the treatment options? Expert Opin Pharmacother. 2010;11:587-96
6. Tallman M, Lo-Coco F, Kwaan H, *et al*. Clinical roundtable monograph. Early death in patients with acute promyelocytic leukemia. Clin Adv Hematol Oncol. 2011;9:1-16.
7. Dimov ND, Medeiros LJ, Kantarjian HM *et al*. Rapid and reliable confirmation of acute promyelocytic leukemia by immunofluorescence staining with an antipromyelocytic leukemia antibody: the M. D. Anderson Cancer Center experience of 349 patients. Cancer. 2010 ;116:369-76
8. Dong HY, Kung JX, Bhardwaj V, *et al*. Flow cytometry rapidly identifies all acute promyelocytic leukemias with high specificity independent of underlying cytogenetic abnormalities. Am J Clin Pathol. 2011;135:76-84
9. Di Noto R, Mirabelli P, Del Vecchio L. Flow cytometry analysis of acute promyelocytic leukemia: the power of 'surface hematology'. Leukemia. 2007;21:4-8
10. Lin P, Hao S, Medeiros LJ, *et al*. Expression of CD2 in acute promyelocytic leukemia correlates with short form of PML-RARalpha transcripts and poorer prognosis. Am J Clin Pathol. 2004;121:402–7.
11. Albano F, Mestice A, Pannunzio A, *et al*. The biological characteristics of CD34+ CD2+ adult acute promyelocytic leukemia and the CD34 CD2 hypergranular (M3) and microgranular (M3v) phenotypes. Haematologica. 2006;91:311–6
12. Ferrara F, Morabito F, Martino B, *et al*. CD56 expression is an indicator of poor clinical outcome in patients with acute promyelocytic leukemia treated with simultaneous all-trans-retinoic acid and chemotherapy. J Clin Oncol. 2000;18:1295–300
13. Takenokuchi M, Kawano S, Nakamachi Y *et al*. FLT3/ITD associated with an immature immunophenotype in PML-RARα leukemia. Hematol Rep. 2012;4:e22.
14. Grimwade D, Mistry AR, Solomon E, *et al*. Acute promyelocytic leukemia: a paradigm for differentiation therapy. Cancer Treat Res. 2010;145:219-35
15. Mi JQ, Li JM, Shen ZX, *et al*. How to manage acute promyelocytic leukemia. Leukemia. 2012;26:1743-51
16. Lo-Coco F, Ammatuna E. Front line clinical trials and minimal residual disease monitoring in acute promyelocytic leukemia. Curr Top Microbiol Immunol. 2007;313:145-56
17. Cazzaniga G, Gaipa G, Rossi V, *et al*. Monitoring of minimal residual disease in leukemia, advantages and pitfalls. Ann Med. 2006;38:512-21
18. Freeman SD, Jovanovic JV, Grimwade D. Development of minimal residual disease-directed therapy in acute myeloid leukemia. Semin Oncol. 2008;35:388-400
19. Sanz MA, Lo-Coco F. Modern approaches to treating acute promyelocytic leukemia. J Clin Oncol. 2011;29:495-503.
20. Sanz MA, Lo Coco F, Martín G *et al*. Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. Blood. 2000;96:1247-53.
21. Gallagher RE, Moser BK, Racevskis J *et al*. Treatment-influenced associations of PML-RARα mutations, FLT3 mutations, and additional chromosome abnormalities in relapsed acute promyelocytic leukemia. Blood. 2012;120:2098-10
22. Kutny MA, Moser BK, Laumann K *et al*. FLT3 mutation status is a predictor of early death in pediatric acute promyelocytic leukemia: a report from the Children's Oncology Group. Pediatr Blood Cancer. 2012;59:662-7
23. Schnittger S, Bacher U, Haferlach C, *et al*. Clinical impact of FLT3 mutation load in acute promyelocytic leukemia with t(15;17)/PML-RARA. Haematologica. 2011;96:1799-807
24. Barragán E, Montesinos P, Camos M *et al*. Prognostic value of FLT3 mutations in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline monochemotherapy. Haematologica. 2011;96:1470-7
25. Callens C, Chevret S, Cayuela JM, *et al*. Prognostic implication of FLT3 and Ras gene mutations in patients with acute promyelocytic leukemia (APL): A retrospective study from the European APL Group. Leukemia. 2005;19:1153–1160
26. Beitinjaneh A, Jang S, Roukoz H, *et al*. Prognostic significance of FLT3 internal tandem duplication and tyrosine kinase domainmutations in acute promyelocytic leukemia: A systematic review. Leuk Res. 2010;34:831–836.
27. Ablain J, de The H. Revisiting the differentiation paradigm in acute promyelocytic leukemia. Blood. 2011;117:5795-802
28. de Thé H, Le Bras M, Lallemand-Breitenbach V. The cell biology of disease: Acute promyelocytic leukemia, arsenic, and PML bodies. J Cell Biol. 2012;198:11-21.
29. Lo-Coco F, Ammatuna E. The biology of acute promyelocytic leukemia and its impact on diagnosis and treatment. Hematology Am Soc Hematol Educ Program. 2006:156-61
30. Stein, Tallman MS. Provocative pearls in diagnosing and treating acute promyelocytic leukemia. Oncology (Williston Park). 2012 ;26:636-41
31. Schlenk RF, Fröhling S, Hartmann F *et al*. Phase III study of all-trans retinoic acid in previously untreated patients 61 years or older with acute myeloid leukemia. Leukemia. 2004;18:1798-803
32. McClellan JS, Kohrt HE, Coutre S *et al*. Treatment advances have not improved the early death rate in acute promyelocytic leukemia. Haematologica. 2012;97:133-6
33. Lehmann S, Ravn A, Carlsson L *et al*. Continuing high early death rate in acute promyelocytic leukemia: a population-based report from the Swedish Adult Acute Leukemia Registry. Leukemia. 2011 Jul;25:1128-34
34. Park JH, Qiao B, Panageas KS *et al*. Early death rate in acute promyelocytic leukemia remains high despite all-trans retinoic acid. Blood. 2011;118:1248-54
35. Lengfelder E, Hanfstein B, Haferlach C *et al*. Outcome of elderly patients with acute promyelocytic leukemia: results of the German Acute Myeloid Leukemia Cooperative Group. Ann Hematol. 2013;92:41-52
36. Ferrara F, Finizio O, D'Arco A, *et al*. Acute promyelocytic leukemia in patients aged over 60 years: multicenter experience of 34 consecutive unselected patients. Anticancer Res. 2010;30:967-71.
37. Breen KA, Grimwade D, Hunt BJ. The pathogenesis and management of the coagulopathy of acute promyelocytic leukaemia. Br J Haematol. 2012;156:24-36
38. Sanz MA, Lo Coco F, Martin G, *et al*. Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. Blood 2000;96:1247-53
39. Ades L, Fenaux P. Is cytarabine required in the treatment of acute promyelocytic leukemia? Updated experience and review of the literature. Clin Adv Hematol Oncol 2006;4:678-82

40. Burnett AK, Hills RK, Grimwade D *et al*. Inclusion of chemotherapy in addition to anthracycline in the treatment of acute promyelocytic leukaemia does not improve outcomes: results of the MRC AML15 trial. Leukemia. 2012 Dec 10 [Epub ahead of print]
41. Breccia M, Lo-Coco F. Arsenic trioxide for management of acute promyelocytic leukemia: current evidence on its role in front-line therapy and recurrent disease. Expert Opin Pharmacother. 2012;13:1031-43
42. Lo coco F, Avvisati G, Orlando SM *et al*. ATRA and Arsenic Trioxide (ATO) Versus ATRA and Idarubicin (AIDA) for Newly Diagnosed, Non High-Risk Acute Promyelocytic Leukemia (APL): Results of the Phase III, Prospective, Randomized, Intergroup APL0406 Study by the Italian-German Cooperative Groups Gimema-SAL-AMLSG. Blood 2012;120:6 (Abs)
43. Sanz MA, Martin G, Gonzalez M, *et al*. Risk-adapted treatment of acute promyelocytic leukemia with all-trans-retinoic acid and anthracycline monochemotherapy: a multicenter study by the PETHEMA group. Blood 2004;103:1237-43
44. Ades L, Sanz MA, Chevret S, *et al*. Treatment of newly diagnosed acute promyelocytic leukemia (APL): a comparison of French-Belgian-Swiss and PETHEMA results. Blood 2008;111:1078-84
45. Lo-Coco F, Avvisati G, Vignetti M *et al*. Front-line treatment of acute promyelocytic leukemia with AIDA induction followed by risk-adapted consolidation for adults younger than 61 years: results of the AIDA-2000 trial of the GIMEMA Group. Blood. 2010;116:3171-9
46. Powell BL, Moser B, Stock W *et al*. Arsenic trioxide improves event-free and overall survival for adults with acute promyelocytic leukemia: North American Leukemia Intergroup Study C9710. Blood. 2010;116:3751-7
47. Marzbani E, Estey E. Who benefits from maintenance therapy in acute promyelocytic leukemia ? J Natl Compr Canc Netw. 2012;10:1023-8
48. Avvisati G, Lo-Coco F, Paoloni FP *et al*. AIDA 0493 protocol for newly diagnosed acute promyelocytic leukemia: very long-term results and role of maintenance. Blood. 2011;117:4716-25
49. Asou N, Kishimoto Y, Kiyoi H, *et al*. A randomized study with or without intensified maintenance chemotherapy in patients with acute promyelocytic leukemia who have become negative for PML-RAR_transcript after consolidation therapy: the Japan Adult Leukemia Study Group (JALSG) APL97 study. Blood 2007;110:59-66
50. Tallman MS. Treatment of relapsed or refractory acute promyelocytic leukemia. Best Pract Res Clin Haematol 2007;20:57-65
51. Specchia G, Lo CF, Vignetti M, *et al*. Extramedullary involvement at relapse in acute promyelocytic leukemia patients treated or not with all-trans retinoic acid: a report by the Gruppo Italiano Malattie Ematologiche dell'Adulto. J Clin Oncol 2001;19:4023-8
52. Estey EH. Treatment options for relapsed acute promyelocytic leukaemia. Best Pract Res Clin Haematol 2003;16:521-34
53. Lengfelder E, Hofmann WK, Nowak D. Impact of arsenic trioxide in the treatment of acute promyelocytic leukemia. Leukemia. 2012;26:433-42
54. Soignet SL, Frankel SR, Douer D, *et al*. United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. J Clin Oncol 2001;19:3852-60
55. Yanada M, Tsuzuki M, Fujita H *et al*. Phase 2 study of arsenic trioxide followed by autologous hematopoietic cell transplantation for relapsed acute promyelocytic leukemia. Blood. 2013 Feb 14. [Epub ahead of print]
56. Ferrara F, Palmieri S, Annunziata M, *et al*. Prolonged molecular remission after autologous stem cell transplantation in relapsed acute promyelocytic leukemia. Haematologica 2004;89:621-2
57. Thirugnanam R, George B, Chendamarai E, *et al*. Comparison of clinical outcomes of patients with relapsed acute promyelocytic leukemia induced with arsenic trioxide and consolidated with either an autologous stem cell transplant or an arsenic trioxide-based regimen. Biol Blood Marrow Transplant 2009;15:1479-84
58. Ferrara F, Finizio O, Izzo T *et al*. Autologous stem cell transplantation for patients with acute promyelocytic leukemia in second molecular remission. Anticancer Res. 2010;30:3845-9.
59. Kharfan-Dabaja MA, Abou Mourad YR, Fernandez HF, *et al*. Hematopoietic cell transplantation in acute promyelocytic leukemia: a comprehensive review. Biol Blood Marrow Transplant 2007;13:997-1004
60. Sanz MA, Labopin M, Gorin NC, *et al*. Acute Leukemia Working Party (ALWP) of European Cooperative Group for Blood and Marrow Transplantation (EBMT). Hematopoietic stem cell transplantation for adults with acute promyelocytic leukemia in the ATRA era: a survey of the European Cooperative Group for Blood and Marrow Transplantation. Bone Marrow Transplant 2007;39:461-9
61. Rogers JE, Yang D. Differentiation syndrome in patients with acute promyelocytic leukemia. J Oncol Pharm Pract. 2012;18:109-14
62. Montesinos P, Sanz MA. The differentiation syndrome in patients with acute promyelocytic leukemia: experience of the pethema group and review of the literature. Mediterr J Hematol Infect Dis. 2011;3:e2011059.
63. Zuckerman T, Ganzel C, Tallman MS, *et al*. How I treat hematologic emergencies in adults with acute leukemia. Blood. 2012 ;120:1993-200
64. Luesink M, Pennings JL, Wissink WM, *et al*. Chemokine induction by all-trans retinoic acid and arsenic trioxide in acute promyelocytic leukemia–triggering the differentiation syndrome. Blood. 2009;114:5512-21
65. Breccia M, Mazzarella L, Bagnardi V *et al*. Increased BMI correlates with higher risk of disease relapse and differentiation syndrome in patients with acute promyelocytic leukemia treated with the AIDA protocols. Blood. 2012;119:49-54
66. Ganzitti L, Fachechi G, Driul L, *et al*. Acute promyelocytic leukemia during pregnancy. Fertil Steril. 2010;94:2330.e5-6
67. Sanz MA, Grimwade D, Tallman MS *et al*. Management of acute promyelocytic leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood. 2009;113:1875-91.
68. Ferrara F, Finizio O, D'Arco A, *et al*. Acute promyelocytic leukemia in patients aged over 60 years: multicenter experience of 34 consecutive unselected patients. Anticancer Res. 2010;30:967-71.
69. Lengfelder E, Hanfstein B, Haferlach C *et al*. Outcome of elderly patients with acute promyelocytic leukemia: results of the German Acute Myeloid Leukemia Cooperative Group. Ann Hematol. 2013;92:41-52
70. Sanz MA, Vellenga E, Rayon C, *et al*. All-trans retinoic acid and anthracyclines monochemotherapy for the treatment of elderly patients with acute promyelocytic leukemia. Blood 2004;104:3490-3
71. Mandelli F, Latagliata R, Avvisati G, *et al*. Treatment of elderly patients (> or = 60 years) with newly diagnosed acute promyelocytic leukemia. Results of the Italian multicenter group GIMEMA with ATRA and idarubicin (AIDA) protocols. Leukemia 2003;17:1085-90
72. Prebet T, Gore SD. Treatment of acute promyelocytic leukemia for older patients. J Natl Compr Canc Netw. 2011;9:337-42.
73. Breccia M, Lo-Coco F. Gemtuzumab ozogamicin for the treatment of acute promyelocytic leukemia: mechanisms of action and resistance, safety and efficacy. Expert Opin Biol Ther. 2011;11:225-34
74. Godley LA, Larson RA. Therapy-related myeloid leukemia. Semin Oncol 2008;35:418-29
75. Duffield AS, Aoki J, Levis M, *et al*. Clinical and pathologic features of secondary acute promyelocytic leukemia. Am J Clin Pathol. 2012;137:395-402
76. Ammatuna E, Montesinos P, Hasan SK *et al*. Presenting features and treatment outcome of acute promyelocytic leukemia arising after multiple sclerosis. Haematologica. 2011;96:621-5
77. Rego EM, Kim HT, Ruiz-Argüelles GJ *et al*. Improving acute promyelocytic leukemia (APL) outcome in developing countries through networking, results of the International Consortium on APL. Blood. 2013;121:1935-43

Further Reading

Please see the abstracts below for further reading on leukaemia. These articles plus others are available in previous editions of *Treatment Strategies - Hematology*. Please see our website for more articles on leukaemia and a range of other topics within haematology.

New Weapons and Strategies in the Battle Against Relapsed Acute Myeloid Leukaemia (AML)

Ellen K. Ritchie
Assistant Clinical Professor of Medicine, Weill Cornell Medical College, New York, New York

Despite recent developments in the diagnosis and treatment of Acute Myeloid Leukaemia (AML), the majority of patients will die from progressive disease after relapse. While 70-80% of patients younger than age 60 will achieve a complete response (CR) to induction chemotherapy, most will relapse and five-year survival is only 40-45%. For those patients older than age 60, the median survival is less than one year and cure rates are 10% or less. Once a patient relapses, the prognosis is uniformly poor. There is a desperate need for new treatment approaches to avoid relapse and to treat it more successfully.

This paper takes an in-depth look at the issues with prognosis and whether these can be improved, what constitutes remission, novel agents for the treatment of AML as well as intensive salvage therapies and drug regimens.

Checkpoint and DNA Repair Inhibition in Leukaemias

Raoul Tibes[1], Megan E. Buechel[2], and **Ruben A. Mesa[1]**
1. Division of Hematology and Oncology, Mayo Clinic, Scottsdale Arizona; 2. The University of Arizona College of Medicine, Phoenix, Arizona

It is estimated that in 2011 12,950 people will be diagnosed with Acute Myeloid Leukaemia (AML) and 9,050 will die. The majority of elderly patients and those with poor cyotegentic features fail standard therapy. Of those >60 years old that have achieved an initial remission, 80% will relapse. Investigation of various chemotherapy combinations, dosing schedules and novel agents over the past 30 years has failed to significantly improve survival in patients >60 years old where the majority of the disease burden remains. Patients whose disease relapses are often treated with the initial regimen if they have sustained a clinical response for >12 months or alternatively are treated with a number of various combinations of salvage cytotoxic regimens or with investigational agents. Some patients can be salvaged on high dose Ara-C, especially those with favourable cytogenetics. However, resistance to cytotoxic agents including resistance to Ara-C has presented limitations to most salvage strategies.

Conceptually, rather than looking at increasing Ara-C concentration and activity within a cell, an alternative approach is to look at what complimentary pathways alter cellular sensitivity to Ara-C. Recently there has been a growing body of evidence to suggest that targeting various components of cell cycle checkpoints and DNA repair machinery may present a therapeutic opportunity to increase sensitivity of chemotherapeutic agents including Ara-C. The goal of this paper is to examine how targeting cell cycle checkpoints and the related DNA repair machinery are currently being applied in clinical trials and their potential to impacting the treatment of AML. Data on chronic leukaemias and solid tumours will be presented as far as clinically pertinent.

B-Lymphocyte Stimylator Role In Chronic Lymphocytic Leukaemia, Waldenström's Macroglobulinaemia and Multiple Myeloma

Marie-Christine Kyrtsonis, Katerina Sarris, Tatiana Tzenou, and **Dimitrios Maltezas**
Haematology Section of the First Department of Propaedeutic Internal Medicine, Laikon University Hospital, Athens

Introduction

B-Lymphocyte Stimulator (BLyS) is a cytokine with crucial role in differentiation, proliferation, survival and immunoglobulin (Ig) production by normal B-cells and plasmablasts.[1-3]

Inadequate BLyS production contributes to the pathogenesis of various diseases. Its decreased levels lead to immunodeficiency syndromes including common variable immunodeficiency. On the contrary, excessive secretion results in high antibody production and the development of autoimmune diseases as shown in BLyS transgenic mice that developed disorders resembling rheumatoid arthritis, systemic lupus erythematosus and Sjogren's syndrome; malignant B-cell lymphoproliferative diseases also emerged.[4]

In malignant B-cell disorders, BLyS controls neoplastic cells growth and survival[5-8] and because of the specificity of its effects to the B-cell lineage, its blockade appears appealing for adjuvant treatment.

In the present context, we will briefly review existing knowledge of BLyS functions and effects in normal and malignant B-cells with a special focus on Chronic Lymphocytic Leukaemia (CLL), Waldenström's Macroglobulinaemia (WM) and Multiple Myeloma (MM). We will also discuss the biologic basis of eventual therapeutic applications.

A. BLyS Functions in Normal and Malignant B-Cells.

BLyS, also known as BAFF, TALL-1, THANK, and zTNF4 is a cytokine that belongs to the tumour necrosis factor (TNF) ligand family.[1] It is mainly produced by myeloid cells, monocytes, dendritic cells and osteoclasts in the bone marrow[9] and is active either in its membrane bound form or as a soluble cytokine after its proeolytic cleavage by the subtilisin-like furin family member proteases.[1,10]

BLyS has been shown to play a critical role in the development of normal B-lymphocytes as well as in maintenance of their homeostasis.[11, 12] It contributes to B-lymphocytes accumulation in the peripheral lymphoid organs to complete their maturation.[13] It co-stimulates B-cell proliferation and Ig secretion.[1] In addition, it sustains peripheral B lymphocytes survival possibly by increasing Bcl-2 protein expression.[14, 15] For the achievement of the aforementioned functions (proliferation and stimulation of B lymphocyte growth and survival), up-regulation of intracellular free Ca (2+) appears necessary.[16]

BLyS exercises its functions through its three receptors, namely B-cell maturation antigen (BCMA), BAFF-R (BR3) and TACI (transmembrane activator and CAML interactor). BAFF-R is exclusively expressed on B lymphocytes whereas TACI and BCMA are found on B- and T-cells.[7] BAFF-R is specific for BLyS but BCMA and TACI can also bind APRIL, another TNF superfamily member[12, 17, 18] BLyS binding is tighter to BAFF-R and TACI than to BCMA.

Of special interest is the fact that the regulation of BLyS-binding receptor expression during B-cell differentiation reflects the maturation stages of normal B-cells.[19] Cells of B-lineage ranging from naive to terminally differentiated plasma cells uniformly express BlyS receptors but not all 3 together, nor at the same time. Moreover, BCMA is expressed on more mature forms than BAFF-R and TACI.[20] B-cell maturation stages consist the basis for B-cell neoplasms classification and nomenclature,[21] given that transformed cells conserve features of their normal counterpart. As it is well known, Ig heavy chain (IgH) genes rearrangement (restructuration) is the turning point of B-cell differentiation.[22] It is possible that BlyS (or APRIL) stimulus is crucial at this step since TACI expression was shown to be indispensible for class switch recombination

Marie-Christine Kyrtsonis graduated in Medicine from Athens Medical School, and completed her Medical Country Service as a general practitioner on the Island of Tinos. After completing her residency in Internal Medicine at 'Tasangaris' General Hospital and 'Evangelismos' General Hopital in Athens and a further residency in Haematology at 'St. Savvas' Anti-cancer Institute, Athens, Dr. Kyrtsonis completed her doctoral thesis on 'the role of cytokines in the prognosis, course and pathogenesis of multiple myeloma.' Having previously worked as Consultant Haematologist at Patras University Hospital, Dr. Kyrtsonis currently works as Consultant Haematologist and Assistant Professor of Haematology at Laikon University Hospital, Athens.

	CLL	WM	MM
Receptors Expression	Yes Decreased	Yes ?	Yes Increased
Soluble Serum Levels	Low	Increased	Increased
Survival In Relation To High Serum Levels	Better	Better in long survivors*	Worse

Table 1. Reported Findings on BLyS IN CLL, WM and MM. * Unpublished obsevations from our group.

(CSR), as it regulates the expression of activation-induced cytidine deaminase (AID), which is the enzyme responsible for both CSR and somatic hypermutation.[23] Malignant B-cell transformation frequently occurs at this point, as risks for genetic derailment are increased during SHM and CSR that are associated with DNA remodelling.

BLyS functions[15, 24] are made possible thanks to its ability to activate, through its receptors, important signalling pathways, including the Akt/mTOR and NFkB ones.[14, 15, 25]

B. BLyS Role In CLL, WM and MM

The main reported findings concerning BlyS in CLL, WM and MM are summarised in table 1 and briefly reported below.

Chronic Lymphocytic Leukaemia

Chronic lymphocytic leukaemia (CLL) is characterised by the accumulation of monoclonal CD5+ B Lymphocytes in blood, bone marrow and lymphatic tissues. The etiology is unknown but familial cases strongly suggest a genetic basis for the disease. CLL is defined, together with small lymphocytic lymphoma (SLL), as a neoplasm of mature B-lymphocytes involving peripheral blood, bone marrow, spleen and lymph nodes.[21] In addition CLL is further characterised by its leukaemic nature, requiring the presence of at least 5×10^9 blood lymphocytes/L[26] that co-express CD5, CD19, CD20, and CD23 antigens with dim CD20 and CD79b expression, and exhibit lower levels of surface Ig.[27] Clinical manifestations include anaemia, peripheral lymphadenopathy, splenomegaly, autoimmune manifestations and others. The disease is usually indolent but some patients have a more aggressive course and shorter overall survival. Prognostic factors mainly include Rai or Binet stage, IgH mutational status, ZAP-70 expression and CD38 expression.[28]

CLL cells were shown to bind soluble BlyS and to express TACI and BAFF-R, while only a subset expressed BCMA.[29] Surprisingly B-CLL cells were found to aberrantly express BlyS mRNA in half of samples tested;[29] on the contrary, another simultaneous study found no or very low levels of BLyS protein in CLL cells, while lower levels of BLyS receptor expression was observed in CLL compared normal controls or other B-lymphomas.[30]

Serum BLyS levels were found decreased in CLL patients compared to healthy individuals.[31, 32] Another interesting finding was that, when BAFF-R that activates the non-canonical NF-kB pathway was blocked, CLL cells continued their duplication normally, but if the canonical NF-kB pathway was inhibited, this ability was lost.[33]

With regard to the relationship between serum BLyS levels and disease characteristics, it was recently shown, in patients with sporadic CLL, that higher levels were associated with favourable disease, female gender, younger age, Rai stage 0, higher platelet count, mutated IgH disease, normal cytogenetic profile or presence of 13q deletion, low ZAP-70 and CD38 expression. With regard to patients' survival, lower soluble BLyS levels were associated with a shorter time to first treatment,[34, 35] although not with overall survival, possibly because a longer patients' follow-up was needed. Nevertheless, the mechanisms by which low serum BLyS levels are related to adverse factors in CLL have yet to be established. As an eventual explanation, Molica *et al*. suggested that in aggressive CLL a greater amount of soluble BLyS might have been bound by its receptors on B-cell surface, sequestrating it from circulation. They supported their assumption by the observation that serum BLyS increased in follicular lymphoma patients after rituximab administration, possibly because it is unbounded by its surface receptors.[36] In any case, explanations are difficult in CLL as it is a heterogeneous disease. It seems that there are many CLL disease subsets, characterised by different normal counterpart precursor cells, among which are rare B-cells unresponsive to BLyS, such as transitional ones.[37] In another CLL patients' category, with familial disease, BLyS serum levels were reported as increased[38] without any specific relationship with disease characteristics or mutational status.

Waldenström's Macroglobulinaemia

WM is a rare B-cell lymphoma carrying the pathological diagnosis of LPL as defined in the WHO classification and characterised by the presence of a serum monoclonal IgM irrespectively of its levels.[21, 39] The disease is usually indolent and presents a wide range of clinical signs and symptoms including those due to the lymphoma (lymph nodes' swelling, organomegaly, bone marrow failure) and the paraprotein driven. WM shares with CLL most of its clinical manifestations and, with MM, the serum paraprotein component.

As far as the role of BlyS in WM is concerned, there are a very limited number of published studies. Elsawa and colleagues[40] showed that malignant B-cells from patients with WM express the receptors for BLyS and can bind soluble BLyS. They also showed that serum BlyS levels are elevated in WM patients compared to healthy controls, and that BLyS, in the presence of cytokines such as, IL-2, IL-6, IL-10, and IL-10, up-regulates Ig secretion. Furthermore, they showed that BLyS protects malignant B-cells from apoptosis and enhances WM cells

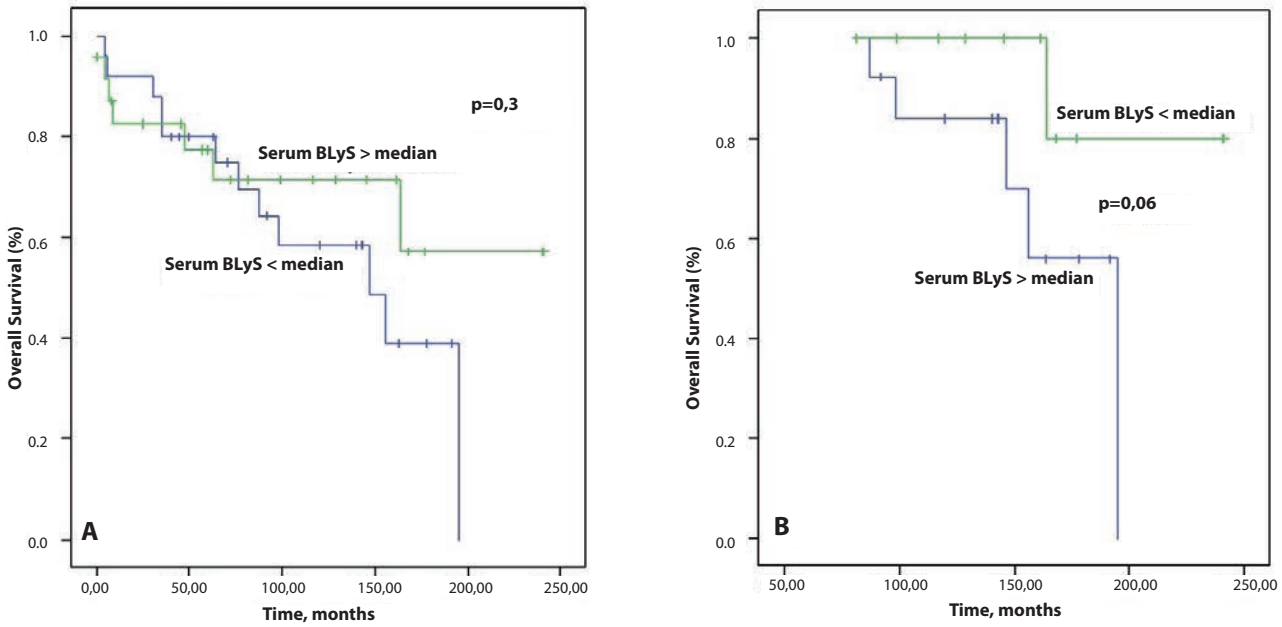


Figure 1. Survival of 48 WM patients according to serum BLyS levels. A) all patients, B) 24 of 48 long WM survivors.

proliferation *in vitro*. Based on these, they suggested a potential role for BlyS inhibition in WM therapeutics. Accordingly, Rossi and colleagues tested the efficacy of a specific inhibitor of BLyS and APRIL in four patients with relapsed or refractory WM; disease stabilisation was obtained in two but none entered partial or complete response.[42]

In our experience, serum BLyS levels were increased in WM patients, compared to healthy individuals. They were however not correlated with survival or other disease parameters, as it was the case for MM patients.[35] In a updated survival analysis concerning 49 WM patients with a median survival of 72 months, serum BLyS levels were not correlated with survival in all patients (Figure 1A), but for the 24 long survivors still alive after 80 months follow-up, the outcome was better in patients with high BLys levels (above median) at diagnosis (Figure 1B), suggesting a dual BLyS impact, MM-like in patients with a more aggressive disease and CLL-like in long survivors.

Multiple Myeloma

MM is characterised by the uncontrolled proliferation of monoclonal plasma cells (PCs) in the bone marrow that secrete a monoclonal Ig. Symptomatic disease is potentially aggressive with significant morbidity resulting from frequent disease manifestations such as extreme fatigue due to anaemia, bone pains or spontaneous fractures, recurrent infections and others. Survival of MM patients is usually reduced compared to the one enjoyed by patients with CLL or WM, in spite of significant therapeutic improvements in the new agents era.

The role of BLyS in MM pathogenesis and prognosis has been evaluated in numerous studies. BLyS and its receptors are expressed on PCs' membrane, while it can be detected in its soluble form in patients' serum.[43] It was shown that serum BLyS levels are significantly elevated in MM patients at diagnosis[44, 45] and that they increase with advancing disease stage while they decrease when the disease enters remission after treatment.[45] BLyS constitutes a MM cell growth factor. Its secretion is induced by interaction between PCs and the bone marrow milieu resulting into NF-κB activation[43] while it rescued myeloma cells from dexamethasone-induced apoptosis.[6] Several studies have reported a strong correlation between BLyS and established factors of MM activity such as albumin, LDH, b2-microglobulin and CRP but also with other biomarkers such TNF-α, bone marrow microvascular density (MVD), proliferating cell nuclear antigen (PCNE) and IL6.[44-47] Moreover it was shown that patients with elevated BLyS levels at diagnosis have poor prognosis.[35, 44, 45]

Preclinical experiments with anti-BLyS agents showed interesting results. Moreover fusion proteins composed of the human IgG and the extracellular, ligand-binding portion of the BLyS receptor neutralises BLyS, while their addition to MM cells has antiproliferative effects and promotes apoptosis.[48] Furthermore, such agents administered to relapsed/refractory MM patients produced promising results.[42, 49, 50] A phase I clinical trial[51] of human anti-BLyS antibody, in combination with bortezomib, presented an overall response rate of 55% in patients previously treated with Bortezomib, while clinical trials are going on.

Conclusions

BLyS is a molecule involved in CLL, WM and MM pathophysiology by mechanisms that are not fully elucidated yet. The fact that it is an inhibitor of malignant cells apoptosis, in conjunction with its own and receptors' specificity to the B-cell lineage, renders the idea of using agents with neutralising activity against BLyS or its receptors for adjuvant treatment, very appealing. Its therapeutic use may contribute to patients' outcome improvement, just in the same way

that the monoclonal antibody against CD20, a pan B-cell marker, constituted almost 2 decades ago a breakthrough in the treatment of B-cell malignancies. Anti-BLyS, anti-BR3, anti-BCMA antibodies and BLyS-based immunotoxins have already been manufactured and results of ongoing clinical trials that mostly concern MM patients are awaited with great interest. However with regard to CLL and WM, further studies to better understand BLyS contribution to disease biology are needed.

References

1. Schneider P, MacKay F, Steiner V, *et al*., "BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth". J. Exp. Med. (1999), 189:pp. 1747–56.
2. Moore PA, Belvedere O, Orr A, *et al*., "BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator". Science. (1999), 285:pp. 260-263.
3. Nardelli B, Belvedere O, Roschke V, *et al*., "Synthesis and release of B-lymphocyte stimulator from myeloid cells". Blood (2001), 97:pp.198-204
4. Mackay F, Woodcock SA, Lawton P, *et al*., "Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations". J. Exp. Med. (1999), 190:pp. 1697-1710.
5. He B, Chadburn A, Jou E, *et al*., "Lymphoma B cells evade apoptosis through the TNF family members BAFF/BLyS and APRIL". J Immunol. (2004), 172:pp. 3268-3279.
6. Moreaux J, Legouffe E, Jourdan E, *et al*., "BAFF and APRIL protect myeloma cells from apoptosis induced by interleukin 6 deprivation and dexamethasone". Blood. (2004), 103:pp. 3148-3157.
7. Haiat S, Billard C, Quiney C, *et al*., "Role of BAFF and APRIL in human B-cell chronic lymphocytic leukemia". Immunology (2006), 118: pp. 281-292.
8. Neri P, Kumar S, Fulciniti MT, *et al*., "Neutralizing B-cell Activating Factor Antibody improves survival and inhibits osteoclastogenesis in a Severe Combined immunodeficient human Multiple myeloma model". Clin Cancer Res. (2007), 13:pp. 5903-5909.
9. Craxton A, Magaletti D, Ryan EJ, *et al*., "Macrophage- and dendritic cell dependent regulation of human B-cell proliferation requires the TNF family ligand BAFF". Blood (2003), 101:pp. 4464-4471.
10. Melchers F. *et al*., "Actions of BAFF in B cell maturation and its effects on the development of autoimmune disease". Ann Rheum Dis (2003), 62(Suppl II):pp 25–27.
11. Locksley RM, Killeen N, Lenardo MJ., *et al*., "The TNF and TNF receptor superfamilies: integrating mammalian biology". Cell. (2001), 104:pp. 487-501.
12. Mackay F, Ambrose C., *et al*., "The TNF family members BAFF and APRIL: the growing complexity". Cytokine Growth Factor Rev. (2003), 14:pp. 311-324.
13. Zhang X, Park CS, Yoon SO, *et al*., "BAFF supports human B cell differentiation in the lymphoid follicles through distinct receptors". Int Immunol (2005), 17:pp. 779-88.
14. Woodland RT, Fox CJ, Schmidt MR, *et al*., "Multiple signaling pathways promote B lymphocyte stimulator dependent B-cell growth and survival". Blood (2008), 111:pp. 750-60.
15. Fu L, Lin-Lee YC, Pham LV, *et al*., "BAFF-R promotes cell proliferation and survival through interaction with IKKbeta and NF-kappaB/c-Rel in the nucleus of normal and neoplastic B-lymphoid cells". Blood (2009), 113:pp. 4627-36.
16. Liang JQ, Zhang W, Wen L, *et al*., "hsBAFF-upregulated intracellular free Ca(2+) homeostasis regulates ERK1/2 activity and cell proliferation in B cells in vitro". Physiol Res. (2009), 58:pp. 411-8.
17. Mackay F, Browning JL, *et al*., "BAFF: a fundamental survival factor for B cells". Nat Rev Immunol. (2002), 2:pp. 465-475.
18. Mackay F, Schneider P, Rennert P, *et al*., " BAFF AND APRIL: a tutorial on B cell survival". Annu Rev Immunol. (2003), 21:pp. 231-264.
19. Darce JR, Arendt BK, Wu X, *et al*., "Regulated expression of BAFF-binding receptors during human B cell differentiation". J Immunol.(2007), 179:pp. 7276-86.
20. Treml JF, Hao Y, Stadanlick JE, *et al*., "The BLyS family: toward a molecular understanding of B cell homeostasis". Cell Biochem Biophys (2009), 53: 1–16.
21. Swerdlow SH, Campo E, Harris NE, *et al*., Eds. WHO classification of tumours of haematipoetic and lymphoid tissues. Lyon IARC Press, 2008.
22. Kyrtsonis MC, Koulieris E, Bartzis V, *et al*., "Monoclonal Immunoglobulin In Multiple Myeloma, InTech open access publisher, 2013, in Press.
23. Cattoretti G., Buttner M, Shaknovich R, *et al*., "Nuclear and cytoplasmic AID in extrafollicular and germinal center B cells". Blood (2006) 107:pp. 3967-3975.
24. Warnatz K, Salzer U, Rizzi M, *et al*., "B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans". Proc Natl Acad Sci U S A. (2009), 106:pp. 13945-50.
25. Huang S, Houghton PJ, *et al*., "Targeting mTOR signaling for cancer therapy". Curr Opin Pharmacol (2003), 3:pp. 371-7.
26. Hallek M, Cheson BD, Catovsky D, *et al*., "Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic lymphocytic leukemia updating the National Cancer Institute-Working group 1996 guidelines". Blood (2008), 111:pp. 5446-56.
27. Matutes E, Owusu-Ankomah K, Morilla R, *et al*., "The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL". Leukemia (1994), 8:pp. 1640-1645.
28. Cramer P, Hallek M. "Prognostic factore in Chronic lymphocytic leukemia-what do we need to know?". Nat. Rev.Clin.Oncol. (2011), 8:pp. 38-47.
29. Novak AJ, Bram RJ, Kay NE, et al., "Aberrant expression of B-lymphocyte stimulator by B chronic lymphocytic leukemia cells: a mechanism for survival". Blood. (2002), 100:pp. 2973-2979.
30. Briones J, Timmerman JM, Hilbert DM, *et al*., "BLyS and BLyS receptor expression in non- Hodgkin's lymphoma". Exp Hematol. (2002), 30:pp. 135-141.
31. Ferrer G, Hodgson K, Monserrat E, *et al*., "B-cell activator factor and a proliferation-inducing ligand at the cross-road of chronic lymphocytic leukemia and autoimmunity". Leuk Lymphoma (2009), 50:pp. 1075-82.
32. Planelles L, Castillo-Gutierrez S, Medema JP, *et al*., "APRIL but not BLyS serum levels are increased in chronic lymphocytic leukemia:prognostic relevance of APRIL for survival". Haematologica (2007), 92:pp. 1284-5.
33. Endo T, Nishio M, Enzler T, *et al*., "BAFF and APRIL support chronic lymphocytic leukemia B-cell survival through activation of the canonical NF-B pathway". Blood (2007), 109:pp. 703-710.
34. Molica S, Digiesi G, Battaglia C, *et al*., "BAFF serum level predict time to first treatment in early chronic lymphocytic leukemia". Eur J Hematol (2010), 24(6):pp. 314-320
35. Kyrtsonis MC, Sarris K, Pangalis G, *et al*., "Contradictory impact of serum BLyS levels in Chronic Lymphocytic leukemia, Waldestrom's macroglobulinemia and Multiple Myeloma" EHA (2012) Abstr 741.
36. Ansell SM, Novak AJ, Ziesmer S, *et al*., "Serum BLyS levels increase after rituximab as initial therapy in patients with follicular Grade 1 non-Hodgkin lymphoma". Am J Hematol. (2009), 84:pp. 71-73.
37. Garcia-Munoz R, Galiacho V, Llorente L, *et al*., Ann Hematol (2012), 91:pp. 981-996.
38. Novak AJ, Grote DM, Ziesmer SC, *et al*., "Elevated serum B-lymphocyte stimulator levels in patients with familial lymphoproliferative disorders", J Clin Oncol. (2006), 24: pp 983-7.
39. Owen RG, Treon SP, Al Katib A, *et al*., "Clinicopathological definition of Waldenstrom's macroglobulinemia. Consensus panel recommendations from the 2nd International Workshop on Waldenstrom's macroglobulinemia". Seminars in Oncology (2003), 30:pp 110-115.
40. Elsawa SF, Novak AJ, Grote DM, *et al*. "B-lymphocyte stimulator (BLyS) stimulates immunoglobulin production and malignant B-cell growth in Waldenstrom's macroglobulinemia". Blood. (2006), 107:pp. 2882-2888.
41. Gross JA, Johnston J, Mudri S, *et al*., "TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease". Nature (2000), 404:pp. 995 –999.
42. Rossi J-F, Moreaux J, Rose M, *et al*., "Phase I/II study of atacicept (TACI-Ig) to neutralize APRIL and BLyS in patients with refractory or relapsed multiple myeloma (MM) or active previously treated Waldenstrom's macroglobulinemia (WM)". Blood (ASH Annual Meeting Abstracts)2005, 106:pp. 2566.
43. Novak AJ, Darce JR, Arendt BK, *et al*., "Expression of BCMA, TACI, and BAFF-R in multiple myeloma: a mechanism for growth and survival". Blood. (2004), 103:pp. 689-694.
44. Maltezas D., Tzenou T., Papanikolaou X, *et al*., "Elevated serum B-Lymphocyte stimulator levels in diagnosis are related to disease aggressiveness and adverse survival in Multiple Myeloma". Haematologica (2009), 94:pp. 388.
45. Fragioudaki M, Boula A, Tsirakis G, *et al*., "B cell-activating factor: its clinical significance in multiple myeloma patients". Ann Hematol. (2012), 91:pp. 1413-8.
46. Fragioudaki M, Tsirakis G, Pappa CA, *et al*., "Serum BAFF levels are related to angiogenesis and prognosis in patients with multiple myeloma". Leuk Res. (2012), 36:pp. 1004-8.
47. Jiang P, Yueguo W, Huiming H, *et al*., "B-Lymphocyte stimulator: a new biomarker for multiple myeloma". Eur J Haematol. (2009), 82:pp. 267-76.
48. Yaccoby S, Pennisi A, Li X, *et al*., "Atacicept (TACI-Ig) inhibits growth of TACI(high) primary myeloma cells in SCID-hu mice and in coculture with osteoclasts". Leukemia. (2008), 22:pp. 406-13.
49. Rossi JF, Moreaux J, Hose D, *et al*., "Atacicept in relapsed/refractory multiple myeloma or active Waldenström's macroglobulinemia: a phase I study". Br J Cancer (2011), 101:pp. 1051–1058.
50. Rossi JF.,*et al*., "Phase I study of atacicept in relapsed/refractory multiple myeloma (MM) and Waldenstrom's macroglobulinemia". Clin Lymphoma Myeloma Leuk (2011), 11:pp. 136-8.
51. Raje NS, Hohl Rj, Faber EA, *et al*., "Phase I study of LY2127399, a human anti-BAFF antibody, and bortezomib in patients with previously treated multiple myeloma". J Clin Oncol (2011), 29, suppl; abstr 8012

Helicobacter Pylori Eradication as Exclusive Treatment for Gastric Diffuse Large B-cell Lymphoma

Andrés J. M. Ferreri,[1,3] **Silvia Govi,**[1,3] and **Maurilio Ponzoni**[1,2]
1. Unit of Lymphoid Malignancies, 2. Pathology Unit and 3. Division of Onco-Hematological Medicine; Department of Onco-Hematology, San Raffaele Scientific Institute, Milan

Clinical Presentation and Diagnosis

Non-Hodgkin lymphomas (NHL) represent 3-4% of all malignancies of the gastrointestinal tract. The stomach is the most involved organ, with diffuse large B-cell lymphoma (DLBCL) representing 40-70% of all gastric lymphomas.[1,2] Primary gastric DLBCL is localised in the gastric wall, with or without concomitant perigastric and/or abdominal lymphadenopathies.

Gastric DLBCL occurs more frequently in males, and the median age at presentation is 50–60 years.[1] Symptoms remind to gastric cancer or benign ulcer. Patients present epigastric pain (70% of cases) or dyspepsia (30%), and frequently exhibit weight loss (50%), which is usually a consequence of the dyspepsia. Patients exhibit a good performance status at diagnosis. Bleeding, perforation and palpable epigastric mass are uncommon at presentation.[3,4]

Recently, flexible gastroscopy have improved the diagnostic accuracy[5] and ultrasonography delineated deep infiltration in cases of preservation of the mucosa. The use of endoscopic ultrasonography and high-resolution computed tomography scanning has significantly improved the clinico-radiological staging.[5]

Staging

DLBCL of the stomach is usually a limited-stage disease. Complete staging work-up includes complete haematological and biochemical exams, physical examination with Waldeyer's ring evaluation, contrasted total-body computerised tomography, gastrointestinal tract examination and endoscopic ultrasonography, and bone marrow biopsy.[4]

The Ann Arbor staging system[6] is the most commonly used score for stage definition in NHLs; however, it is inadequate for gastric DLBCL. A valid alternative has been recommended during an International Workshop in 1994.[7]

Patients are classified in two groups according to the presence (B) or absence (A) of systemic symptoms (fever of no evident cause, night sweats and weight loss of more than 10% of body weight), and the presence of a bulky mass, such as a lesion of 10 cm or more in the longest diameter, is signalled as "X".

Pathology

This lymphoma is composed of large cells with vesicular nuclei, prominent nucleoli and moderate-high proliferation rate, with intense cellular infiltration of the lamina propria.

Tumour cells express CD19, CD20, CD22 and CD79a; 50% of cases is bcl-6 positive and 26% is CD10 positive. CD38+ are 47% of cases.[2,8] The rearrangement of bcl-2 is present in about 30% of primary gastric lymphoma.[9]

High-grade lymphoma with the same Ig light chain restriction with co-existing MALT lymphoma are the transformed cases.[10] Transformed MALT lymphomas are CD10-, bcl2- and bcl6 +, while DLBCL *de novo* are bcl6 and CD10+.[11]

DLBCL occurs frequently in males, median age was 50-60 years and clinical presentation is the same reported for MALT lymphoma.[2]

Natural History

Gastric DLBCL has a better prognosis than others DLBCL. Weight loss and compromise of immunological or nutritional status are uncommon at presentation, allowing to indicate more aggressive and opportune

Andrés José María Ferreri is Head of the Unit of Lymphoid Malignancies and vice-director of the Division of Medicine of the Department of Onco-Hematology of the San Raffaele Scientific Institute of Milan. He is a Founder Member of the International Extranodal Lymphoma Study Group and of the International PCNSL Collaborative Group. Dr. Ferreri is an opinion leader in the field of non-Hodgkin lymphomas, and is co-author of 200 selected abstracts, 195 articles on peer-reviewed journals and 18 book chapters, in English, Italian and Spanish. He is Associate Editor of ESO/START Project and of *Hematological Oncology*. Dr. Ferreri is member of ESMO, EORTC, ASCO, ASH, SIE, FIL, and SAKK Group.

therapeutic modalities in these patients. Currently, conservative treatment of stage I/II PGDLCL, with primary chemotherapy alone or in combination with radiation therapy, is standard treatment, with a low incidence of iatrogenic complications.[2]

Independent prognostic factors resulted age, lactate dehydrogenase (LDH) serum level, stage (using both the Ann Arbor and Musshoff's staging systems), and use of chemotherapy.[4, 12] Patients with CD10-positive gastric DLBCL presented a significantly longer survival conversely to patients with CD10-negative lymphomas.

Treatment

Conservative treatment with six to eight courses of CHOP or CHOP-like regimens as exclusive treatment, or four to six courses of the same regimens followed by consolidation radiotherapy produces a response rate of 85-100%, with a 5-year overall survival of 90%,[13] and a 10-year cause-specific survival of 82%. Systemic relapse occurs in 12% of cases. The impact of the addition of rituximab to CHOP has not been adequately investigated,[14] but its use seems to be justified by the results obtained in other DLBCL.

In a retrospective series, patients receiving radiation therapy showed a lower local relapse rate compared with patients treated with chemotherapy alone. A randomised trial documented that involved-field radiation therapy is associated with significantly better local disease control in patients with firstly diagnosed limited-stage gastric DLBCL who achieved complete remission after chemotherapy.[15] In addition to chemotherapy, Hp should always be eradicated in gastric DLBCL.[13] If it is not eradicated, it may relapse as the Hp antigenic drive to the appearance and development of lymphoma is still active. This is especially the case of DLBCL with concomitant MALT areas, which may eventually relapse with a low-grade component unless Hp can be successfully treated.[16]

H. Pylori Eradication

In gastric MALT-type lymphoma a strong association with Hp infection is documented in more than 90%.[17, 18] Epidemiological studies have demonstrated the association between Hp infection and development of gastric lymphoma.[19] Host immune responses also play a role in MALT formation.[20] The detection in cases of Hp-related gastritis of the B-cell clone that eventually gave rise to MALT lymphoma confirmed that this malignancy might result from a multi-step process started by Hp infection.[21] Hp infection induces an immune response of Th1 type mediated by several pro-inflammatory cytokines and is also associated with the production of DNA-damaging reactive oxygen species (ROS) by neutrophils. Hp-related gastritis reproduces features of acquired MALT; development antigens expressed by Hp together with antigen-specific T-cells activate the antigen receptor of polyclonal B-cells and lead to the interaction of BCL10 and MALT1, with activation of NF-kB pathway; during infection, a subclone may acquire one lymphoma-specific translocation and develop a growth advantage; at this point, eradication of the bacterium doesn't revert the disease process.[19] The last evidence regards the 75% lymphoma regression rate observed in patients treated with Hp-eradicating therapy alone.[22]

The role of Hp infection in primary gastric DLBCL is controversial. Hp is detected in 35% of DLBCL, more commonly in cases with concomitant MALT areas with respect to *de novo* cases (65% versus 15%);[23] however, gastric DLBCL are generally believed to be Hp-independent growing tumours. Selected cases of gastric DLBCL received antibiotic therapy alone, resulting in sporadic lymphoma regression.[13, 24] A few small retrospective studies confirmed such results, with a CR rate ranging between 27% and 87%.[6, 25] A prospective trial assessing the activity and efficacy of Hp–eradicating therapy in gastric DLBCL has been performed in Taiwan.[7] Sixteen stage IE "high-grade transformed MALT lymphomas" patients obtained a 62% CR rate; these data were confirmed by the same authors comparing two retrospective series of gastric *de novo* and MALT-related DLBCL, showing that both forms are responsive to antibiotics.[26] Last year, the first multicentre phase II trial assessing the role of Hp-eradicating therapy as exclusive treatment in patients with early-stage DLBCL of the stomach diagnosed in Western countries was reported.[27] In this trial, named HG-L1,[27] this conservative strategy has been associated with a 63% CR rate, and all responders but one resulted relapse-free at a median follow-up of 68 months, with a median PFS of 83+ months. Patients with unresponsive/relapsed lymphoma achieved CR after chemo-radiotherapy and remained relapse-free at 13-90 months (median 55+). No patients died of lymphoma, with a 5-year OS of 94%.[27] Rituximab was used both as consolidation of PRs and as part of salvage therapy.[27] Some previous reports have suggested that the depth of infiltration into the gastric wall and involvement of perigastric lymph nodes are negative predictors of response to antibiotics.[28, 29] To our knowledge, only two cases of DLBCL with perigastric lymphadenopathies (stage II1) responsive to Hp eradication have been reported. Both patients had lymphadenopathies sized ≤1.5 cm, but histological confirmation of lymphomatous infiltration was lacking. A cut-off of 1.5 cm in the diameter of perigastric lymph nodes was used in line with the standardised response criteria for NHL to define lymph-node infiltration when assessed by non-functional exams.[30] This indication should be taken into account with caution since the well-known difficulties in distinguishing reactive and neoplastic nature of perivisceral lymph nodes in gastric lymphomas.

Two-thirds of treated patients achieved long-term lymphoma remission without chemotherapy, a critical issue considering that most of these patients are older than 65 years. Lymphoma responses were equally documented both in patients with MALT-related DLBCL or with *de novo* DLBCL, in patients with "germinal-centre-like" or "activated-B-cell-like" lymphomas and in patients with or without small perigastric lymph nodes (size <1.5 cm; endoscopic ultrasonography).

These data consent us to recommend Hp eradication as upfront treatment for patients with limited-stage Hp-associated DLBCL of the

stomach, using chemo-radiotherapy only in unresponsive patients. This recommendation should equally regard patients with MALT-related or *de novo* DLBCL, and with "germinal-centre-like" or "activated-B-cell-like" lymphomas.

Conclusions

H. pylori eradication is a fast, safe, cheap, and effective upfront treatment in limited-stage gastric DLBCL, with or without MALT-areas. Obtaining significant remissions without toxicities is an important goal, especially in elderly patients. Antibiotic activity in different DLBCLs subtypes, categorised according to the ontogenetic subclassification proposed by Hans *et al*.,[31] produced similar results. Responses were equally documented in patients with GCB and non-GCB DLBCL, with similar PFS in both subgroups.[27] The identification of predictors of response to antibiotics, like nuclear expression of BCL10 and BAFF signal transduction[32, 33] remains an important step forward a rational conservative treatment of gastric Hp-associated DLBCL. An international trial will allow us to better understand the role of antibiotics in these patients and to identify the best candidates for this conservative treatment.

References

1. Tondini C, Giardini R, Bozzetti F, *et al*. Combined modality treatment for primary gastrointestinal non-Hodgkin's lymphoma: the Milan Cancer Institute experience. Ann Oncol (1993);4(10):831-837.
2. Ferreri AJ, Montalban C. Primary diffuse large B-cell lymphoma of the stomach. Crit Rev Oncol Hematol (2007);63(1):65-71.
3. Tondini C, Balzarotti M, Santoro A, *et al*. Initial chemotherapy for primary resectable large-cell lymphoma of the stomach. Ann Oncol (1997);8(5):497-499.
4. Ferreri AJ, Cordio S, Ponzoni M, *et al*. Non-surgical treatment with primary chemotherapy, with or without radiation therapy, of stage I-II high-grade gastric lymphoma. Leuk Lymphoma (1999) ;33(5-6):531-541.
5. D'Amore F, Christensen BE, Thorling K, *et al*. Incidence, presenting features and prognosis of low-grade B-cell non-Hodgkin's lymphomas. Population-based data from a Danish lymphoma registry. Leuk Lymphoma (1993);12(1-2):69-77.
6. Carbone PP, Kaplan HS, Musshoff K, *et al*. Report of the Committee on Hodgkin's Disease Staging Classification. Cancer Res (1971);31(11):1860-1861.
7. Rohatiner A, d'Amore F, Coiffier B, *et al*. Report on a workshop convened to discuss the pathological and staging classifications of gastrointestinal tract lymphoma. Ann Oncol (1994);5(5):397-400.
8. Ponzoni M, Ferreri AJ, Pruneri G, *et al*. Prognostic value of bcl-6, CD10 and CD38 immunoreactivity in stage I-II gastric lymphomas: identification of a subset of CD10+ large B-cell lymphomas with a favorable outcome. Int J Cancer (2003) ;106(2):288-291.
9. Weiss LM, Warnke RA, Sklar J, *et al*. Molecular analysis of the t(14;18) chromosomal translocation in malignant lymphomas. N Engl J Med (1987);317(19):1185-1189.
10. Peng HZ, Du MQ, Koulis A, *et al*. Nonimmunoglobulin gene hypermutation in germinal center B cells. Blood (1999);93(7):2167-2172.
11. Yoshino T, Akagi T. Gastric low-grade mucosa-associated lymphoid tissue lymphomas: their histogenesis and high-grade transformation. Pathol Int (1998);48(5):323-331.
12. Aviles A, Delgado S, Huerta-Guzman J. Marginal zone B cell lymphoma of the parotid glands: results of a randomised trial comparing radiotherapy to combined therapy. Eur J Cancer B Oral Oncol (1996);32B(6):420-422.
13. Raderer M, Chott A, Drach J, *et al*. Chemotherapy for management of localised high-grade gastric B-cell lymphoma: how much is necessary? Ann Oncol (2002);13(7):1094-1098.
14. Wohrer S, Troch M, Raderer M. Treatment of localized primary gastric lymphoma. J Clin Oncol (2006);24(17):2682; author reply 2682-3.
15. Martinelli G, Gigli F, Calabrese L, *et al*. Early stage gastric diffuse large B-cell lymphomas: results of a randomized trial comparing chemotherapy alone versus chemotherapy + involved field radiotherapy (IELSG 4). Leuk Lymphoma (2009);50(6):925-931.
16. Boot H, de Jong D, van Heerde P, *et al*. Role of Helicobacter pylori eradication in high-grade MALT lymphoma. Lancet (1995);346(8972):448-449.
17. Wotherspoon AC, Doglioni C, Isaacson PG. Low-grade gastric B-cell lymphoma of mucosa-associated lymphoid tissue (MALT): a multifocal disease. Histopathology (1992);20(1):29-34.
18. Doglioni C, Wotherspoon AC, Moschini A, *et al*. High incidence of primary gastric lymphoma in northeastern Italy. Lancet (1992);339(8797):834-835.
19. Farinha P, Gascoyne RD. Helicobacter pylori and MALT lymphoma. Gastroenterology (2005);128(6):1579-1605.
20. Delchier JC, Lamarque D, Levy M, *et al*. Helicobacter pylori and gastric lymphoma: high seroprevalence of CagA in diffuse large B-cell lymphoma but not in low-grade lymphoma of mucosa-associated lymphoid tissue type. Am J Gastroenterol (2001);96(8):2324-2328.
21. Zucca E, Bertoni F, Roggero E, *et al*. Molecular analysis of the progression from Helicobacter pylori-associated chronic gastritis to mucosa-associated lymphoid-tissue lymphoma of the stomach. N Engl J Med (1998);338(12):804-810.
22. Wotherspoon AC, Doglioni C, Diss TC, *et al*. Regression of low grade B-cell gastric lymphoma of MALT type following eradication of Helicobacter pylori (Meeting abstract). J Pathol (1993);170(Suppl):335A.
23. Binn M, Ruskone-Fourmestraux A, Lepage E, *et al*. Surgical resection plus chemotherapy versus chemotherapy alone: comparison of two strategies to treat diffuse large B-cell gastric lymphoma. Ann Oncol (2003);14(12):1751-1757.
24. Horning SJ, Weller E, Kim K, *et al*. Chemotherapy with or without radiotherapy in limited-stage diffuse aggressive non-Hodgkin's lymphoma: Eastern Cooperative Oncology Group study 1484. J Clin Oncol (2004);22(15):3032-3038.
25. Ferreri AJ, Freschi M, Dell'Oro S, *et al*. Prognostic significance of the histopathologic recognition of low- and high-grade components in stage I-II B-cell gastric lymphomas. Am J Surg Pathol (2001);25(1):95-102.
26. Kuo SH, Yeh KH, Wu MS, *et al*. Helicobacter pylori eradication therapy is effective in the treatment of early-stage H pylori-positive gastric diffuse large B-cell lymphomas. Blood (2012);119(21):4838-4844.
27. Ferreri AJ, Govi S, Raderer M, *et al*. Helicobacter pylori eradication as exclusive treatment for limited-stage gastric diffuse large B-cell lymphoma: results of a multicenter phase 2 trial. Blood (2012);120(18):3858-3860.
28. Tari A, Asaoku H, Kashiwado K, *et al*. Predictive value of endoscopy and endoscopic ultrasonography for regression of gastric diffuse large B-cell lymphomas after Helicobacter pylori eradication. Dig Endosc (2009);21(4):219-227.
29. Cavanna L, Pagani R, Seghini P, *et al*. High grade B-cell gastric lymphoma with complete pathologic remission after eradication of Helicobacter pylori infection: report of a case and review of the literature. World J Surg Oncol (2008);6:35.
30. Cheson BD, Horning SJ, Coiffier B, *et al*. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol (1999);17(4):1244.
31. Hans CP, Weisenburger DD, Greiner TC, *et al*. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood (2004);103(1):275-282.
32. Kuo SH, Chen LT, Wu MS, *et al*. Long-term follow-up of gastrectomized patients with mucosa-associated lymphoid tissue lymphoma: need for a revisit of surgical treatment. Ann Surg (2008);247(2):265-269.
33. Kuo SH, Yeh PY, Chen LT, *et al*. Overexpression of B cell-activating factor of TNF family (BAFF) is associated with Helicobacter pylori-independent growth of gastric diffuse large B-cell lymphoma with histologic evidence of MALT lymphoma. Blood (2008);112(7):2927-2934.

CAMBRIDGE RESEARCH CENTRE

Visit the publications online and view in our eBook format

Submit manuscripts to editor@cambridgeresearchcentre.co.uk

All articles included in Treatment Strategies are available as reprints

Advertise your products and services within the Treatment Strategies series and appeal to today's marketplace

WWW.CAMBRIDGERESEARCHCENTRE.CO.UK

Novel Agents Being Investigated for the Management of Relapsed/Refractory Systemic Anaplastic Large Cell Lymphoma

Annalisa Guida,[1] **Irene Biasoli**[2] and **Massimo Federico**[1]
1. Department of Diagnostic Medicine, Clinical and Public Health, University of Modena and Reggio Emilia, Modena;
2. Federal University of Rio de Janeiro, Rio de Janeiro

Systemic Anaplastic Large Cell Lymphoma (ALCL) is an aggressive T-cell lymphoma characterised by the presence of large anaplastic cells that are strongly CD30 positive and the presence of a variable proportion of cells with eccentric, horseshoe- or kidney-shaped nuclei often with an eosinophilic region near the nucleus (hallmark cells). ALCL accounts for 2% to 8% of non-Hodgkin lymphomas in adults and 20% - 30% of large cell lymphoma in children.[1-2] During the last thirty years, the definition of ALCL has undergone a substantial modification, with the incorporation of morphological, immunophenotypic and genetic knowledge which has led to a better characterisation of the distinct clinical entities of ALCL.[3-5] In 1985, Stein demonstrated the expression of the lymphoid activation antigen CD30/Ki-1 by neoplastic cells.[3] Lymphomas expressing this antigen were defined as Ki-1/CD30+ ALCL and were incorporated into the updated Kiel classification as a separate entity in 1989.[4] In 1994, the NPM- anaplastic lymphoma kinase (ALK) fusion protein due to the t(2; 5) chromosomal translocation was first identified.[6-10] By immunohistochemistry, ALK staining of large cells positivity can be detected in the cytoplasm and nucleus in the majority of the cases that exhibit the t(2;5)/NPM-ALK translocation.[11]

The expression of ALK protein allowed the distinction of systemic ALCL in two categories: ALCL ALK-positive and ALCL ALK-negative. ALCL-ALK- is now a provisional entity in the WHO 2008.[2] These two entities present different clinical and prognostic features.[2] ALCL ALK-positive mainly occurs in young patients (median age 35), with a predominance in males, frequently presents in advanced stages with B symptom as well as extranodal involvement (skin, bone, soft tissue). On the other hand, ALCL ALK-negative occurs mostly in older patients (median age 55 years-old), also with B symptoms and advanced-stage disease. Extranodal involvement is less frequent than in ALK-positive cases.[1, 12-15]

Annalisa Guida is a follower in Oncology at Università di modena e Reggio Emilia. Università di modena e Reggio Emilia is one of the oldest universities in Italy and was founded in 1175. It now has a population of more than 20,000 students. The Faculty of Medicine and Surgery has always been recognised both nationally and internationally as a prestigious institution. Numerous scientific collaborations with leading institutes from all over the world have only helped to confirm this reputation.

The prognosis of ALCL ALK-positive is remarkably better than other T-cells lymphomas and ALCL ALK-negative. The 5-year overall survival for ALCL ALK-positive and ALCL ALK-negative is 70% vs 49%, respectively. 5-year failure-free-survival is 60% for ALCL ALK- positive and 36% for ALCL ALK-negative.[14, 16] In general, International Prognostic Index (IPI) and T-cell prognostic index (PIT) have been applied in ALCL patients, and seem to predict survival.[14, 17]

Standard first-line treatment for ALCL consists of doxorubicin-containing regimens, including CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), CHOEP (cyclophosphamide, doxorubicin, vincristine, prednisone, etoposide) and MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin). CHOP and CHOEP are associated with an overall response rate of 90%,[13, 18] a 5-year relapse-free survival of 60%, and a 5-year overall survival of 70%. The 3-year EFS and OS were 46% and 62%, respectively, in patients with ALCL-ALK–.[19] The Alemtuxumab-CHOP regimen was also associated with excellent remission rate, but increased toxicity.[20] Consolidative high-dose chemotherapy and autologous stem cell transplantation (HDC/ASCT) has also been evaluated in patients in first remission with favourable results, however, it has not proved superior to standard chemotherapy and the approach remains investigational. HDC/ASCT can effectively salvage a proportion of patients with relapsed or refractory ALK+ ALCL. The role of allergenic transplantation in patients with relapsed/refractory ALCL remains undefined but there are data to support the contention that a graft-versus-lymphoma effect does exist. Myeloablative conditioning has been associated with 5-year PFS and OS of 40% and 41%, respectively, but a 5-year TRM of 33% was reported. Allo-SCT can be an option for relapsed/refractory ALCL in younger patients, preferably in the setting of a clinical trial. Recently, the development of novel therapies targeting CD30 and ALK appear promising.[21]

Advances in understanding the biology, immunophenotype and genetics of lymphoma have led to the identification of a number of

potential therapeutic targets. In the case of ALCL, the development of new drugs targeting CD30 and ALK is a substantial improvement. Besides, new agents such as histone deacetylase inhibitors (e.g. romidepsin, vorinostat), antifols (e.g. pralatrexate), monoclonal antibodies (e.g. CD25 and CD4), and immunotoxins (e.g. denileukin diftitox) are under investigation in T-cell lymphomas in general.

Brentuximab vedotin (BV) is antibody-drug conjugate (anti-CD30), which consists of a chimeric monoclonal antibody and targets cell membrane protein CD30, which is linked to the antitubulin agent monomethyl auristatin E (MMAE). The activity of BV is due to the binding of the antibody to CD30-expressing cells, followed by the internalisation of the molecule, and the release of MMAE that destructs the microtubule network inducing apoptotic death of the cells. *In vitro*, the drug was found to be potent and selective against CD30-positive tumour-cell lines, and activity was observed in models of Hodgkin lymphoma and ALCL in mice.[22-24] In the phase I study that predominantly included patients with relapsed and refractory Hodgkin Lymphoma and 2 patients with ALCL, it has been demonstrated to induce durable response, and tumour regression was described in 86% of cases. The median duration of response was at least 9.7 months. BV was administrated intravenously at doses of 0.1 to 3.6 mg per kilogram of body weight every 3 weeks (one cycle). The study used a traditional dose-escalation design, followed by a cohort expansion phase. The maximum tolerated dose was 1.8 mg per kilogram. The most common adverse events were fatigue, pyrexia, diarrhoea, nausea, neutropenia, and peripheral neuropathy. Thus, in phase I studies, BV demonstrated significant activity with a favourable safety profile.[25]

Recently, the results of a phase II study with BV were published. Of note, it was the largest prospective trial reported in patients with recurrent ALCL, and it showed that the majority of patients responded. A total of 58 patients were included in the study. BV 1.8 mg/Kg was administrated intravenously once every 3 weeks for up to 16 total doses. The median number of the cycle was seven (range 1 to 16); among patients with an objective response, the median number of cycles was eight (range 1 to 6 cycles). Objective response was observed in 50 patients (86%), 57% achieved a complete remission (CR), and 17 patients (29%) achieved a partial remission. The median duration of overall response was 13.2 months. These improvements were independent of ALK status or number of prior therapies, suggesting that responses observed with BV are not limited to a specific subgroup of patients. The study results support a CD30-targeted approach in a disease with uniform antigen expression. The CR rate and reasonable safety profile achieved in this study indicate that brentuximab vedotin could be useful as a potential treatment strategy for aggressive, CD30-positive, T-cell lymphomas.[21]

Another potential target is ALK protein. The constitutive activation of ALK fusion protein leads to cellular transformation through a complex signalling network. Among the potential combinations of proteins phosphorylated by ALK kinase activity, it has been postulated that the most important effects include activation of STAT3, AKT/PI3K, and RAS/ERK pathways, which control cell proliferation, survival, and cell cycling.[26-27]

Crizotinib is an oral inhibitor of ALK tyrosine kinase that has been approved for the treatment of ALK+ non-small cell lung cancer.[28] Studies of crizotinib in animal models of ALCL suggest that its inhibitory effect on ALK phosphorylation is sufficient for significant anti-tumour efficacy in patients.[29] In case reports of patients with relapsed ALCL treated with oral crizotinib at a dose of 250 mg twice daily, both patients showed complete remission after 1 month, and this kind of response persisted for 6 months in one patient.[30] Successful treatment of ALK ALCL with crizotinib has been reported in paediatric patients and small case series leading to ongoing trials in relapsed/refractory ALCL.[31] The use of crizotinib in combination with conventional chemotherapy is under investigation in a phase I study for relapsed or refractory solid tumours or ALCL.

Praletrexate is an antifolate that was designed to be efficiently internalised by the reduced folate carrier (RFC), a protein that is overexpressed on some cancer cells compared to normal cells. It is hypothesised that the high affinity of pralatrexate for RFC leads to selective tumour cell accumulation. Preclinical data have clearly established the superiority of pralatrexate over other antimetabolites.[32-34] The results of PROPEL study led to FDA approval of pralatrexate for the treatment of patients with relapsed or refractory PTCL. Pralatrexate was administrated as an intravenous push at 30 mg/m^2/wk for 6 weeks followed by 1 week of rest (7-week cycle). Treatment was continued until progressive disease, unacceptable toxicity or patient/physician discretion. The median duration of treatment was 70 days; the median duration of treatment among responders was 186 days. In this trial 111 were enrolled, 17 with ALCL, the ORR was respectively of 29% and 35%. The treatment was well tolerated in the majority of patients. The most common adverse events were mucositis, nausea, thrombocytopenia and fatigue. Additional areas of clinical development are focused on integrating pralatrexate into first-line PTCL treatment programmes, and exploring its clinical benefit in B-cell and cutaneous T-cell lymphomas.[35]

Histone deacetylase (HDAC) inhibitors are an emerging class of promising anticancer drugs. HDAC inhibitors have been shown to induce the acetylation of both histones and other proteins resulting in antitumour activity.[36] At present, vorinostat and depsipeptide (romidepsin) have received approval from FDA for treatment of refractory cutaneous T-cell lymphoma (CTCL), and more recently, depsipeptide has gained FDA approval for PTCL.[37-38] Recently, Coiffier *et al*.[39] published the results of a phase II trial of romidepsin in relapsed or refractory PTCL. Patients received romidepsin 14 mg/m as an intravenous infusion on days 1, 8 and 15 of each 28-day cycle for up to six cycles; patients with stable disease, partial response or complete response could elect to offered therapy until progressive disease. Fifty patients (38%) were treated for at least four cycles, and 36 patients (28%) were treated for more than six cycles. It showed an ORR of 25% (15% with CR/CRu) in PTCL, and 24% in ALCL (19% with CR/CRu). The duration of response was of 17 months and 12 months respectively for patients

with PTCL and ALCL. These results demonstrated significant single-agent activity of romidepsin in patients with relapsed or refractory PTCL. The authors concluded that the results warrant further investigation of romidepsin in the first-line setting and in combination with CHOP or other experimental agents.

Denileukin diftitox (DD), a fusion protein that combines an interleukin-2 receptor binding domain with diphtheria toxin has demonstrated activity in patients with ALCL in a multicentre phase II trial to determinate the safety and efficacy of DD and CHOP in patients with newly diagnosed PTCL.[40] Patients received six 21-day cycles of therapy, up to a maximum of eight cycles. Each cycle comprised DD 18µg/Kg/day on days 1 and 2, plus CHOP chemotherapy, as classical schedule on day 3. DD was infused IV after an orally pretreatment. All patients were evaluated for response after two cycle of DD-CHOP treatment. Patients achieving a complete response would continue treatment for two additional cycles. Patients with progressive disease following a minimum of two cycles were discontinued. Forty-nine patients with PTCL were enrolled, eight of them with ALCL. The ORR was respectively 65% and 100%. This study demonstrated that the combination of DD with CHOP is an effective and well tolerated regimen for the initial treatment of aggressive T-cell lymphoma.

Heat-shock protein (HSP) 90 is an ATP-dependent molecular chaperone essential for oncogenic transformation. HSP90 are essential for the creation and the maintenance as well as the destruction of a variety of signal transduction proteins, including the NPM-ALK oncogenic protein observed in ALCL cells.[41-42] Tanespimycin (17-AAG), targeting HSP90 function, may provide a novel therapeutic strategy for ALCL, either as single-agent activity or by combining 17-AAG with conventional or targeted therapeutic schemes.

Daclizumab is a monoclonal antibody (anti CD25) that targets interleukin 2 (IL-2) receptor subunit alpha, which is expressed by the abnormal T-cells in patients with lymphoid malignancies and also blocks the interaction of this cytokine with its growth factor.[43-44] Successful cases have been reported of paediatric patients with relapsed/refractory ALCL treated with daclizumab. The longest clinical remission reported was 45 months.[45-46]

Most PTCLs, including ALCL, have a T-helper cell phenotype expressing CD4 on the cell surface.[18] Zanolimumab, an anti-CD4 monoclonal antibody, prevents signalling by the T-cell receptor and induces killing of CD4+ cells by antibody-dependent cellular cytotoxicity. In a phase II trial of zanolimumab in relapsed or refractory PTCL objective tumour responses were obtained in 24% of the patients, one patient with ALCL.[47]

In conclusion, patients with refractory/relapsed ALCL can now benefit from several novel promising therapies, although results from prospective phase 3 trials are lacking, and will probably continue to do so in the future due to the rarity of these diseases. In the meantime, BV is by far the most tested new agent and represents an effective treatment option. Of course, additional studies are needed to determine the relative value of this and other novel therapies in development.

The therapeutic challenges are the assessment of the potential benefit of combining these new drugs with the existing ones, including conventional chemotherapy and Autologous or Allogeneic Transplantation, for offering the best salvage treatment strategies to patients with refractory/relapsed ALCL.

References

1. Stein, H., *et al*., CD30(+) anaplastic large cell lymphoma: a review of its histopathologic, genetic, and clinical features. Blood, 2000. 96(12): p. 3681-95.
2. Swerdlow, S., *et al*., , World Health Organization Classification: Tumours of Hematopoietic and Lymphoid Tissues. 2008, Lyon: IARC Press.
3. Stein, H., *et al*., The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood, 1985. 66(4): p. 848-58.
4. Stansfeld, A.G., *et al*., Updated Kiel classification for lymphomas. Lancet, 1988. 1(8580): p. 292-3.
5. Harris, N.L., *et al*., A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood, 1994. 84(5): p. 1361-92.
6. Fischer, P., *et al*., A Ki-1 (CD30)-positive human cell line (Karpas 299) established from a high-grade non-Hodgkin's lymphoma, showing a 2;5 translocation and rearrangement of the T-cell receptor beta-chain gene. Blood, 1988. 72(1): p. 234-40.
7. Rimokh, R., *et al*., A translocation involving a specific breakpoint (q35) on chromosome 5 is characteristic of anaplastic large cell lymphoma ('Ki-1 lymphoma'). Br J Haematol, 1989. 71(1): p. 31-6.
8. Mason, D.Y., *et al*., CD30-positive large cell lymphomas ('Ki-1 lymphoma') are associated with a chromosomal translocation involving 5q35. Br J Haematol, 1990. 74(2): p. 161-8.
9. Bitter, M.A., *et al*., Morphology in Ki-1(CD30)-positive non-Hodgkin's lymphoma is correlated with clinical features and the presence of a unique chromosomal abnormality, t(2;5)(p23;q35). Am J Surg Pathol, 1990. 14(4): p. 305-16.
10. Morris, S.W., *et al*., Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science, 1994. 263(5151): p. 1281-4.
11. Benharroch, D., *et al*., ALK-positive lymphoma: a single disease with a broad spectrum of morphology. Blood, 1998. 91(6): p. 2076-84.
12. Fornari, A., *et al*., Anaplastic large cell lymphoma: one or more entities among T-cell lymphoma? Hematol Oncol, 2009. 27(4): p. 161-70.
13. Falini, B., *et al*., ALK+ lymphoma: clinico-pathological findings and outcome. Blood, 1999. 93(8): p. 2697-706.
14. Vose, J., J. Armitage, and D. Weisenburger, International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. J Clin Oncol, 2008. 26(25): p. 4124-30.
15. Ferreri, A.J., *et al*., Anaplastic large cell lymphoma, ALK-positive. Crit Rev Oncol Hematol, 2012. 83(2): p. 293-302.
16. Longo, G., *et al*., Clinical characteristics, treatment outcome and survival of 36 adult patients with primary anaplastic large cell lymphoma. Gruppo Italiano per lo Studio dei Linfomi (GISL). Haematologica, 1999. 84(5): p. 425-30.
17. Savage, K.J., Prognosis and primary therapy in peripheral T-cell lymphomas. Hematology Am Soc Hematol Educ Program, 2008: p. 280-8.
18. Savage, K.J., *et al*., ALK- anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK+ ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the International Peripheral T-Cell Lymphoma Project. Blood, 2008. 111(12): p. 5496-504.
19. Ferreri, A.J., *et al*., Anaplastic large cell lymphoma, ALK-negative. Crit Rev Oncol Hematol, 2013. 85(2): p. 206-15.

20. Gallamini, A., *et al*., Alemtuzumab (Campath-1H) and CHOP chemotherapy as first-line treatment of peripheral T-cell lymphoma: results of a GITIL (Gruppo Italiano Terapie Innovative nei Linfomi) prospective multicenter trial. Blood, 2007. 110(7): p. 2316-23.
21. Pro, B., *et al*., Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: results of a phase II study. J Clin Oncol, 2012. 30(18): p. 2190-6.
22. Sun, M.M., *et al*., Reduction-alkylation strategies for the modification of specific monoclonal antibody disulfides. Bioconjug Chem, 2005. 16(5): p. 1282-90.
23. McDonagh, C.F., *et al*., Engineered antibody-drug conjugates with defined sites and stoichiometries of drug attachment. Protein Eng Des Sel, 2006. 19(7): p. 299-307.
24. Oflazoglu, E., *et al*., Combination of the anti-CD30-auristatin-E antibody-drug conjugate (SGN-35) with chemotherapy improves antitumour activity in Hodgkin lymphoma. Br J Haematol, 2008. 142(1): p. 69-73.
25. Younes, A., *et al*., Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med, 2010. 363(19): p. 1812-21.
26. Chiarle, R., *et al*., The anaplastic lymphoma kinase in the pathogenesis of cancer. Nat Rev Cancer, 2008. 8(1): p. 11-23.
27. Tabbo, F., *et al.*, ALK Signaling and Target Therapy in Anaplastic Large Cell Lymphoma. Front Oncol, 2012. 2: p. 41.
28. Kwak, E.L., *et al*., Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med, 2010. 363(18): p. 1693-703.
29. Yamazaki, S., *et al*., Pharmacokinetic/pharmacodynamic modeling of crizotinib for anaplastic lymphoma kinase inhibition and antitumor efficacy in human tumor xenograft mouse models. J Pharmacol Exp Ther, 2012. 340(3): p. 549-57.
30. Gambacorti-Passerini, C., C. Messa, and E.M. Pogliani, Crizotinib in anaplastic large-cell lymphoma. N Engl J Med, 2011. 364(8): p. 775-6.
31. Foyil, K.V. and N.L. Bartlett, Brentuximab vedotin and crizotinib in anaplastic large-cell lymphoma. Cancer J, 2012. 18(5): p. 450-6.
32. Wang, E.S., *et al*., Activity of a novel anti-folate (PDX, 10-propargyl 10-deazaaminopterin) against human lymphoma is superior to methotrexate and correlates with tumor RFC-1 gene expression. Leuk Lymphoma, 2003. 44(6): p. 1027-35.
33. Toner, L.E., *et al*., The schedule-dependent effects of the novel antifolate pralatrexate and gemcitabine are superior to methotrexate and cytarabine in models of human non-Hodgkin's lymphoma. Clin Cancer Res, 2006. 12(3 Pt 1): p. 924-32.
34. Marchi, E., *et al*., Pralatrexate is synergistic with the proteasome inhibitor bortezomib in in vitro and in vivo models of T-cell lymphoid malignancies. Clin Cancer Res, 2010. 16(14): p. 3648-58.
35. O'Connor, O.A., *et al*., Pralatrexate in patients with relapsed or refractory peripheral T-cell lymphoma: results from the pivotal PROPEL study. J Clin Oncol, 2011. 29(9): p. 1182-9.
36. Ververis, K., *et al*., Histone deacetylase inhibitors (HDACIs): multitargeted anticancer agents. Biologics, 2013. 7: p. 47-60.
37. Marks, P.A. and R. Breslow, Dimethyl sulfoxide to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. Nat Biotechnol, 2007. 25(1): p. 84-90.
38. Campas-Moya, C., Romidepsin for the treatment of cutaneous T-cell lymphoma. Drugs Today (Barc), 2009. 45(11): p. 787-95.
39. Coiffier, B., *et al*., Results from a pivotal, open-label, phase II study of romidepsin in relapsed or refractory peripheral T-cell lymphoma after prior systemic therapy. J Clin Oncol, 2012. 30(6): p. 631-6.
40. Foss, F.M., *et al*., A multicenter phase II trial to determine the safety and efficacy of combination therapy with denileukin diftitox and cyclophosphamide, doxorubicin, vincristine and prednisone in untreated peripheral T-cell lymphoma: the CONCEPT study. Leuk Lymphoma, 2013.
41. Neckers, L. and P. Workman, Hsp90 molecular chaperone inhibitors: are we there yet? Clin Cancer Res, 2012. 18(1): p. 64-76.
42. Georgakis, G.V., *et al*., The HSP90 inhibitor 17-AAG synergizes with doxorubicin and U0126 in anaplastic large cell lymphoma irrespective of ALK expression. Exp Hematol, 2006. 34(12): p. 1670-9.
43. Waldmann, T.A., Daclizumab (anti-Tac, Zenapax) in the treatment of leukemia/lymphoma. Oncogene, 2007. 26(25): p. 3699-703.
44. El-Mallawany, N.K., *et al*., Pediatric T- and NK-cell lymphomas: new biologic insights and treatment strategies. Blood Cancer J, 2012. 2(4): p. e65.
45. Linden, O., Remission of a refractory, anaplastic large-cell lymphoma after treatment with daclizumab. N Engl J Med, 2004. 351(14): p. 1466-7.
46. Costa, V., T. Oliva, and L. Norton, Successful treatment with daclizumab of refractory anaplastic lymphoma. Pediatr Blood Cancer, 2009. 53(6): p. 1130-1.
47. d'Amore, F., *et al*., Phase II trial of zanolimumab (HuMax-CD4) in relapsed or refractory non-cutaneous peripheral T cell lymphoma. Br J Haematol, 2010. 150(5): p. 565-73.

Intensive Treatment Approaches in Frontline Therapy of Advanced Follicular Lymphoma: Effective or Toxic?

Vít Procházka
Department of Hemato-Oncology, University Hospital in Olomouc, Olomouc

Introduction

Follicular lymphoma (FL) accounts for about 20–30% of non-Hodgkin lymphomas, with the median age being under sixty.[1] Clinical behaviour and overall prognosis are highly variable, ranging from indolent forms with occasional spontaneous remissions to rapidly progressive disease.[2] Modern treatment strategies have shifted from a primarily "palliative" approach to more intensive CHOP (cyclophosphamide, vincristine, prednisone, doxorubicin) - like regimens with the intention of achieving and maintaining complete long-term remission. New targeted treatment with monoclonal antibodies (MoAb) and radioimmunoconjugates has resulted in unprecedented improvements in treatment outcome.[3] Despite this fact, there is still a proportion of very high-risk patients who are at risk of early treatment failure, disease transformation and death.[4] We review current first-line treatment opportunities in risky FL.

Treatment Goals in FL

Prior to the introduction of rituximab, FL was considered a chronically relapsing condition. This was reflected by a treatment strategy using low-dose chemotherapy irrespective of the particular patient's risk. Despite evidence that patient survival is influenced by the quality of remission, those claiming that high-risk patients need intensive therapy were in the minority. The therapeutic success of MoAb showed that long-term remission may be induced. There were even optimistic predictions that these patients could be cured by conventional therapy. In the rituximab era, the treatment goal has changed significantly. Primary therapy, as non-toxic as possible, should induce long-term complete remission, preferably supported by maintenance (MoAb) or consolidation (radioimmuno-) therapy.

Vít Procházka is a specialist in Haemato-Oncology. His current position is as Senior House Officer at Department of Hemato-Oncology, University Hospital, Olomouc. He is a member of the Czech Lymphoma Study Group as an Executive committee member and participates as principal investigator in clinical trials on malignant lymphomas. Dr. Procházka has authored and co-authored 38 papers in peer-reviewed journals together with more than 100 scientific abstracts. Major topics of interest includes prognostic factors in follicular lymphoma, the role of PET/CT in lymphoma and autologous transplant in lymphomas.

Standard Treatment in Advanced FL

Most of the published evidence-based guidelines for FL treatment are in agreement concerning the criteria to start chemotherapy. Treatment is usually recommended in patients who have marrow failure due to lymphoma, develop organ compression, B symptoms and serous effusions, that is generally in patients with symptomatic disease with high tumour burden.[5] Many studies confirmed a superiority of adding rituximab to combination with chemotherapy, and this concept has been accepted as an evidence-based treatment standard.[6-8]

The successful concept of chemoimmunotherapy is overshadowed by the fact the there is still no agreement as to the optimal initial chemotherapy protocol and individual European and American professional groups prefer particular (or their own) protocols.[9-12]

So far, no prospective randomised study comparing a larger number of induction chemotherapy schemes has been carried out. Only one large (but not randomised) comparison of induction regimens was provided in the PRIMA study.[13] One of three regimens was selected based on the decision of a particular study group; these were CVP (cyclophosphamide, vincristine, prednisone), CHOP or FCM (fludarabine, cyclophosphamide, mitoxantrone), all combined with rituximab. R-CHOP was identified as superior compared to CVP (higher efficacy of CHOP) and FCM (comparable efficacy but lower toxicity of CHOP).

The published data suggest that the benefit of adding rituximab to induction therapy is not comparable for all risk subgroups. The progression-free survival (PFS)/overall survival (OS) were substantially more influenced in high-risk patients than in low-risk groups.[7, 13] However, patients with a high risk according to the Follicular Lymphoma International Prognostic Index (FLIPI) still have relatively unsatisfactory results after conventional chemoimmunotherapy. In this group, the proportion of patients free from lymphoma progression is about 30% and the proportion of survivors is 50-60% at 5 years.[7-9]

Risk Assessment in FL: A Background for an Identification of Poor Responders?

A standard in risk assessment in FL is most frequently based upon a calculation of two prognostic indices, the FLIPI and the more recently published FLIPI 2. The FLIPI was calculated in 2004 and retrospectively analysed the OS of 4,162 patients treated between 1985 and 1992. Eight independent prognostic parameters were identified. Of those, the following five parameters were selected to produce the index: age over 60 years, lactate dehydrogenase level above the upper limit of normal, involvement of more than 4 lymph node groups, haemoglobin level of less than 120 g/L and advanced stage of the disease (Ann Arbor classification). The final tool, referred to as the FLIPI, is used to evenly distribute patients into three groups: low, intermediate and high risk.[14] The predictive value of the FLIPI was confirmed by a large number of prospective studies of both untreated and relapsed patients. A high FLIPI score was found to predict unfavourable outcome after autologous stem cell transplantation (ASCT)[15-17] and is associated with a high risk of lymphoma transformation.[17] Although the FLIPI score is used globally as the gold standard, it has some historical and methodological limitations. It was created in pre-rituximab era, most of the patients were treated with reduced-intensity regimens (without anthracyclines) and the primary endpoint was the OS. It is possible that if the PFS was assessed, the score would include other variables as well. Moreover, from a current perspective, the PFS is a much more practical tool for comparing individual treatment modalities in indolent lymphoma. With better (longer) survival of FL patients, studies with the OS as the endpoint would take a rather long time.

The above reasons led to an effort to create a new index comprising all of the relevant modern prognostic factors and primarily concerned with the PFS. In 2009, a working group led by Professor Solal-Céligny, one of the authors of the original FLIPI, published a draft of a new index, FLIPI 2.[19] Prospective data from 832 patients were analysed. Univariate analysis of 15 clinical and laboratory parameters identified five independent variables. These included two factors already contained in the FLIPI (age more than 60 years and serum haemoglobin less than 120 g/l) and three new factors: the longest diameter of the largest involved node, bone marrow involvement and beta-2 microglobulin level. Compared with the FLIPI, the FLIPI 2 places stress on as precise an assessment of tumour mass as possible. The FLIPI 2 also stratifies patients into three groups, albeit according to a different number of risk factors: low (0), intermediate (1-2) and high (3 or more) risk. This score was validated in an independent series of follicular lymphoma patients.[20]

Besides the FLIPI/FLIPI 2 scores, the last few years have brought an explosion of novel non-FLIPI related prognostic factors which reflects lymphoma microenviroment, tumour biology and genetic variability of the host.[21, 22] The most influential methods in the prediction of outcome in particular patient is assessment of treatment response using PET/CT and with molecular techniques. PET/CT is the most sensitive method in the detection of initial extent of the disease both in nodal and extranodal localisations.[23-26] PET performed at the end of R-CHOP therapy was found strongly predictive of outcome in two prospective studies.[27, 28] One study also confirmed a predictive potential of "interim" PET performed after 4 cycles of therapy.[28]

Per analogiam, achieving of molecular complete remission – clearance of the tumour cells bearing bcl-2/IgH transcript from peripheral blood or bone marrow – have a significant influence on survival.[29-32]

Intensive Front-line Treatment: Surely Not Recommended?

Many recently published guidelines and meta-analyses consider front-line intensive treatment with ASCT as not recommended. The main reason for this statement is the results of four prospective randomised trials, which do not prove the OS advantage in the transplant arm.[33-36]

The OS, as discussed above, is a rather general and unspecific parameter which does not reflect the number of subsequent relapses, number of treatments and quality of life. More detailed analysis of these trials could provide some important messages for routine practice. Firstly, patients in the ASCT arm had an overwhelming advantage in terms of the PFS which was about 10-33%. Data from meta-analyses showed a risk of relapse or death reduced by 58% (HR 0.42, P<0.001). Secondly, when analysing pooled data, none of the studies showed a higher incidence of secondary tumours, myelodysplastic syndrome or secondary acute myeloid leukaemia. Surprisingly, there was no difference in treatment-related mortality between both arms.[37] Thirdly, the PFS advantage was not turned into a survival benefit, mainly due to the application of ASCT in standard arm patients who failed the therapy.

Besides the above trials, numerous remarkable non-randomised trials have been carried out in the rituximab era. German group published data of the retrospective analysis done in rituximab era: ten-year overall survival, progression-free survival and freedom from progression (FFP) after first-line ASCT were 79%, 57% and 64% after second-line ASCT 41%, 35% and 42%, respectively. Prognostic factors for FFP were treatment line and FLIPI (Follicular Lymphoma International Prognostic Index). Remarkably, no relapses occurred after 6 years following ASCT.[38] Our retrospective study showed superiority of up-front ASCT compared to chemotherapy in terms of PFS and OS. Five-year PFS was 76% and 56% in ASCT and chemotherapy group, respectively. Five-year overall survival of 95% in ASCT group was superior to that of 84% (p=0.024) in CHT arm.[39] Up front, ASCT is notably beneficial in patients who develop histological transformation, and two series published in last two years have confirmed the superiority of this approach.[40, 41]

Conclusion

In physically fit patients with high-risk disease according to the FLIPI/FLIPI 2 and large tumour mass (bulk), intensive consolidation should be considered even in the first-line. Patients who do not respond well

to induction chemoimmunotherapy and remain positron emission tomography-positive both in interim or final scans or do not achive a CR share extremely unfavourable outcome. Those should be also candidates for intensive consolidation. Modern conditioning regimens (BEAM 200) without total body irradiation have no higher long-term toxicity in terms of secondary malignancies or treatment-related deaths.[42] Rituximab improves the results of ASCT without increasing toxicity, and could also be safely administered in the maintenance setting.[43] In conclusion, the benefit of ASCT could be seen in well selected, high-risk, fit patients. An intensive approach may help us overcome tumour mass, prevent the risk of disease transformation and achieve a very long disease-free period with a good quality of life.[44]

Acknowledgement

Supported by the grant of Faculty of Medicine and Dentistry, Palacky University Olomouc (LF-2013-004). We would like to thank Mrs Kateřina Sičová for a technical support.

References

1. Trneny M, Vasova I, Pytlik R, *et al.*, "The Non-Hodgkin's lymphoma subtypes distribution and survival in Czech Republic", Klinicka onkologie. (2007), 20: pp. 341–348.
2. Swenson WT, Wooldridge JE, Lynch CF, *et al.*, "Improved survival of follicular lymphoma patients in the United States", J Clin Oncol. (2005), 23: pp. 5019–26.
3. Salles G, Ghesquières H, "Current and future management of follicular lymphoma", Int J Hematol. (2012), 96: pp. 544–51.
4. Wong E, Dickinson M, "Transformation in follicular lymphoma: biology, prognosis, and therapeutic options", Curr Oncol Rep. (2012), 14: pp. 424–32.
5. Decaudin D, Lepage E, Brousse N, *et al.*, "Lowgrade stage III-IV follicular lymphoma: multivariate analysis of prognostic factors in 484 patients-a study of the groupe d'Etude des lymphomes de l'Adulte", J Clin Oncol. (1999), 17: pp. 2499–505.
6. Marcus R, Imrie K, Solal-Celigny P, *et al.*, "Phase III study of R-CVP compared with cyclophosphamide, vincristine, and prednisone alone in patients with previously untreated advanced follicular lymphoma", J Clin Oncol. (2008), 26: pp. 4579–86.
7. Hiddemann W, Kneba M, Dreyling M, *et al.*, "Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group", Blood. (2005), 106: pp. 3725–32.
8. van Oers MH, Van Glabbeke M, Giurgea L, *et al.*, "Rituximab maintenance treatment of relapsed/resistant follicular non-Hodgkin's lymphoma: long-term outcome of the EORTC 20981 phase III randomized intergroup study", J Clin Oncol. (2010), 28: pp. 2853–8.
9. Buske C, Hoster E, Dreyling M, *et al.*, "The Follicular Lymphoma International Prognostic Index (FLIPI) separates high-risk from intermediate- or low-risk patients with advanced-stage follicular lymphoma treated frontline with rituximab and the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) with respect to treatment outcome", Blood. (2006), 108: pp. 1504–8.
10. Ghielmini M, Vitolo U, Kimby E, *et al.*, "ESMO Guidelines consensus conference on malignant lymphoma 2011 part 1: diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) and chronic lymphocytic leukemia (CLL)", Ann Oncol. (2013), 24: pp. 561-76.
11. Zelenetz AD, Wierda WG, Abramson JS, *et al.*, "Non-Hodgkin's Lymphomas, version 3.2012", J Natl Compr Canc Netw. (2012), 10: pp. 1487-98.
12. McNamara C, Davies J, Dyer M, *et al.*, "Guidelines on the investigation and management of follicular lymphoma", Br J Haematol. (2012), 156: pp. 446-67.
13. Salles G, Seymour JF, Offner F, *et al.*.„Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial", Lancet. (2011), 377: pp. 42-51.
14. Solal-Celigny P, Roy P, Colombat P, et al., "Follicular lymphoma international prognostic index", Blood. (2004), 104: pp. 1258–65.
15. Keeney GE, Gooley TA, Pham RN, *et al.*, "The pretransplant Follicular Lymphoma International Prognostic Index is associated with survival of follicular lymphoma patiens undergoing autologous hematopoietic stem cell transplantation", Leuk Lymphoma. (2007), 48: pp. 1961–7.
16. Montoto S, Lopez-Guillermo A, Altes A, *et al.*, "Predictive value of Follicular Lymphoma International Prognostic Index (FLIPI) in patients with follicular lymphoma at first progression", Ann Oncol. (2004), 15: pp. 1484–9.
17. Vose JM, Bierman PJ, Loberiza FR, *et al.*, "Long-term outcomes of autologous stem cell transplantation for follicular non-Hodgkin lymphoma: effect of histological grade and Follicular International Prognostic Index", Biol Blood Marrow Transplant. (2008), 14: pp. 36–42.
18. Gine E, Montoto S, Bosch F, *et al.*, "The Follicular Lymphoma International Prognostic Index (FLIPI) and the histological subtype are the most important factors to predict histological transformation in follicular lymphoma", Ann Oncol. (2006), 17: pp. 1539–45.
19. Federico M, Bellei M, Marcheselli L, *et al.*, "Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project", J Clin Oncol. (2009), 27: pp. 4555–62.
20. Arcaini L, Merli M, Passamonti F, *et al.*, "Validation of follicular lymphoma international prognostic index 2 (FLIPI 2) score in an independent series of follicular lymphoma patients", Br J Haematol. (2010), 149: pp. 455–7.
21. Solal-Celigny P, Cahu X, Cartron G, "Follicular lymphoma prognostic factors in the modern era: what is clinically meaningful?", Int J hematol. (2010), 92: pp. 246–54.
22. Yang ZZ, Ansell SM, "The tumor microenviroment in follicular lymphoma", Clin Lymphoma Myeloma Leuk. (2012), 10: pp. 810–8.
23. Wohrer S, Jaeger U, Kletter K, *et al.*, "18F-fluoro-deoxy-glucose positron emission tomography (18F-FDG-PET) visualizes follicular lymphoma irrespective of grading", Ann Oncol. (2006), 17: pp. 780–4.
24. Papajik T, Myslivecek M, Sedova Z, *et al.*, "Standardised uptake value of (18) F-FDG on staging PET/CT in newly diagnosed patients with different subtypes of non-Hodgkin's lymphoma", Eur J Haematol. (2011), 86: pp. 32–7.
25. Paes FM, Kalkanis DG, Sideras PA, *et al.*, "FDG PET/CT of extranodal involvement in non-Hodgkin lymphoma and Hodgkin disease", Radiographics. (2010), 30: pp. 269–91.
26. Abou-Nassar KE, Vanderplas A, Friedberg JW, *et al.*,„Patterns of use of 18-fluoro-2-deoxy-D-glucose positron emission tomography for initial staging of grade 1-2follicular lymphoma and its impact on initial treatment strategy in the National Comprehensive Cancer Network Non-Hodgkin Lymphoma Outcomes database", Leuk Lymphoma. 2013 Feb 25. [Epub ahead of print]
27. Trotman J, Fournier M, Lamy T, *et al.*, "Positron emission tomography-computed tomography (PET-CT) after induction therapy is highly predictive of patient outcome in follicular lymphoma: analysis of PET-CT in a subset of PRIMA trial participants", J Clin Oncol. (2011), 29: pp. 3194–200.
28. Dupuis J, Berriolo-Riedinger A, Julian A, *et al.*, „Impact of [(18)F]fluorodeoxyglucose positron emission tomography response evaluation in patients with high-tumor burden follicular lymphoma treated with immunochemotherapy: a prospective study from the Groupe d'Etudes des Lymphomes de l'Adulte and GOELAMS", J Clin Oncol. (2012), (35): pp. 4317-22.
29. Rambaldi A, Lazzari M, Manzoni C, *et al.*, "Monitoring of minimal residual disease after CHOP and rituximab in previously untreated patients with follicular lymphoma", Blood. (2002), 99: pp. 856–62.
30. Hirt C, Schuler F, Kiefer T, *et al.*, "Rapid and sustained clearance of circulating lymphoma cells after chemotherapy plus rituximab: clinical significance of quantitative t(14;18) PCR monitoring in advanced stage follicular lymphoma patients", Br J Haematol. (2008), 141: pp. 631–40.
31. Goff L, Summers K, Iqbal S, *et al.*, "Quantitative PCR analysis for Bcl-2/IgH in a phase III study of Yttrium-90 Ibritumomab Tiuxetan as consolidation of first remission in patients with follicular lymphoma", J Clin Oncol. (2009), 27: pp. 6094–100.
32. Papajik T, Jedlickova K, Kriegova E, *et al.*, "Polymerase chain reaction detection of cells carrying t(14;18) in bone marrow of patients with follicular and diffuse large B-cell lymphoma: the importance of analysis at diagnosis and significance of long-term follow-up", Neoplasma. (2001), 48: pp. 501–5.
33. Lenz G, Dreyling M, Schiegnitz E, *et al.*, "Myeloablative radiochemotherapy followed by autologous stem cell transplantation in first remission prolongs progression-free survival in follicular lymphoma: results of a prospective, randomized trial of the German Low-Grade Lymphoma Study Group", Blood. (2004), 104: pp. 2667–2674.
34. Deconinck E, Foussard C, Milpied N, *et al.*, "High-dose therapy followed by autologous purged stem-cell transplantation and doxorubicin-based chemotherapy in patients with advanced follicular lymphoma: a randomized multicenter study by GOELAMS", Blood. (2005), 105: pp. 3817–3823.
35. Sebban C, Mounier N, Brousse N, *et al.*, "Standard chemotherapy with interferon compared with CHOP

followed by high-dose therapy with autologous stem cell transplantation in untreated patients with advanced follicular lymphoma: the GELF-94 randomized study from the Groupe d'Etude des Lymphomes de l'Adulte (GELA)", Blood. (2006), 108: pp. 2540–2544.
36. Ladetto M, De Marco F, Benedetti F, *et al*., "Prospective, multicenter randomized GITMO/IIL trial comparing intensive (R-HDS) versus conventional (CHOP-R) chemoimmunotherapy in high-risk follicular lymphoma at diagnosis: the superior disease control of R-HDS does not translate into an overall survival advantage", Blood. (2008), 111: pp. 4004–4013.
37. Schaaf M, Reiser M, Borchmann P, "High-dose therapy with autologous stem cell transplantation versus chemotherapy or immuno-chemotherapy for follicular lymphoma in adults", Cochrane Database Syst Rev. (2012), 18: 1:CD007678. doi: 10.1002/14651858.CD007678.pub2.
38. Metzner B, Pott C, Müller TH, *et al*., "Long-term clinical and molecular remissions in patients with follicular lymphoma following high-dose therapy and autologous stem cell transplantation", Ann Oncol. 2013 Feb 7. [Epub ahead of print]
39. Procházka V, Papajík T, Raida L *et al*. „UP-FRONT AUTOLOGOUS STEM CELL TRANSPLANTATION BRINGS SURVIVAL BENEFIT TO PATIENTS WITH POOR-RISK FOLLICULAR LYMPHOMA", Haematologica (2012), 97(s1), abstract no 449.
40. Reddy N, Oluwole O, Greer JP, "Superior long-term outcome of patients with early transformation of non-Hodgkin lymphoma undergoing stem cell transplantation", Clin Lymphoma Myeloma Leuk. (2012), 12: pp. 406–11.
41. Villa D, Crump M, Panzarella T, "Autologous and Allogeneic Stem-Cell Transplantation for Transformed Follicular Lymphoma: A Report of the Canadian Blood and Marrow Group", J Clin Oncol. (2013), Feb 11. [Epub ahead of print]
42. Ladetto M, Vallet S, Benedetti F, *et al*., "Prolonged survival and low incidence of late toxic sequelae in advanced follicular lymphoma treated with a TBI-free autografting program: updated results of the multicenter consecutive GITMO trial", Leukemia. (2006), 20: pp. 1840–1847.
43. Morschhauser F, Recher C, Milpied N, *et al*., "A 4-weekly course of rituximab is safe and improves tumor control for patients with minimal residual disease persisting 3 months after autologous hematopoietic stem- cell transplantation: results of a prospective ulticenter phase II study in patient with follicular lymphoma", Ann Oncol. (2012), 23: pp. 2687–95.
44. Andresen S, Brandt J, Dietrich S, *et al*., "The impact of high-dose chemotherapy, autologous stem cell transplant and conventional chemotherapy onquality of life of long-term survivors with follicular lymphoma", Leuk Lymphoma. (2012), 53: pp. 386-93.

MALT Lymphomas - Novel Treatment Options

Antonio Salar[1] and **Carlos Montalbán**[2]
1. Autonomous University of Barcelona, Department of Clinical Hematology, Hospital del Mar, Parc de Salut Mar, Barcelona;
2. University of Alcalá, Department of Internal Medicine, Hospital Ramón y Cajal, Madrid

Introduction

Marginal zone lymphomas (MZL) are indolent B-cell neoplasms arising from the marginal zone of B-cell follicles, which can be found in the lymph nodes, spleen and mucosal lymphoid tissues. According to the World Health Organization (WHO), MZLs are currently categorised into 3 clinicopathologic entities: nodal marginal zone lymphoma, splenic marginal zone lymphoma and extranodal marginal zone lymphoma of the mucosa-associated lymphoid tissue (MALT).[1] The theme of this review will focus in those MZL originating in the extranodal tissue.

In western populations, MALT lymphomas are the third most common type of non-Hodgkin lymphomas, comprising of 7-10%.[2] These lymphomas originate in sites normally devoid of lymphoid tissues but, paradoxically, these affected sites accumulate B-cells in response to chronic infection or inflammation. In the WHO classification, MALT lymphomas are described as extranodal lymphomas comprised of morphologically heterogeneous small B-cells including marginal zone cells, cells resembling monocytoid cells, small lymphocytes, and scattered immunoblasts and/or plasma cell differentiation. The neoplastic infiltration originates in the marginal zone of reactive B-cell follicles and extends into the interfollicular region. It is characteristic to find "lymphoepithelial lesions" consisting on neoplastic cells infiltrating the epithelium.

MALT lymphomas can arise at any site.[3] At least one third occurs in the stomach, but other common extranodal sites include: skin, salivary gland, ocular adnexa, respiratory tract and others. It is important to remember that many MALT lymphomas might have multifocal involvement at presentation and at relapse.

Antonio Salar is a specialist in haematology. His current positions are Head of Section of the Department of Clinical Hematology at the Hospital del Mar, Barcelona and Associate Professor of Hematology in the Universidad Autónoma de Barcelona. He is member of the GELTAMO group (Grupo Español de Linfomas y Trasplante de Médula Ósea) and participates as Principal Investigator in clinical trials on lymphomas. Dr. Salar has contributed to approximately 80 publications in national and international medical journals. His main topic of interest is clinical and translational research in indolent lymphomas, and in particular in MALT lymphomas.

Pathogenesis and Genetics

Chronic antigen-dependent immune stimulation, with a microbial pathogen acting as the antigenic source in most cases, is under the pathogenesis of many MALT lymphomas.[4] This stimulation initially drives lymphoid hyperplasia and, in addition, these polyclonal B-cells trigger inflammatory responses by attracting neutrophils. The release of reactive oxygen species by these later cells are genotoxic and may produce additional oncogenic events, such as chromosomal translocations leading to constitutive activation of signalling pathways that ultimately result in antigen-independent lymphoproliferation. Also, regulatory T-cells might have a role in modulating host responses against the MALT lymphomas.[5]

An increasing number of antigens have been identified so far. Among infections, we describe: *Helicobacter pylori* and *Helicobacter heilmannii* in the stomach,[6] *Borrelia burgdorferi* in the skin,[7] *Chlamydia psittaci* in the ocular adnexal,[8] *Campylobacter jejuni* in the small intestine[9] and, recently, *Achromobacter (Alcaligenes) xylosidans* in the lung. Hepatitis C virus (HCV)[10] and parasites have also been associated with MALT lymphomas. In addition, autoimmune conditions are linked with an increased risk of non-gastric MALT, such as Sjögren syndrome in the lymphomas of salivary glands[11] and lung, and chronic (Hashimoto's) thyroiditis in lymphomas of the thyroid.[12]

Several genetic abnormalities can be found in MALT lymphomas. The most common structural cytogenetic abnormalities are trisomies 3 and 18, that can be found in approximately 30% and 18% of MALT lymphomas respectively. The most common recurrent translocation is translocation t(11;18)(q21;q21) that can be found in 30% of gastric MALT lymphomas.[13] This translocation juxtaposes the *AIP2* gene and the *MALT1* gene generating a chimeric protein that induces aberrant nuclear expression of bcl-10 and NF-κB activation, promoting cell survival and proliferation. Much less frequent (1-2%) is t(1;14)(p22;q32) transferring

the *bcl-10* gene to the *IgH* chain region, which also results in nuclear bcl-10 expression and NF-κB activation.[14] Cytogenetic abnormalities seem to influence the development of gastric MALT lymphomas along different pathogenetic pathways and condition differences in clinical behaviour.[15] Other translocations have been described in MALT lymphomas from non-gastric sites: t(14;18)(q32;q21),[16] t(1;2)(p22;p12) and t(3;14)(p14.1;q32).[17] Recently, new translocations have been described,[18, 19] providing evidence of the genetic heterogeneity of MALT lymphomas.[20, 21]

Clinical Features

The classical concept of MALT lymphoma assumes that it is a distinctive indolent extranodal lymphoma.[22] These patients usually have a prolonged clinical course before diagnosis, with non-specific symptoms mainly related to the primary site of disease. In gastric MALT lymphomas,[23-25] clinical features at presentation include epigastric pain or burning, nausea or vomiting, and quite rarely upper gastrointestinal bleeding or perforation. Characteristically, gastric MALT lymphoma is often multifocal within the stomach. It is also assumed that the disease follows a protracted and indolent course, and is restricted to the stomach for long periods of time. However, it is currently well-known that a considerable number of patient's MALT lymphoma can disseminate not only to MALT sites, but also to lymph nodes and bone marrow. In non-gastric MALT lymphomas,[25-26] the presenting symptoms are related to the primary localisation of the disease. However, advanced disease at diagnosis is not uncommon with dissemination to multiple mucosal sites. Up to 50% of patients with extra-gastric MALT lymphoma present with disseminated disease, but these figures vary according to the exhaustive of the work-up.[27]

Staging is challenging in MALT lymphomas. In addition to standard lymphoma work-up, some additional specific studies should be performed based on the primary site of origin. For instance, in gastric MALT lymphoma, upper endoscopy with multiple random biopsies is recommended and endoscopic ultrasound of the stomach is clearly useful in order to have an accurate imaging of the gastric wall and surrounding structures.[24, 28] In MALT lymphomas from other sites, local ultrasound or magnetic resonance imaging may help to better characterise local stage and also to evaluate response to treatment. The role of PET scans for staging is scarce, mainly due to its low sensitivity.[29] PET scans are not recommended for daily practice, but they can be useful in selected cases.

Treatment

The prognosis of patients with MALT lymphoma is excellent regardless of the site of origin and treatment modality. It is said that these are often the most indolent of the indolent lymphomas and it is important to avoid over-treatment. However, it is also convenient not to succumb to the temptation to under-treat patients that can eventually have multifocal relapse or even transformation to large-cell lymphoma.

As explained above, extranodal MALT lymphomas have important site-specific and pathogen-trigger associations; thus, when considering treatment of MALT lymphomas, the main principles to consider are the location of the disease and its association to a specific pathogen. Therefore, it is conceptually useful to distinguish gastric MALT lymphomas from non-gastric MALT lymphomas.[30]

Anti-infectious Therapy

In 1993 Wotherspoon *et al*. reported that eradication of *H. pylori* in 6 patients with gastric MALT lymphoma in stage I resulted in the histological disappearance of the lymphoma.[31] This result was confirmed in successive studies.[32, 33] Following eradication of *H. pylori*,[34, 35] responses are achieved in 60-90% of patients with a median time from treatment to remission between 2 and 6 months. However, some responses are seen at long-term, and for this reason, it is sensible to wait for at least 12 months before starting another treatment. Among complete responders, almost 15% will relapse within 3 years, with or without infection of *H. pylori*. Currently, several predictive factors for inferior response to antibiotic treatment have been described (translocations t(11;18) or t(1;14), perigastric lymph nodes, etc).[36, 37] Detection of monoclonality can be detected in 19-84% of histologic remissions after eradiation therapy;[38, 39] currently, it is accepted that there is no higher relapse risk in this setting. The role of additional chemotherapy after antibiotics did not show benefit in terms of progression-free or overall survival in a prospective study.[40]

Lymphoma regression following antibiotics has been recently reported in MALT lymphomas from the ocular adnexa associated to *Chlamydia psittaci* infection.[41] In a phase II study, Ferrari *et al*. treated 34 patients with stage I MALT lymphoma with doxycycline for 3 weeks. Chlamydia eradication was achieved in 28% at 3 months and 48% at 12 months. The overall response rate was 65%, being higher among those in which Chlamydia was eradicated.

Some cases successfully treated with interferon and ribavirin have been reported in HCV-positive patients with extranodal MALT lymphoma from several sites.[42]

Surgery

Surgical resection of the stomach has been successfully used to treat gastric MALT lymphoma, being the classical therapeutic approach for localised gastric MALT in the past.[43, 44] Despite wide surgical excisions, residual disease can be observed in the margins or relapses in the gastric stump, and these situations require additional radiation and/or chemotherapy. It is important to consider the severely impairing quality of life of total or subtotal gastrectomy with or without lymphadenectomy. Therefore, in the last two decades, the role of surgery in gastric lymphoma has progressively declined and is currently abandoned.[45]

In patients with extra-gastric localised MALT lymphomas, surgery is a suitable option not only for treatment but also as a diagnostic procedure.

Patients with involvement of the skin, conjunctiva, lachrymal glands, etc. can achieve an excellent control of the disease either with a low morbidity and mortality rate.

Radiation

MALT lymphomas are very sensitive to low doses of radiation. In patients with localised gastric MALT lymphoma, radiation of the stomach and perigastric nodes at moderate doses (median of 30 Gy) is the treatment of choice in some centres, mainly due to the following reasons: organ preserving approach, excellent local control of disease, low rate of relapses, quite low morbidity and mortality.[46-48]

Although there is no consensus on the role of radiotherapy for non-gastric MALT lymphoma, this approach is often used in patients with limited stage disease from some sites as ocular adnexa, salivary glands, skin and others.[49-51] Patients usually achieve very high local control rates that should be balanced against treatment-related toxicity and the higher risk of distant recurrence in comparison with gastric MALT lymphoma.

Chemo/Immunotherapy

Treatment with chemotherapy or immunotherapy is usually reserved for patients with disseminated disease or for those relapsing to antibiotics, surgery or radiotherapy. Patients are usually treated according to the same principles as for other advanced-stage indolent lymphomas.[24] Conventional chemotherapy has demonstrated activity in gastric and extra-gastric MALT lymphomas, with response rates of over 90%. Purine analogue-based therapy appears to have more efficacy but also more toxicity than alkylating-based therapy.[52-56] Rituximab have demonstrated activity in patients with (relapsed or *de novo*) gastric or extra-gastric MALT lymphomas, but with a relative short time to progression.[57-59] It is important to note that the presence of translocation t(11;18) in gastric MALT lymphoma is predictive of resistance to oral alkylating agents, with less than 10% of durable remission at long-term follow-up.[60] However, purine analogs and rituximab are effective in both negative and positive cases for t(11;18).[61]

Immunochemotherapy combining rituximab with alkylating or purine analogs have been tested. In a phase 2 study, rituximab plus fludarabine was very effective (95% complete remission) as first-line treatment of patients with MALT lymphoma of gastric or extra-gastric origin. As expected, the main toxicity was haematologic, with 41% of cases having grade 3-4 neutropenia, although grade 3-4 infectious events were very rare.[62]

A randomised trial was launched in 2003 by the International Extranodal Lymphoma Study Group (IELSG) to compare chlorambucil alone versus combination of chlorambucil plus rituximab in the treatment of MALT lymphomas.[63] Later, a third arm with rituximab alone was added. The results have recently been reported showing a very good response rate in both arms (87% vs 90%, p value not significant). Complete remission rate was significantly higher in the arm with chlorambucil plus rituximab (78% vs 65%, p = 0.017), as was the EFS at 5 years (68% vs 50%, p= 0.002). However, this improvement did not translate into improved overall survival.

Recently, in an abstract form, bendamustine plus rituximab has shown a very high activity in first-line MALT lymphoma (gastric and extra-gastric) with and overall response rate of 100% and complete remission rate of 97% at the end of therapy.[64] The safety profile of the combination was favourable, with grade 3-4 neutropenia in only 3% of cycles. More time is needed for the data to mature and to draw firm conclusions. Other novel agents as lenalidomide,[65] bortezomib[66] or 90Y-ibritumomab have shown some antitumour activity in MALT lymphomas, but it clinical usefulness should be better defined.

Outcome

MALT lymphomas typically have a favourable prognosis, with most studies showing 5-year OS more than 85%. The median time to progression is better for gastric than for non-gastric lymphomas, but with similar overall survival between both groups. Histologic transformation to large-cell lymphoma has been reported to occur in approximately 10-15% of cases, usually as a late event, and in these scenarios aggressive treatment is required for curative intention.

References

1. Swerdlow S, Campo E, Harris NL, eds; International Agency for Research on Cancer. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue. Geneva, Switzerland: World Health Organization; 2008.
2. Salar A, Fernández de Sevilla A, Romagosa V, *et al*. "Distribution and incidence rates of lymphoid neoplasms according to the REAL classification in a single institution. A prospective study of 940 cases". Eur J Haematol. (1997), 59: 231-237.
3. Isaacson PG, Wright DH. "Malignant lymphomas of mucosa-associated lymphoid tissue. A distinctive type of B-cell lymphoma". Cancer. (1983), 52: 1410-1416.
4. Suarez F, Lortholary O, Hermine O, *et al*. "Infection associated lymphomas derived from marginal zone B cells:a model of antigen-driven lymphoproliferation". Blood. (2006),107: 3034-3044.
5. García M, Bellosillo B, Sánchez-González B, *et al*. "Study of Regulatory T-Cells in Patients with Gastric Malt Lymphoma: Influence on Treatment Response and Outcome". PLoS ONE. (2012), 7 (12): e51681.
6. Parsonnet J, Hansen S, Rodriguez L, *et al*. "Helicobacter pylori infection and gastric lymphoma". New Eng J Med (1994), 330:1267-1271.
7. Roggero E, Zucca E, Mainetti C, *et al*. "Eradication of Borrelia burgdorferi infection in primary marginal zone B-cell lymphoma of the skin". Hum Pathol. (2000), 31: 263-268.
8. Ponzoni M, Ferreri AJ, Guidoboni M, *et al*. "Chlamydia infection and lymphomas: association beyond ocular adnexal lymphomas highlighted by multiple detection methods". Clin Cancer Res. (2008),14: 5794-5800.
9. Lecuit M, Abachin E, Martin A, *et al*. "Immunoproliferative small intestinal disease

associated with Campylobacter jejuni". N Engl J Med. (2004); 350: 239-248.
10. Zucca E, Roggero E, Maggi-Solca N, *et al*. "Prevalence of Helicobacter pylori and hepatitis C virus infections among non-Hodgkin's lymphoma patients in Southern Switzerland". Haematologica. (2000); 85: 147-153.
11. Manganelli P, Fietta P, Quaini F. "Hematologic manifestations of primary Sjögren's syndrome". Clin Exp Rheumatol. (2006), 24: 438-448.
12. Derringer GA, Thompson LD, Frommelt RA, *et al*. "Malignant lymphoma of the thyroid gland: a clinicopathologic study of 108 cases". Am J Surg Pathol. (2000), 24: 623-639.
13. Dierlamm J, Baens M, Wlodarska I, *et al*. "The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa associated lymphoid tissue lymphomas". Blood. (1999), 93: 3601-3609.
14. Willis TG, Jadayel DM, Du MQ, *et al*. "Bcl10 is involved in t(1;14)(p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types". Cell. (1999), 96: 35-45.
15. Starostik P, Patzner J, Greiner A. *et al*. Gastric Marginal zone B-cell lymphomas of MALT type develop along 2 distinct pathogenetic pathways. Blood. (2002), 99: 3-9.
16. Streubel B, Lamprecht A, Dierlamm J, *et al*. "T(14;18)(q32;q21) involving IGH and MALT1 is a frequent chromosomal aberration in MALT lymphoma". Blood. (2003), 101: 2335-2339.
17. Streubel B, Vinatzer U, Lamprecht A, *et al*. "T(3;14)(p14.1;q32) involving IGH and FOXP1 is a novel recurrent chromosomal aberration in MALT lymphoma". Leukemia. (2005), 19: 652-658.
18. Vinatzer U, Gollinger M, Mullauer L, *et al*. "Mucosa-associated lymphoid tissue lymphoma: novel translocations including rearrangements of ODZ2, JMJD2C, and CNN3". Clin Cancer Res. (2008), 14: 6426-6431.
19. Ansell SM, Akasaka T, McPhail E, *et al*. "t(X;14)(p11;q32) in MALT lymphoma involving GPR34 reveals a role for GPR34 in tumor cell growth". Blood. (2012), 120: 3949-3957.
20. Farinha P, Gascoyne R. "Molecular pathogenesis of mucosa-associated lymphoid tissue lymphoma". J Clin Oncol. (2005), 23:6370-6378.
21. Kwee I, Rancoita PM, Rinaldi A, *et al*. "Genomic profiles of MALT lymphomas: variability across anatomical sites". Haematologica. (2011), 96: 1064-1066.
22. Fisher RI, Dahlberg S, Nathwani BN, *et al*. "A clinical analysis of two indolent lymphoma entities: mantle cell lymphoma and marginal zone lymphoma (including the mucosa-associated lymphoid tissue and monocytoid B-cell subcategories): a Southwest Oncology Group study". Blood. (1995),85:1075-1082.
23. Zucca E, Bertoni F, Roggero E, *et al*. "The gastric marginal zone B-cell lymphoma of MALT type", Blood. (2000), 96: 410-419.
24. Ruskone-Fourmestraux A, Fischbach W, Aleman BMP, *et al*. "EGILS consensus report. Gastric extranodal marginal zone B-cell lymphoma of MALT". Gut (2011), 60: 747–758.
25. Thieblemont C, Bastion Y, Berger F, *et al*. "Mucosa-associated lymphoid tissue gastrointestinal and non-gastrointestinal lymphoma behavior: analysis of 108 patients". J Clin Oncol. (1997), 15: 1624-1630.
26. Zinzani PL, Magagnoli M, Galieni P, *et al*. "Non-gastrointestinal low-grade mucosa-associated lymphoid tissue lymphoma: analysis of 75 patients". J Clin Oncol. (1999), 17: 1254-1258.
27. Raderer M, Wohrer S, Streubel B, *et al*. "Assessment of disease dissemination in gastric compared with extragastric mucosaassociated lymphoid tissue lymphoma using extensive staging: a single-center experience". J Clin Oncol. (2006), 24: 3136-3141.
28. Palazzo L, Roseau G, Ruskone-Fourmestraux A, *et al*. "Endoscopic ultrasonography in the local staging of primary gastric lymphoma". Endoscopy. (1993), 25:502-508.
29. Beal KP, Yeung KW, Yahalom J. "FGD-PET scanning for detection and staging of extranodal marginal zone lymphomas of the MALT type". Ann Oncol. (2005), 16: 473-480.
30. Isaacson PG. "Gastric MALT lymphoma: from concept to cure". Ann Oncol. (1999), 10: 637-645.
31. Wotherspoon A, Doglioni C, Diss TC, *et al*. "Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori". Lancet. (1993), 342:575-577.
32. Bayerdörffer E, Neubauer A, Rudolph B, *et al*. "Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of helicobacter pylori infection". Lancet. (1995), 345:1591-1594.
33. Montalban C, Manzanal A, Boixeda D, *et al*. "Helicobacter pylori eradication for the treatment of low grade gastric MALT lymphoma. Follow up together with sequential molecular studies". Ann Oncol. (1997), 8(Suppl 2);S37-S39.
34. Malfertheiner P, Megraud F, O'Morain C, *et al*. "Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report". Gut. (2007), 56: 772-781.
35. Chey WD Wong BC. "American College of Gastroenterology guideline on the management of Helicobacter pylori infection". Am J Gastroenterol. (2007), 102: 1808-1825.
36. Ruskoné-Fourmestraux A, Lavergne A, Aegerter PH, *et al*. "Predictive factors for regression of gastric MALT lymphoma after anti-Helicobacter pylori treatment". Gut. (2001), 48: 297-303.
37. Liu H, Ye H, Ruskoné-Fourmestraux A, *et al*. "t(11;18) is a marker for all stage gastric MALT lymphomas that will not respond to H.pylori eradication". Gastroenterology. (2002), 122: 1286-1294.
38. Santón A, García-Cosio M, Bellosillo B, *et al*. "Persistent monoclonality after histological remission in gastric mucosa-associated lymphoid tissue lymphoma treated with chemotherapy and/or surgery: influence of t(11;18)(q21;q21)". Leuk Lymphoma. (2008), 49:1516–1522.
39. Salar A, Bellosillo B, Serrano S, *et al*. "Persistent residual disease in t(11;18)(q21;q21) positive gastric mucosa-associated lymphoid tissue lymphoma treated with chemotherapy or rituximab". J Clin Oncol. (2005), 23: 7361-7362.
40. Hancock B, Qian W, Linch D, *et al*. "Chlorambucil versus observation after anti-Helicobacter therapy in gastric MALT lymphomas: results of the international randomised LY03 trial". Br J Haematol. (2009), 144: 367-375.
41. Govi S, Dolcetti R, Ponzoni M, *et al*. "Final results of a multicenter phase II trial with translational elements to investigate the possible infective causes of ocular adnexal marginal zone B-cell lymphoma (OAMZL) with particular reference to Chlamydia species and the efficacy of doxycycline as first-line lymphoma treatment (the IELSG#27 trial) [abstract]". Blood (ASH Annual Meeting Abstracts). (2011), 118: 267
42. Arcaini L, Merli M, Volpetti S, *et al*. "Indolent B-Cell Lymphomas Associated with HCV Infection: Clinical and Virological Features and Role of Antiviral Therapy". Clin Dev Immunol. (2012): 638185.
43. Koch P, del Valle F, Berdel W, *et al*. "Primary gastrointestinal non-Hodgkin's lymphoma: II. Combined surgical and conservative or conservative management only in localized gastric lymphoma. Results of the prospective German Multicenter Study GIT NHL 01/92". J Clin Oncol. (2001), 18: 3874-3883.
44. Zinzani PL, Tani M, Barbieri E, *et al*. "Utility of surgical resection with or without radiation therapy in patients with low-grade gastric mucosa-associated lymphoid tissue lymphoma". Haematologica. (2003), 88: 830-831.
45. Coiffier B, Salles G. "Does surgery belong to medical history for gastric lymphomas?" Ann Oncol. (1997), 8: 419-421.
46. Schechter N, Portlock CS, Yahalom J. "Treatment of mucosa-associated lymphoid tissue lymphoma of the stomach with radiation alone". J Clin Oncol. (1998), 16: 1916-1921.
47. Yahalom J. "MALT lymphomas: a radiation oncology viewpoint". Ann Hematol. (2001), 80(Suppl 3):B100-5.
48. Tsang RW, Gospodarowicz MK, Pintilie M, *et al*. "Localized Mucosa-Associated Lymphoid Tissue lymphoma treated with radiation therapy has excellent clinical outcome". J Clin Oncol. (2003), 22: 4157-4164.
49. Fung CY, Tarbell NJ, Lucarelli MJ, *et al*. "Ocular adnexal lymphoma: clinical behavior of distinct World Health Organization classification subtypes". Int J Radiat Oncol Biol Phys. (2003),57: 1382-91.
50. Suh CO, Shim SJ, Lee SW, *et al*. "Orbital marginal zone B-cell lymphoma of MALT: radiotherapy results and clinical behavior". Int J Radiat Oncol Biol Phys. (2006), 65: 228-33.
51. Son SH, Choi BO, Kim GW, *et al*. "Primary radiation therapy in patients with localized orbital marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT Lymphoma)". Int J Radiat Oncol Biol Phys. (2010), 77: 86-91.
52. Hammel P, Haioum C, Chaumette MT, *et al*. "Efficacy of single agent chemotherapy in low grade B-cell mucosa-associated lymphoid tissue lymphoma with prominent gastric expression". J Clin Oncol. (1995), 13: 2524-2529.
53. Jager G, Neumeister P, Brezinschek R, *et al*. "Treatment of extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT)-type with Cladribine (2-Chlorodeoxyadenosine): A phase II study". J Clin Oncol. (2002), 18: 3872-3877.
54. Wöhrer S, Drach J, Hejna M, *et al*. "Treatment of extranodal marginal zone B-cell lymphoma of Mucosa-Associated Lymphoid Tissue (MALT lymphoma) with mitoxantrone, chlorambucil and prednisone (MCP)". Ann Oncol. (2003), 14: 1758-1761.
55. Zinzani PL, Stefoni V, Musuraca G, *et al*. "Fludarabine-containing chemotherapy as frontline treatment of nongastrointestinal Mucosa-Associated lymphoid tissue". Cancer (2004), 100: 2190-2194.
56. Raderer M, Wohrer S, Bartsch R, *et al*. "Phase II Study of oxaliplatin for treatment of patients with mucosa-associated lymphoid tissue lymphoma". J Clin Oncol. (2005), 23: 8442-8446.
57. Raderer M, Jager G, Brugger S, *et al*. "Rituximab for treatment of advanced extranodal marginal zone B cell lymphoma of the Mucosa-Associated Lymphoid Tissue lymphoma". Oncology. (2003), 65: 306-310.
58. Conconi A, Martinelli G, Thieblemont C, *et al*. "Clinical activity of rituximab in extranodal marginal zone B-cell lymphoma of MALT type". Blood. (2003), 102: 2741-2745.
59. Martinelli G, Laszlo D, Ferreri AJM, *et al*. "Clinical activity of Rituximab in gastric marginal zone lymphoma non-Hodgkin's lymphoma resistant to or not eligible for anti-Helicobacter pylori therapy". J Clin Oncol. (2005), 23:1979-1983.
60. Levy M, Copie-Bergman C, Gameiro C, *et al*. "Prognosis value of translocation t(11;18) in tumoral response of low-grade gastric lymphoma of Mucosa-Associated Lymphoid t tissue type to oral chemotherapy". J Clin Oncol. (2005), 22: 5061-5066.
61. Streubel B, Ye H, Du MG, et al. "Translocation t(11;18))(q21;q21) is not predictive of response to chemotherapy with 2CdA in patients with gastric MALT lymphoma". Oncology. (2004), 100: 2190-2194.
62. Salar A, Domingo-Domenech E, Estany C, *et al*. "Combination therapy with rituximab and intravenous or oral fludarabine in the first-line, systemic treatment of patients with extranodal marginal zone B cell lymphoma of the mucosa-associated lymphoid tissue type". Cancer. (2009), 115: 5210-5217.
63. Zucca E, Conconi A, Laszlo D, *et al*. "Addition of Rituximab to Chlorambucil Produces Superior Event-Free Survival in the Treatment of Patients With

Extranodal Marginal-Zone B-Cell Lymphoma: 5-Year Analysis of the IELSG-19 Randomized Study." J Clin Oncol. (2013), 31: 565-572.
64. Salar A, Domingo-Domenech E, Panizo C, *et al*. "Final Results of a Multicenter Phase II Trial with Bendamustine and Rituximab As First Line Treatment for Patients with MALT Lymphoma (MALT-2008-01)" Blood (ASH Annual Meeting Abstracts). (2012), Abstract 3691.
65. Kiesewetter B, Troch M, Dolak W, *et al*. "A phase II study of lenalidomide in patients with extranodal marginal zone B-cell lymphoma of the mucosa associated lymphoid tissue (MALT lymphoma)". Haematologica. (2013), 98: 353-356.
66. Conconi A, Martinelli G, Lopez-Guillermo A, *et al*; "International Extranodal Lymphoma Study Group (IELSG). Clinical activity of bortezomib in relapsed/refractory MALT lymphomas: results of a phase II study of the International Extranodal Lymphoma Study Group (IELSG)". Ann Oncol. (2011), 22: 689-695.

Further Reading

Please see the abstracts below for further reading on lymphoma. These articles plus others are available in previous editions of *Treatment Strategies - Hematology*. Please see our website for more articles on lymphoma and a range of other topics within haematology.

Hodgkin Lymphoma in Older Patients: Prior, Current, and Future Treatment Strategies

Andrew M. Evens
Division of Hematology/Oncology, UMass Memorial Cancer Center, The University of Massachusetts Medical School, University Hospital, Worcester, Massachusetts

Older patients with Hodgkin lymphoma (HL), commonly defined as age ≥60 years, is a disease entity in which survival rates are disproportionately and markedly inferior compared with younger HL patients. Further, it represents a population that is under-represented in HL clinical studies. Generally, treatment of older HL patients should be given with curative intent for all disease stages, however caution should be given to potential serious treatment-related toxicity including treatment-related mortality. Inclusion of anthracycline therapy appears important, however bleomycin-containing regimens (e.g., ABVD) are often associated with prohibitive pulmonary toxicity and more intensive therapy such as BEACOPP is too toxic. The impact of patient co-morbidities and assessment of functional status needs to be incorporated into prospective studies, while the integration of novel therapeutic agents into treatment paradigms is warranted. Additionally, multi-centre and multi-national collaborations will be critical in helping to refine and tailor therapy in order to improve survival for older HL patients.

New Agents in the Treatment of B-cell Non-Hodgkin Lymphomas and Future Expectations

Alexandre Christinat, Anastasios Stathis, Emanuele Zucca and **Franco Cavalli**
Oncology Institute of Southern Switzerland (IOSI), International Extranodal Lymphoma Study Group, Ospedale San Giovanni, Bellinzona

Major progress has been achieved over the past decade in the treatment of non-Hodgkin lymphomas (NHL) of B-cell origin. In particular, Rituximab in combination with standard chemotherapy has improved response rate (RR) and survival in both follicular (FL) and diffuse large B-cell lymphoma (DLBCL). Nevertheless, patients with follicular or other indolent lymphomas will invariably relapse. On the other hand, for patients with DLBCL, despite the disease being potentially curable, relapses are frequent, while some patients remain refractory to standard chemoimmunotherapy regimens. Salvage approaches based on high-dose chemotherapy with stem cell transplantation are only helpful for selected patients and it is understood that alternative treatments are needed. With respect to this essential, the past decade has seen an expansion of pre-clinical laboratory investigation resulting in improved knowledge of the molecular lymphomagenesis and relevant insights on the complex relationship between lymphoma cells and their microenvironment. Meanwhile, several pathways active in lymphomas have been discovered, and components of these pathways may represent targets for the development of new agents.

Recent clinical trials in lymphoma patients are evaluating new targeted agents and there is hope that active treatments can be developed to overcome the resistance to standard therapy. In this article we review the most recent clinical developments in patients with B-cell non-Hodgkin lymphomas and outline future treatment directions.

New Insights into the Biology of Multiple Myeloma Bone Disease and Future Treatment Targets

Ida Bruun Kristensen and **Niels Abildgaard**
Department of Hematology at Odense University Hospital, Odense

Clinical Observations

Multiple myeloma is a disease caused by clonal expansion of malignant plasma cells in the bone marrow. The incidence is about 8 in every 100,000 in the UK (http://www.cancerresearchuk.org/cancer-info/cancerstats/types/myeloma/incidence/uk-multiple-myeloma-incidence-statistics) and higher in African Americans. It is mainly a disease of the elderly, with a median age of 69 years. Multiple myeloma is an incurable cancer. In the elderly, myeloma is treated with chemotherapy, including newer drugs, whereas physically fit younger patients (≤65-70 years) are often treated with high-dose chemotherapy and stem-cell support.

One of the most important clinical problems for patients is lytic bone disease, causing bone pain, fractures and hypercalcaemia. Conventional radiographs reveal abnormalities in 79% of patients at the time of diagnosis. Lytic lesions are seen in 2/3 of patients, and osteoporosis, pathologic fractures and compression of the spine are each observed with a frequency of approximately 20%. At least 25% of the patients without osteolytic lesions at the time of diagnosis develop lesions during the course of the disease,[1] but some to a much greater extent than others. During periods of disease control and complete remission most patients are unable to heal the bone lesions, indicating some degree of persistent imbalance in bone remodelling.

Ida Bruun Kristensen is a MD at the Department of Haematology at Odense University Hospital. She studied medicine at the University of Copenhagen and has recently finished her PhD thesis on the expression of osteoblast and osteoclast regulators in the micro-environment in the bone marrow in multiple myeloma. Dr. Kristensen is currently Post-Doc at the Haemato-Pathology Research Laboratory at Odense University Hospital, where she is completing gene expression profiling studies of mesenchymal stromal cells from multiple myeloma patients. She is an active member of the Danish Myeloma Study Group.

Niels Abildgaard is Clinical Professor of Haematology, Chief Physician, DMSc and Head of the Clinical Research Unit at the Department of Haematology at Odense University Hospital. His particular research area is multiple myeloma. Dr. Abildgaard is represented in various networks and is a member of the International Myeloma Working Group (IMWG), European Myeloma Network (EMN), Chairman of the Danish Myeloma Study Group, and since 2012 he is also Chairman of the Nordic Myeloma Study Group. Dr. Abildgaard is also the supervisor for several PhD-students.

Data from the British Medical Research Council Myeloma-IX study shows that intensive osteoclast inhibition with zolendronic acid every four weeks compared to inhibition with the less potent clodronic acid results in both fewer skeletal related events (SRE) and improved overall survival.[2-4] This indicates that targeting the stroma is of importance for disease control.

Normal Bone Remodelling

Normal bone remodelling is a delicate coupling between 1) bone resorption of monocyte-derived osteoclasts, 2) cleaning-up by reversal cells of osteoblast lineage, 3) followed by the production of organic osteoid by active osteoblasts that derive from mesenchymal stromal cells (MSC) under Wnt or Bone Morphogenetic Protein (BMP)-stimulation (for reviews see [5,6]), and finally 4) mineralisation of the osteoid into mature hard bone. The osteoblasts are transformed into osteocytes imbedded in the bone tissue. The balance between bone resorption and bone formation is important for the maintenance of normal bone structure. Two well-known transcription factors for osteoblast maturation are RUNX2, thought to be of importance in early recruitment and maturation, and osterix, thought to determine the maturation to a functional osteoblast (for review see [7]).

The Patophysiology of Multiple Myeloma Bone Remodelling

Overt myeloma lytic bone disease is characterised by a marked uncoupling of bone remodelling with increased bone resorption and decreased bone formation. Histomorphometric studies by Bataille *et al.*[8] in early stage multiple myeloma showed an increased number of osteoblasts and increased bone formation rate together with an increased number of osteoclasts and increased bone resorption. by contrast, in patients with overt myeloma and lytic bone disease the osteoclasts showed the same number of cells and levels of bone resorption, but significantly lower bone formation rates due to a decreased number of osteoblasts and decreased activity per osteoblast. Patients without lytic bone disease retained a balanced bone remodelling.

Osteoclast Hyperactivation

In 1991 Bataille *et al.* described hyperactive osteoclasts, and in 2007

hyperactive fusion cells of myeloma plasma cells and osteoclasts were reported.[9] Furthermore, increased markers of bone resorption were described in newly diagnosed multiple myeloma patients with lytic bone disease.[10] The hyperactivation is caused by an imbalance in the OPG (osteoprotegerin)/RANK (receptor activator of nuclear factor kappa-beta)/RANKL (RANK-ligand) system. Under normal circumstances RANKL, secreted by mature osteoblasts (or in multiple myeloma the myeloma plasma cells itself, which is debatable[11]) binds to RANK on osteoclast-precursors, thus promoting osteoclast fusion and activation.[12] This is counter-balanced by OPG that acts as a decoy receptor for RANKL, thus preventing the RANK/RANKL interaction. In myeloma patients a diminished OPG/RANKL ratio has been found.[13] Some osteoclast activators, e.g. MIP1alfa, have been found to be overexpressed in patients with lytic bone disease, and patients with MGUS compared to healthy volunteers,[14, 15] but MIP1alfa also correlates to advanced disease stages (e.g. as defined by ISS[16]). Whether MIP1alfa is a marker of disease severity rather than lytic bone disease requires further investigation. In general, osteoclast activators have not been shown to be constitutively up-regulated in multiple myeloma patients,[17-19] and even though osteoclasts are inhibited by bisphosphonates, current optimal bisphosphonate treatment still results in an incidence of skeletal related events of 27%.[3] Thus, osteoclast hyperactivation is not the sole explanation for lytic bone disease and this has brought osteoblast inactivation into focus.

Osteoblast Inactivation

The study of Tian *et al*.[18] on gene-expression of magnetic-activated cell-sorting isolated myeloma plasma cells brought attention to osteoblast inhibitors, since they found overexpression of the wnt-inhibitor DKK1 in association with lytic bone disease. Following this, other wnt-inhibitors have been investigated: the SFRP2 and SFRP3 by Giuliani *et al*.,[20] who found that SFRP2 was not expressed by the myeloma plasma cells, and that 60% of myeloma plasma cell patient samples expressed SFRP3 at the mRNA level and 76% in BM plasma; and more recently sclerostin, a wnt-inhibitor thought to be secreted by osteocytes, has been found to be up-regulated in serum from patients with multiple myeloma and lytic bone disease by Terpos *et al*.[21]

Another pathway thought to be involved in the lytic bone disease is the Hepatocyte Growth Factor (HGF) pathway (for a review of HGF see [22]). Standal *et al*.[23] found that HGF induced a maturation stop in osteoblast-lineage cells in culture, and HGF has been shown to be elevated in myeloma serum.[24] Also, HGF is known to be involved in angiogenesis.[25] Its receptor is *c-Met* which is expressed on approximately 40% of myeloma plasma cells,[26-28] and c-Met is up-regulated in multiple myeloma.[28] Syndecan-1, expressed solely on plasma cells, is known to work as a co-receptor for HGF, modulating its effects on c-Met.[29, 30] Recently an upregulation of both HGF and the c-Met receptor in association with the lytic bone disease in multiple myeloma has been published.[31] HGF is secreted in an inactive pro-form, which is activated by several proteases, one of which is the HGF activator, and HGF expression is induced by heparanase.[32] Heparanase has also recently been shown to upregulate RANKL expression,[33] through upregulation of RUNX2 expression.[34] The biological effect of HGF-cMET interaction can be modulated by a partial antagonist of the c-MET receptor, *decorin*. Comparison of isolated mesenchymal stromal cells from multiple myeloma patients with and without lytic bone disease has shown lower gene expression of decorin in the former.[35] Thus, multiple factors related to the HGF pathway may contribute to the mechanisms involved in osteoblast inhibition. For further review of the mechanisms of multiple myeloma lytic bone disease see [36].

Current and Future Treatment Strategies of Myeloma Bone Disease

Zoledronic Acid

The Z-Mark study (NCT00622505)[37] reported that bone marker-directed dosing of zoledronic acid after 1-2 years of prior treatment (a minimum of 4 prior doses) guided after urinary NTX levels is feasible and safe. However, due to a high drop-out rate and reported reasons for drop-out the results are difficult to translate into clinical practice, and further studies are needed to evaluate whether bone-marker directed treatment is applicable in the beyond two years perspective in multiple myeloma.

Interim analysis of a study evaluating zoledronic acid treatment for anti-myeloma effects in asymptomatic myeloma patients with biochemical M-component relapse shows significantly fewer SRE in the zoledronic acid arm compared to the untreated, but so far no difference in progression-free or overall survival.[38]

The anti-RANKL antibody denosumab is currently under investigation in a non-inferiority study of zoledronic acid in multiple myeloma patients with the focus on overall survival (NCT01345019), as the large study including several cancers showed unfavourable survival for myeloma patients in the denosumab arm.[39]

Osteoclast Targeting

Targeting the Bruton Kinase with an inhibitor (PCI-32765, currently under clinical investigation in the CLL setting) shows *in vitro* efficacy on myeloma cell growth, but also inhibits the interaction between myeloma plasma cells and stromal cells, the osteoclastic bone resorption, and the release of myeloma growth factors by osteoclasts. Thus, multiple parts of the vicious cycle in the myeloma bone marrow microenvironment are inhibited.[40] The clinical effects are currently under evaluation in a phase II study in relapsed/refractory myeloma patients (NCT01478581). No clinical efficacy data has been reported, only a reduction in certain cytokines involved in myeloma bone disease.[41]

Activin A was recently identified as a treatment target for myeloma bone disease due to its ability to activate osteoclasts and probably to inhibit osteoblasts.[42] Sotatercept is a novel fusion protein working as a soluble trap of activin receptor IIA ligands, and in the pipeline for treatment of cancer-induced anaemia.[43] It has also been evaluated in the relapsed/refractory multiple myeloma in combination with standard chemotherapy (MPT).[44] However, current ongoing studies are only evaluating the

anti-anaemia effect (clinicaltrials.gov).[45]

Pre-clinical testing of a CCR1 inhibitor (CCX721) in myeloma bone disease, working through inhibition of one of two MIP1alfa receptors blocking osteoclast function, show similar results as zoledronic acid in a mouse model, and showed efficacy in both a prophylactic and treatment setting. The human analogue, CCX354-C, is currently under clinical testing in a phase II setting in rheumatoid arthritis (NCT01242917, clinicaltrials.gov).

Osteoblast Targeting

The proteasome inhibitor bortezomib has shown bone anabolic effects in *in vitro* studies. Casuistic reports of bone healing on sensitive imaging have been reported in bortezomib treated patients, but no statistically significant differences in bone related clinical end-points have been reported from the VISTA trial comparing MP with bortezomib-MP in the elderly.[46] Increased levels of serum bone formation markers were observed in bortezomib treated patients in the VISTA trial. However, apparently the increase in bone formation markers is only transient, and returns to baseline within 3 to 4 months of initiation of treatment.[46, 47]

DKK1, known since the studies by Tian *et al.*[18] to be involved in myeloma bone disease, is under evaluation as a therapeutic target in multiple myeloma. An anti-DKK1 antibody, shown to affect bone metabolism in a mouse model,[48, 49] but with variable effects on patient derived osteoclasts *in vitro*,[50] is in clinical test in the relapsed/refractory myeloma setting (NCT00741377), in high risk smouldering myeloma (NCT01302886), and as first-line treatment for lytic bone disease in patients ineligible for zoledronate treatment due to renal failure (NCT01337752). Data from the high-risk smouldering myeloma study has been reported with no observed anti-myeloma effect, no observed differences in DXA scans, but with an observed 3% increase in bone strength measured by quantitative compound tomography with finite element analysis (qCT with FEA).[51] An anti-sclerostin antibody (AMG785) showing promising results in the phase I study,[52] is under evaluation for efficacy on fracture healing in a phase II study in a non-myeloma population (NCT01081678). Evaluation of the drug in multiple myeloma raises concerns due to potential wnt-driven survival mechanisms in the myeloma plasma cells,[53] and currently there are no myeloma studies.

The HGF/c-Met pathway may be a potential target for intervention in myeloma bone disease. Several drugs targeting the HGF/c-Met-pathway are being evaluated in solid tumours (for review see [54]), as this pathway seems to control the "invasion and metastasis" hallmark of cancer.[55] So far, only one phase II study of single-agent ARQ-197 (Tivantinib) has been reported in multiple myeloma, being well-tolerated and inducing stable disease as the best response in 63% of the evaluable patients with relapsed myeloma.[56]

In conclusion, a lot of drugs are in the pipeline for treatment of myeloma bone disease challenging the current bisphosphonate treatment of myeloma lytic bone disease. Historically, fewer than 10 percent of agents entering phase I testing in cancer ends up with FDA approval.[57] As with anti-myeloma therapy in general the challenge is now for the clinicians to design appropriate studies, well-powered to reveal actual treatment responses even in subsets of patients and to evaluate predictive markers in a prospective setting. In choosing drugs for further investigation, it is important to consider how essential the target is (to identify "drivers" and not just "passengers") and in which patient subsets the drug would be most likely have an effect. Relevant *in vitro* screening methods including stromal cells (e.g. as described by Misund *et al.*[58]) should be further evaluated and studies including analyses of the importance of hereditary factors in evaluation of treatment responses[59] should also be included.

References

1. Kyle RA, Gertz MA, Witzig TE *et al.* Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin.Proc. 2003;78:21-33.
2. Morgan GJ, Davies FE, Gregory WM *et al.* First-line treatment with zoledronic acid as compared with clodronic acid in multiple myeloma (MRC Myeloma IX): a randomised controlled trial. Lancet 2010;376:1989-1999.
3. Morgan GJ, Child JA, Gregory WM *et al.* Effects of zoledronic acid versus clodronic acid on skeletal morbidity in patients with newly diagnosed multiple myeloma (MRC Myeloma IX): secondary outcomes from a randomised controlled trial. Lancet Oncol. 2011;12:743-752.
4. Morgan GJ, Davies FE, Gregory WM *et al.* Effects of induction and maintenance plus long-term bisphosphonates on bone disease in patients with multiple myeloma: the Medical Research Council Myeloma IX Trial. Blood 2012;119:5374-5383.
5. Baron R, Rawadi G. Targeting the Wnt/beta-catenin pathway to regulate bone formation in the adult skeleton. Endocrinology 2007;148:2635-2643.
6. Crockett JC, Rogers MJ, Coxon FP, *et al.* Bone remodelling at a glance. J.Cell Sci. 2011;124:991-998.
7. Westendorf JJ, Kahler RA, Schroeder TM. Wnt signaling in osteoblasts and bone diseases. Gene 2004;341:19-39.
8. Bataille R, Chappard D, Marcelli C *et al.* Recruitment of new osteoblasts and osteoclasts is the earliest critical event in the pathogenesis of human multiple myeloma. J.Clin.Invest 1991;88:62-66.
9. Andersen TL, Boissy P, Sondergaard TE *et al.* Osteoclast nuclei of myeloma patients show chromosome translocations specific for the myeloma cell clone: a new type of cancer-host partnership? J.Pathol. 2007;211:10-17.
10. Abildgaard N, Nielsen JL, Heickendorff L. Connective tissue components in serum in multiple myeloma: analyses of propeptides of type I and type III procollagens, type I collagen telopeptide, and hyaluronan. Am.J.Hematol. 1994;46:173-178.
11. Schmiedel BJ, Scheible CA, Nuebling T *et al.* RANKL Expression, Function, and Therapeutic Targeting in Multiple Myeloma and Chronic Lymphocytic Leukemia. Cancer Res 2013;73:683-694.
12. Yasuda H, Shima N, Nakagawa N *et al.* Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc.Natl.Acad.Sci.U.S.A 1998;95:3597-3602.
13. Seidel C, Hjertner O, Abildgaard N *et al.* Serum osteoprotegerin levels are reduced in patients with multiple myeloma with lytic bone disease. Blood 2001;98:2269-2271.
14. Ng AC, Khosla S, Charatcharoenwitthaya N *et al.* Bone microstructural changes revealed by high-resolution peripheral quantitative computed tomography imaging and elevated DKK1 and MIP-1alpha levels in patients with MGUS. Blood 2011;118:6529-6534.
15. Terpos E, Politou M, Szydlo R *et al.* Serum levels of macrophage inflammatory protein-1 alpha (MIP-1alpha) correlate with the extent of bone disease and survival in patients with multiple myeloma. Br.J.Haematol. 2003;123:106-109.
16. Roussou M, Tasidou A, Dimopoulos MA *et al.* Increased expression of macrophage inflammatory protein-1alpha on trephine biopsies correlates with extensive bone disease, increased angiogenesis and advanced stage in newly diagnosed patients with multiple myeloma. Leukemia 2009;23:2177-2181.

17. Haaber J, Abildgaard N, Knudsen LM *et al*. Myeloma cell expression of 10 candidate genes for osteolytic bone disease. Only overexpression of DKK1 correlates with clinical bone involvement at diagnosis. Br.J.Haematol. 2008;140:25-35.
18. Tian E, Zhan F, Walker R *et al*. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N.Engl.J.Med. 2003;349:2483-2494.
19. Zhan F, Hardin J, Kordsmeier B *et al*. Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. Blood 2002;99:1745-1757.
20. Giuliani N, Morandi F, Tagliaferri S *et al*. Production of Wnt inhibitors by myeloma cells: potential effects on canonical Wnt pathway in the bone microenvironment. Cancer Res. 2007;67:7665-7674.
21. Terpos E, Christoulas D, Katodritou E *et al*. High Serum Sclerostin Correlates with Advanced Stage, Increased Bone Resorption, Reduced Osteoblast Function, and Poor Survival in Newly-Diagnosed Patients with Multiple Myeloma. [abstract]. Blood 2009;114:
22. Mahtouk K, Tjin EP, Spaargaren M, *et al*. The HGF/MET pathway as target for the treatment of multiple myeloma and B-cell lymphomas. Biochim.Biophys.Acta 2010;1806:208-219.
23. Standal T, Abildgaard N, Fagerli UM *et al*. HGF inhibits BMP-induced osteoblastogenesis: possible implications for the bone disease of multiple myeloma. Blood 2007;109:3024-3030.
24. Seidel C, Borset M, Turesson I *et al*. Elevated serum concentrations of hepatocyte growth factor in patients with multiple myeloma. The Nordic Myeloma Study Group. Blood 1998;91:806-812.
25. Andersen NF, Standal T, Nielsen JL *et al*. Syndecan-1 and angiogenic cytokines in multiple myeloma: correlation with bone marrow angiogenesis and survival. Br.J.Haematol. 2005;128:210-217.
26. Kristensen IB, Haaber J, Lyng MB *et al*. Myeloma plasma cell expression of osteoblast regulatory genes: overexpression of SFRP3 correlates with clinical bone involvement at diagnosis. Leuk.Lymphoma 2013;54:425-427.
27. Rocci A, Gambella M, Trusolino L *et al*. Expression of MET mRNA as prognostic marker in multiple myeloma patients treated with novel drugs [abstract]. Haematologica 2010;95:134.
28. Wader KF, Fagerli UM, Borset M *et al*. Immunohistochemical analysis of hepatocyte growth factor and c-Met in plasma cell disease. Histopathology 2012;60:443-451.
29. Seidel C, Borset M, Hjertner O *et al*. High levels of soluble syndecan-1 in myeloma-derived bone marrow: modulation of hepatocyte growth factor activity. Blood 2000;96:3139-3146.
30. Seidel C, Sundan A, Hjorth M *et al*. Serum syndecan-1: a new independent prognostic marker in multiple myeloma. Blood 2000;95:388-392.
31. Kristensen IB, Christensen JH, Lyng MB *et al*.: HGF pathway upregulation in the bone marrow microenvironment in multiple myeloma is associated with lytic bone disease. Br.J.Haematol. In press.
32. Ramani VC, Yang Y, Ren Y, *et al*. Heparanase plays a dual role in driving hepatocyte growth factor (HGF) signaling by enhancing HGF expression and activity. J.Biol.Chem. 2011;286:6490-6499.
33. Yang Y, Ren Y, Ramani VC *et al*. Heparanase enhances local and systemic osteolysis in multiple myeloma by upregulating the expression and secretion of RANKL. Cancer Res. 2010;70:8329-8338.
34. Nan L, Chen H, Ruan J *et al*. Runx2 Transcription Factor Regulates Heparanase-Induced Bone Resorption in Multiple Myeloma. ASH Annual Meeting Abstracts 2012;120:567.
35. Todoerti K, Lisignoli G, Storti P *et al*. Distinct transcriptional profiles characterize bone microenvironment mesenchymal cells rather than osteoblasts in relationship with multiple myeloma bone disease. Exp.Hematol. 2010;38:141-153.
36. Edwards CM, Zhuang J, Mundy GR. The pathogenesis of the bone disease of multiple myeloma. Bone 2008
37. Raje N, Vescio R, Montgomery CW *et al*. Bone Marker-Directed Dosing of Zoledronic Acid for the Prevention of Skeletal Complications in Patients with Multiple Myeloma: Final Results of the Z-MARK Study. ASH Annual Meeting Abstracts 2012;120:4077.
38. Garcia-Sanz R, Oriol A, De La Rubia J *et al*. Analysis of Zoledronic ACID Therapy for Patients with Multiple Myeloma with Asymptomatic Biochemical Relaps. ASH Annual Meeting Abstracts 2012;120:2967.
39. Henry DH, Costa L, Goldwasser F *et al*. Randomized, double-blind study of denosumab versus zoledronic acid in the treatment of bone metastases in patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma. J.Clin.Oncol. 2011;29:1125-1132.
40. Tai YT, Chang BY, Kong SY *et al*. Bruton tyrosine kinase inhibition is a novel therapeutic strategy targeting tumor in the bone marrow microenvironment in multiple myeloma. Blood 2012;120:1877-1887.
41. Vij R, Chang BY, Berdeja JG *et al*. Early Changes in Cytokines, Chemokines and Indices of Bone Metabolism in a Phase 2 Study of the Bruton Tyrosine Kinase (Btk) Inhibitor, Ibrutinib (PCI-32765) in Patients with Relapsed or Relapsed/Refractory Multiple Myeloma (MM). ASH Annual Meeting Abstracts 2012;120:4039.
42. Vallet S, Mukherjee S, Vaghela N *et al*. Activin A promotes multiple myeloma-induced osteolysis and is a promising target for myeloma bone disease. Proceedings of the National Academy of Sciences 2010;107:5124-5129.
43. Fields SZ, Parshad S, Anne M *et al*. Activin receptor antagonists for cancer-related anemia and bone disease. Expert.Opin.Investig.Drugs 2013;22:87-101.
44. Abdulkadyrov KM, Salogub GN, Khuazheva NK *et al*. ACE-011, a Soluble Activin Receptor Type Iia IgG-Fc Fusion Protein, Increases Hemoglobin (Hb) and Improves Bone Lesions in Multiple Myeloma Patients Receiving Myelosuppressive Chemotherapy: Preliminary Analysis. ASH Annual Meeting Abstracts 2009;114:749.
45. Chen N, Laadem A, Sherman ML *et al*. Exposures and Erythropoietic Responses to Sotatercept (ACE-011) in Healthy Volunteers and Cancer Patients: Implications for Mechanism of Action. ASH Annual Meeting Abstracts 2012;120:3454.
46. Delforge M, Terpos E, Richardson PG *et al*. Fewer bone disease events, improvement in bone remodeling, and evidence of bone healing with bortezomib plus melphalan-prednisone vs. melphalan-prednisone in the phase III VISTA trial in multiple myeloma. Eur.J.Haematol. 2011;86:372-384.
47. Haidl F, Plesner T, Lund T. Treatment with bortezomib in multiple myeloma is associated with only a transient and brief increase of bone specific alkaline phosphatase. Leuk.Res. 2012;36:1296-1299.
48. Fulciniti M, Tassone P, Hideshima T *et al*. Anti-DKK1 mAb (BHQ880) as a potential therapeutic agent for multiple myeloma. Blood 2009;114:371-379.
49. Yaccoby S, Ling W, Zhan F *et al*. Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. Blood 2007;109:2106-2111.
50. Pozzi S, Fulciniti M, Yan H *et al*. In vivo and in vitro effects of a novel anti-Dkk1 neutralizing antibody in multiple myeloma. Bone
51. Munshi NC, Abonour R, Beck JT *et al*. Early Evidence of Anabolic Bone Activity of BHQ880, a Fully Human Anti-DKK1 Neutralizing Antibody: Results of a Phase 2 Study in Previously Untreated Patients with Smoldering Multiple Myeloma At Risk for Progression. ASH Annual Meeting Abstracts 2012;120:331.
52. Padhi D, Jang G, Stouch B, *et al*. Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. J Bone Miner Res 2011;26:19-26.
53. Sukhdeo K, Mani M, Zhang Y *et al*. Targeting the beta-catenin/TCF transcriptional complex in the treatment of multiple myeloma. Proc.Natl.Acad. Sci.U.S.A 2007;104:7516-7521.
54. Blumenschein GR, Jr., Mills GB, Gonzalez-Angulo AM. Targeting the hepatocyte growth factor-cMET axis in cancer therapy. J.Clin.Oncol. 2012;30:3287-3296.
55. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-674.
56. Dorkhom SJ, Zaman S, Thomas SK *et al*. Phase II Study of the c-Met Inhibitor ARQ 197 (Tivantinib) in Patients with Relapsed Multiple Myeloma. ASH Annual Meeting Abstracts 2012;120:2976.
57. Mitsiades CS, Davies FE, Laubach JP *et al*. Future directions of next-generation novel therapies, combination approaches, and the development of personalized medicine in myeloma. J.Clin.Oncol. 2011;29:1916-1923.
58. Misund K, Baranowska KA, Holien T *et al*. A Method for Measurement of Drug Sensitivity of Myeloma Cells Co-Cultured with Bone Marrow Stromal Cells. ASH Annual Meeting Abstracts 2012;120:1373.
59. Vangsted AJ, Klausen TW, Abildgaard N *et al*. Single nucleotide polymorphisms in the promoter region of the IL1B gene influence outcome in multiple myeloma patients treated with high-dose chemotherapy independently of relapse treatment with thalidomide and bortezomib. Ann.Hematol. 2011;90:1173-1181.

Primary and Secondary Prophylaxis of Pregnancy-related Venous Thromboembolism

Valerio De Stefano
Institute of Hematology, Catholic University, Rome

Introduction

During pregnancy, haemostasis is shifted towards hypercoagulability.[1, 2] The annual incidence of venous thromboembolism (VTE) among women of childbearing age is 0.18 per 1,000,[3] which increases to 0.71-1.3 per 1,000 when pregnancy-related.[4-6] The approximately 4-fold increased risk of a first VTE in pregnancy increases up to 14-fold during puerperium,[7] and is further increased in carriers of inherited thrombophilia.[8, 9] Age >35 years and caesarean section are well-established factors which further enhance the thrombotic risk.[10] A recent population-based study has shown that the risk of VTE was increased 5-fold during pregnancy and increased 60-fold during the first 3 months after delivery compared with non-pregnant women.[11]

Primary Antithrombotic Prophylaxis

Previous VTE and thrombophilia are two well-established risk factors for pregnancy-related VTE, as detailed below. However, pregnant women should be assessed for the presence of other additional risk factors for VTE[12, 13] (Table 1). According to the Royal College of Obstetricians and Gynaecologists (RCOG) 2009 Guidelines,[12] any woman with three or more additional risk factors should be considered for antenatal prophylaxis with low molecular weight heparin (LMWH). After delivery, the presence of two or more persisting risk factors, other than previous VTE or thrombophilia, should encourage LMWH prophylaxis for at least 7 days; class-III obesity (BMI >40) or emergency caesarean section are considered sufficient reasons to give postpartum prophylaxis.[12]

In most guidelines, special attention is paid to asymptomatic women with a family history of VTE and/or a familial antithrombin (AT), protein C (PC), protein S (PS) deficiency, homozygous factor V Leiden (FVL) or prothrombin (PT) 20210A, or double heterozygous FVL and PT20210A[12, 14-20] (Table 2). In general, the recommendations for antepartum and postpartum prophylaxis for asymptomatic women with thrombophilia are of low-grade evidence. A meta-analysis reported a risk of pregnancy-related VTE 26.4- and 34.4-fold increased in homozygous FVL or PT20210A, respectively, and 3.2- to 8.3-fold increased in carriers of AT, PC, and PS deficiency, and in heterozygous FVL or PT20210A;[21] considering a baseline VTE incidence of 1.4 per 1,000 pregnancies, the estimated absolute risk of pregnancy-related VTE was 4.8 and 3.7 percent pregnancies in homozygous FVL or PT20210A, respectively, and 0.5 to 1.2 percent pregnancies in the remaining cases (AT, PC, or PS deficiency, or heterozygous FVL or PT20210A).[19] Moreover, the 2012 American College of Chest Physicians (ACCP) Guidelines analysed some family-based cohort studies in which the observed absolute risk of a first pregnancy-related VTE was 14 percent pregnancies in homozygous for FVL, and between 1.7 and 6.6 percent pregnancies in the remaining cases.[19]

Antenatal clinical surveillance is suggested in heterozygotes for FVL or PT20210A, due to the low rate of first antepartum VTE[22, 23] (Table 2). In these patients, LMWH should be considered only in the presence of additional risk factors (e.g. family history of VTE, immobility, obesity, age >35 years, gross varicose veins).[12, 14-17, 20]

On the other hand, antenatal LMWH prophylaxis is recommended for carriers of AT deficiency[12, 14-18] or homozygous FVL or PT20210A or carriers of multiple thrombophilia abnormalities[12, 14-18] even in the

Valerio De Stefano is Full Professor of Haematology at the Catholic University in Rome, and is Director and Head Physician of the Institute of Hematology of the Academic Hospital "Agostino Gemelli" in Rome, Italy. His main research interests relate to epidemiology, pathogenetic basis, clinical manifestations and treatment of thrombophilic syndromes and Philadelphia-negative chronic myeloproliferative neoplasms. Dr. De Stefano has been actively involved in the development of National Guidelines formulation on women's health issues such as prevention of venous thromboembolism due to pregnancy (produced by the Italian Society on Haemostasis and Thrombosis, SISET) and oral contraception (produced by the National Institute of Health). He has served as a member of the Executive Committee of the SISET (2000-2002 and 2010-2012) and as a member of the Chronic Myeloproliferative Neoplasms Board of the Italian Group for Adult Hematologic Diseases (GIMEMA) (2007-2009). Currently, he is the Chairman of the Hemostasis and Thrombosis Working Party of the GIMEMA. He has also served as a member of the Advisory or Editorial Board of peer-reviewed journals as *Hematologica*, *Journal of Thrombosis and Haemostasis*, *Thrombosis* and *Haemostasis*. He has authored 200 full-papers in peer-reviewed international journals.

Risk factor for VTE	Adjusted OR	95% CI
Previous VTE	24.8	17.1 – 36
Immobility If combined with BMI > 25	7.7 62	3.2 – 19
BMI >30	5.3	2.1 – 13.5
Smoking	2.7	1.5 – 4.9
Weight gain >21 Kg (vs 7-21 Kg)	1.6	1.1 – 2.6
Parity >1	1.5	1.1 – 1.9
Age >35 years	1.3	1.0 – 1.7
Preeclampsia	3.1	1.8 – 5.3
Preeclampsia with fetal growth restriction	5.8	2.1 – 16
Assisted reproductive techniques	4.3	2.0 – 9.4
Twin pregnancy	2.6	1.1 – 6.2
Antepartum haemorrhage	2.3	1.8 – 2.8
Postpartum haemorrhage	4.1	2.3 – 7.3
Cesarean section	3.6	3.0 – 4.3
Medical conditions such as systemic lupus erythematosus, heart disease, anemia, active infection, or varicose veins	2.0 – 8.7	
Blood transfusion	7.6	6.2 – 9.4

Table 1. Risk factors other than thrombophilia and their odds ratio (OR) for pregnancy-related VTE, reproduced from Greer IA, "Thrombosis in pregnancy: updates in diagnosis and management", Hematology Am. Soc. Hematol. Educ. Program, (2012) ; 2012: 203-207.

absence of additional risk factors. Some guidelines suggest antenatal LMWH prophylaxis in women with a PC or PS deficiency,[14, 16, 18] whereas others judge clinical surveillance to be sufficient in the absence of additional risk factors.[12, 15, 17, 20]

After delivery, LMWH prophylaxis is recommended for 6 weeks in all carriers of inherited thrombophilia.[14-18, 20] For asymptomatic women with PC or PS deficiency or heterozygous FVL or PT20210A, some guidelines suggest LMWH prophylaxis after delivery for at least 7 days,[12] extended for 6 weeks only in cases with additional risk factors.[12, 18]

In contrast with the aforementioned recommendations, the recent 2012 ACCP Guidelines suggest antenatal clinical surveillance in almost all asymptomatic women with inherited thrombophilia, considering antenatal LMWH prophylaxis only for homozygotes for FVL or PT20210A with a family history of VTE. LMWH for 6 weeks after delivery is suggested for homozygous FVL or PT20210A carriers, regardless of the family history, and for women with the other inherited thrombophilias if they have a family history of VTE; in such cases (except women with a PC or PS deficiency), anti-vitamin K agents targeted at INR range from 2.0 to 3.0 are also suggested.[19]

Secondary Antithrombotic Prophylaxis

In the 2012 ACCP Guidelines, the recommendation for antenatal prophylaxis of recurrent VTE was driven by the circumstances of the first event, suggesting clinical surveillance for pregnant women with a single episode of VTE associated with a non-hormonal transient risk factor (independently of the presence of thrombophilia), and antenatal prophylaxis with LMWH for those with a previous single unprovoked, oestrogen- or pregnancy-related VTE or multiple episodes of VTE.[19]

The lack of importance given to thrombophilia as a risk factor for pregnancy-related recurrent VTE in women with previous provoked VTE is consistent with some guidelines,[24, 25] but not with others.[12, 16, 18, 20] Unfortunately evidences on this issue are scarce. The rate of antenatal recurrent VTE in the absence of antithrombotic prophylaxis in one prospective study that excluded women with known thrombophilia was 2.4%,[26] and in two large retrospective studies was 4% and 5.8%, respectively.[27, 28] In the latter studies, women with a previous oestrogen- or pregnancy-related VTE had a rate of antenatal recurrent VTE higher than that of those with a previous unprovoked or non-hormonal provoked VTE (9.5-10% vs 2.7-4.2%). In three such studies, no thrombophilia was found in the unique woman out of the pooled 106 cases, with a first non-hormonal provoked VTE who had recurrent VTE,[26-28] supporting the 2012 ACCP Guidelines. Indeed, the paucity of the overall cases analysed renders a firm recommendation of not administering antithrombotic prophylaxis during pregnancy to women with thrombophilia and a previous non-hormonal provoked VTE problematic. At least, the risk associated with thrombophilia should be weighed as for women without history of VTE.

Finally, a family history of VTE has been consistently reported as a risk factor for VTE in both the general population and in the carriers of thrombophilia.[29-32] In 2009 the RCOG Guidelines recommended antenatal prophylaxis with LMWH for all women with previous VTE and history of VTE in a first-degree relative.[12] However, it should be kept in

	Antenatal prophylaxis with LMWH	Postpartum prophylaxis with LMWH for 6 weeks
International Consensus Statement, 2005[14]	AT or PC or PS deficiency Heterozygous FVL or PT20210A § Multiple abnormalities or homozygotes	AT or PC or PS deficiency Heterozygous FVL or PT20210A Multiple abnormalities and homozygotes
Pregnancy and Thrombosis Working Group, 2007[15]	AT deficiency PC or PS deficiency § Heterozygous FVL or PT20210A § Multiple abnormalities or homozygotes	The consensus panel did not make a formal recommendation.
Royal College of Obstetricians and Gynaecologists (RCOG), 2009[12]	AT deficiency PC or PS deficiency § Heterozygous FVL or PT20210A § Multiple abnormalities or homozygotes	AT deficiency PC or PS deficiency § Heterozygous FVL or PT20210A § Multiple abnormalities or homozygotes In women with a PC or PS deficiency or heterozygous for FVL or PT20210A without family history of VTE or other risk factors, duration of prophylaxis can be 7 days.
Italian Society for Haemostasis and Thrombosis (SISET), 2009[16]	AT or PC or PS deficiency Heterozygous FVL or PT20210A § Multiple abnormalities or homozygotes	AT or PC or PS deficiency Heterozygous FVL or PT20210A Multiple abnormalities or homozygotes
Scottish Intercollegiate Guidelines Network (SIGN), 2010[17]	AT deficiency PC or PS deficiency ¶ Heterozygous FVL or PT20210A ¶ Multiple abnormalities Homozygous FVL	AT or PC or PS deficiency Heterozygous FVL or PT20210A Multiple abnormalities or homozygotes
American College of Obstetricians and Gynecologists (ACOG), 2011[18]	AT deficiency PC or PS deficiency Heterozygous FVL or PT20210A Multiple abnormalities or homozygotes In women with a PC or PS deficiency or heterozygous for FVL or PT20210A surveillance without anticoagulation can be an alternative	AT deficiency PC or PS deficiency § Heterozygous FVL or PT20210A § Multiple abnormalities or homozygotes
American College of Chest Physicians (ACCP) Guidelines, 2012[19]	Homozygous FVL or PT20210A ¶	AT or PC or PS deficiency ¶ Heterozygous FVL or PT20210A ¶ Multiple abnormalities ¶ Homozygous FVL or PT20210A Anti-vitamin K agents (INR 2.0 to 3.0) can be an alternative (except for women with a PC or PS deficiency)
Society of Obstetric Medicine of Australia and New Zealand (SOMANZ) and Australasian Society of Thrombosis and Haemostasis (ASTH), 2012[20]	AT deficiency § PC or PS deficiency § Heterozygous FVL or PT20210A ₸ Multiple abnormalities § Homozygous FVL §	AT or PC or PS deficiency Heterozygous FVL or PT20210A Multiple abnormalities Homozygous FVL

Table 2. Guidelines for prophylaxis during pregnancy and puerperium in asymptomatic women with inherited thrombophilia.
§ antithrombotic prophylaxis is recommended only if family history of VTE or other risk factors are present.
¶ antithrombotic prophylaxis is recommended only if family history of VTE is present.
₸ antithrombotic prophylaxis is recommended only if other risk factors are present.
LMWH: low molecular weight heparin¸ AT: antithrombin; PC: protein C; PS: protein S; FVL: factor V Leiden; PT: prothrombin.

mind that sensitivity of family history (presence of disease in relatives of the informant) is less accurate than specificity (absence of disease), and that accuracy is higher for information related to first-degree relatives than more distant relatives.[33]

In contrast with other recommendations,[12, 16, 18, 20] the family history of VTE is the only risk factor considered by the 2012 ACCP Guidelines that can discriminate the management of pregnant women[19] (Table 2). However, the aforementioned limitations of family history could produce an underestimation of risk, failing to report VTE events occurred in relatives. Moreover, the magnitude of increased risk due to family history of VTE appears quite similar to that reported for other risk factors such as obesity, varicose veins, hyperemesis, or lower than that reported for immobility (7-fold increased) (Table 1), so that it appears contradictory emphasising it as the only risk factor to be considered for making decisions in women with thrombophilia abnormalities other than homozygous FVL and PT20210A, without first considering other risk factors.

Finally, all women with previous VTE should be offered antithrombotic prophylaxis for 6 weeks after delivery, independently of the circumstances of the first VTE.[16-20]

However, the efficacy of prophylaxis with LMWH has been recently debated, reporting in a cohort of 85 women with previous VTE and receiving low-dose LMWH (nadroparin 2850 IU) during their first pregnancy after VTE one antepartum recurrence (1.1%) and 4 postpartum recurrences (4.7%).[34] On the other hand, in a nationwide prospective study using body weight-adjusted LMWH in 326 pregnant women, only two antepartum (0.6 %) and two postpartum (0.6%) recurrences were recorded.[35] In a retrospective cohort of 84 women with a history of DVT, 40 received antenatal b.w.-adjusted LMWH (nadroparin/enoxaparin 3800/4000 IU for b.w. <70 kg or 5700/6000 IU o.d. for b.w. >70 kg). The rate of ante-partum recurrent VTE was significantly reduced by 86% among the women receiving prophylaxis in comparison with women not prophylaxed (2.5% vs. 13.6%).[36]

References

1. Kjellberg U, Andersson NE, Rosen S, *et al*., "APC resistance and other haemostatic variables during pregnancy and puerperium", Thromb. Haemost. (1999), 81: pp. 527-531
2. Hellgren M. "Hemostasis during normal pregnancy and puerperium", Semin. Thromb. Hemost. (2003), 29: pp. 125-130.
3. Nordstrom M, Lindblad B, Bergqvist D, *et al*., "A prospective study of the incidence of deep-vein thrombosis within a defined urban population", J. Intern. Med. (1992), 232: pp. 155-160.
4. McColl MD, Ramsay JE, Tait RC, *et al*., "Risk factors for pregnancy associated venous thromboembolism", Thromb. Haemost. (1997), 78:1183-1188.
5. Andersen BS, Steffensen FH, Sorensen HT, *et al*. "The cumulative incidence of venous thromboembolism during pregnancy and puerperium - an 11 year Danish population-based study of 63,300 pregnancies", Acta Obstet. Gynecol. Scand. (1998), 77: pp. 170-173.
6. Lindqvist P, Dahlback B, Marsal K, "Thrombotic risk during pregnancy: a population study", Obstet. Gynecol. (1999), 94: pp. 595-599.
7. Rosendaal FR, "Risk factors for venous thrombotic disease", Thromb. Haemost. (1999), 82: 610-619.
8. Martinelli I, Legnani C, Bucciarelli P, *et al*. "Risk of pregnancy-related venous thrombosis in carriers of severe inherited thrombophilia", Thromb. Haemost. (2001), 86: 800-803.
9. Martinelli I, De Stefano V, Taioli E, *et al*. "Inherited thrombophilia and first venous thromboembolism during pregnancy and puerperium", Thromb. Haemost. (2002), 87: pp. 791-795.
10. Greer IA, "Thrombosis in pregnancy:maternal and fetal issues", Lancet (1999); 353: pp. 1258–1265.
11. Pomp ER, Lenselink AM, Rosendaal FR, *et al*., "Pregnancy, the postpartum period and prothrombotic defects: risk of venous thrombosis in the MEGA study", J. Thromb. Haemost. (2008), 6: pp. 632 - 637 .
12. Royal College of Obstetricians and Gynaecologists, . "Thrombosis and embolism during pregnancy and the puerperium. Reducing the risk", Green-top Guideline No. 37a (2009), www.rcog.org.uk.
13. Greer IA, "Thrombosis in pregnancy: updates in diagnosis and management", Hematology Am. Soc. Hematol. Educ. Program, (2012), 2012: pp. 203-207.
14. European Genetics Foundation; Cardiovascular Disease Educational and Research Trust; International Union of Angiology; *et al*., "Thrombophilia and venous thromboembolism. International consensus statement. Guidelines according to scientific evidence", Int. Angiol. (2005), 24: pp. 1-26.
15. Duhl AJ, Paidas MJ, Ural SH, *et al*.; Pregnancy and Thrombosis Working Group. "Antithrombotic therapy and pregnancy: consensus report and recommendations for prevention and treatment of venous thromboembolism and adverse pregnancy outcomes", Am. J. Obstet. Gynecol. (2007), 197: pp. 457.e1-21.
16. Lussana F, Dentali F, Abbate R, *et al*.; Italian Society for Haemostasis and Thrombosis, "Screening for thrombophilia and antithrombotic prophylaxis in pregnancy: Guidelines of the Italian Society for Haemostasis and Thrombosis (SISET)", Thromb. Res. (2009), 124: pp. e19-25.
17. Scottish Intercollegiate Guidelines Network, "Prevention and management of venous thromboembolism. A national clinical guideline", SIGN publication no. 122 (2010), www.sign.ac.uk.
18. Lockwood C, Wendel G; Committee on Practice Bulletins— Obstetrics, "Practice bulletin no. 124: inherited thrombophilias in pregnancy", Obstet. Gynecol. (2011), 118: pp. 730-740.
19. Bates SM, Greer IA, Middeldorp S, *et al*.; American College of Chest Physicians, "VTE, thrombophilia, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines", Chest (2012), 141(2 Suppl): pp. e691S-736S.
20. McLintock C, Brighton T, Chunilal S, *et al*.; Councils of the Society of Obstetric Medicine of Australia and New Zealand; Australasian Society of Thrombosis and Haemostasis, "Recommendations for the prevention of pregnancy-associated venous thromboembolism", Aust. N. Z. J. Obstet. Gynaecol. (2012), 52: pp. 3-13.
21. Robertson L, Wu O, Langhorne P, *et al*.; Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) Study, "Thrombophilia in pregnancy: a systematic review", Br. J. Haematol. (2006), 132: pp. 171-196.
22. Tormene D, De Stefano V, Grandone E, *et al*., "The G20210A prothrombin variant and the risk of venous thromboembolism or fetal loss in pregnant women: a family study", J. Thromb. Haemost. (2007), 5: pp. 2193-2196.
23. Martinelli I, Battaglioli T, De Stefano V, *et al*.; GIT (Gruppo Italiano Trombofilia), "The risk of first venous thromboembolism during pregnancy and puerperium in double heterozygotes for factor V Leiden and prothrombin G20210A", J. Thromb. Haemost. (2008), 6: pp. 494-498.
24. Baglin T, Gray E, Greaves M, *et al*.; British Committee for Standards in Haematology, "Clinical guidelines for testing for heritable thrombophilia", Br. J. Haematol. (2010), 149: pp. 209-220.
25. Scottish Intercollegiate Guidelines Network, "Prevention and management of venous thromboembolism. A national clinical guideline", SIGN publication no. 122, (2010), www.sign.ac.uk.
26. Brill-Edwards P, Ginsberg JS, Gent M, *et al*.; Recurrence of Clot in This Pregnancy Study Group. "Safety of withholding heparin in pregnant women with a history of venous thromboembolism. Recurrence of Clot in This Pregnancy Study Group", N. Engl. J. Med. (2000), 343: pp. 1439-1444.
27. Pabinger I, Grafenhofer H, Kaider A, *et al*., "Risk of pregnancy-associated recurrent venous thromboembolism in women with a history of venous thrombosis", J. Thromb. Haemost. (2005), 3: pp. 949-954.
28. De Stefano V, Martinelli I, Rossi E, *et al*., "The risk of recurrent venous thromboembolism in pregnancy and puerperium without antithrombotic prophylaxis", Br. J. Haematol. (2006), 135: pp. 386-391.
29. Bezemer ID, van der Meer FJ, Eikenboom JC, *et al*, "The value of family history as a risk indicator for venous thrombosis", Arch. Intern. Med. (2009), 169: pp. 610-615.
30. Zöller B, Li X, Sundquist J, *et al*, "Parental history and venous thromboembolism: a nationwide study of age-specific and sex-specific familial risks in Sweden", J. Thromb. Haemost. (2011), 9: pp. 64-70.
31. Sørensen HT, Riis AH, Diaz LJ, *et al*, "Familial risk of venous thromboembolism: a nationwide cohort study", J. Thromb. Haemost. (2011), 9: pp. 320-324.
32. Rossi E, Ciminello A, Za T, *et al*., "In families with inherited thrombophilia the risk of venous

thromboembolism is dependent on the clinical phenotype of the proband", Thromb. Haemost. (2011), 106: pp. 646–654.
33. Wilson BJ, Qureshi N, Santaguida P, *et al*., "Systematic review: family history in risk assessment for common diseases", Ann. Intern. Med., (2009), 151: pp. 878-885.
34. Roeters van Lennep JE, Meijer E, Klumper FJ, *et al*, "Prophylaxis with low-dose low-molecular-weight heparin during pregnancy and postpartum: is it effective?", J. Thromb. Haemost. (2011), 9: pp. 473-80.
35. Lindqvist PG, Bremme K, Hellgren M; Working Group on Hemostatic Disorders (Hem-ARG), Swedish Society of Obstetrics and Gynecology, "Efficacy of obstetric thromboprophylaxis and long-term risk of recurrence of venous thromboembolism", Acta Obstet. Gynecol. Scand. (2011), 90: pp. 648-53.
36. De Stefano V, Betti S, Za T, *et al*., "Efficacy of low molecular weight heparin in preventing pregnancy-related recurrent venous thromboembolism", Haematologica (2012), 97 (suppl 1): p. 190 (abstract).

CAMBRIDGE
RESEARCH CENTRE

Sign-up now for our FREE monthly eNewsletter

www.cambridgeresearchcentre.co.uk

Hepatic Veno-occlusive Disease Following Haematopoietic Cell Transplantation: Pathogenesis, Diagnosis, Risk Factors, Prophylaxis and Treatment

Enric Carreras[1, 2]
1.Senior Consultant, Hospital Clinic Barcelona; 2. Director, Spanish Bone Marrow Donor Program / José Carreras Foundation, Barcelona

Introduction

Veno-occlusive disease of the liver (VOD) was first described in patients who drank infusions made with plants containing pyrrolizidine alkaloids. Later, VOD was also observed in association with other pathogenic factors including alcohol, contraceptives, toxic oil, liver radiation and antineoplastic drugs.[1] The first case of VOD after haematopoietic cell transplantation (HCT) was reported in 1979.[2] Since then, HCT has been identified as the main cause of VOD, and VOD is one of the leading causes of morbidity and mortality after HCT.[3-5]

Pathogenesis

Experimental models show that the first events after endothelial injury caused by toxic metabolites are loss of fenestrae in sinusoidal endothelial cells (SEC), formation of gaps within and between SEC and rounding up or swelling of SEC. This results in subendothelial oedema, extravasation of red blood cells into the space of Disse and dissection of the sinusoidal lining, which embolises downstream and blocks the sinusoids, reducing the hepatic venous outflow and producing post-sinusoidal hypertension. There is also fibrin deposition and expression of factor VIII and Von Willebrand factor (vWF) within venular walls and liver sinusoids. Based on all these observations, some authors have proposed the term "sinusoidal obstruction syndrome" for this complication. In severe cases of VOD, these initial processes are followed by the appearance of progressive collagen deposition and sinusoidal fibrosis, which contribute to portal hypertension, hepatocellular necrosis and liver failure.[6-10]

The characteristics of hepatic metabolism of drugs explain why the predominant liver damage occurs around centrilobular veins. Some drugs (e.g., cyclophosphamide [Cy]) are metabolised by the cytochrome P450 enzymatic system, producing several toxic metabolites (e.g., acrolein). These toxic metabolites are converted into stable (non-toxic) metabolites by the glutathione (GSH) enzymatic system and then eliminated. When this process occurs in patients with reduced GSH activity caused by previous liver disease or by the action of agents such as busulfan (Bu), BCNU, or total body irradiation (TBI), which consume GSH, the toxic metabolites are not metabolised. As zone 3 of the hepatic acinus surrounds the centrilobular veins and is very rich in P450 and poor in glutathione, toxic metabolites mainly accumulate in this area, which leads to it suffering the most damage.[6-8]

Clinical Features

Clinical manifestations of VOD are characteristic but indistinguishable from those produced by other morphological changes of zone 3 of the liver acinus. For this reason, in the HCT setting, the term VOD is used to designate a clinical syndrome of painful liver enlargement, weight gain and jaundice appearing in the first 35–40 days after HCT, whenever other possible causes of these clinical manifestations have been excluded.[11-12]

The onset of classical VOD usually occurs within the first 20 days after transplantation, but some cases appear later (up to day 40–60). These cases are usually seen after conditioning regimens that include multiple alkylating agents (e.g., busulfan, melphalan, thiotepa).[13] Weight gain, a consequence of hepato-renal syndrome, is usually the first symptom. In the following days, some patients develop oedema (60–70%) and ascites (20–25%) to a variable degree. Simultaneously, most patients present with hepatomegaly (occasionally demonstrated by image studies) and liver tenderness. Several days later, jaundice is observed in almost all adult patients.[3-5] In children, it is not uncommon to see no jaundice or a late onset of symptoms (in 32% and 28% of cases, respectively, in a large recent series).[14] Most patients with severe VOD show a rapid consumption of transfused platelets. Patients with severe VOD can develop progressive multi-organ failure (MOF), with renal insufficiency being the most frequent manifestation.[3, 4]

Diagnosis

As for any syndrome, the diagnosis of VOD must be established clinically. All HCT teams use one of the following two sets of clinical criteria.[11, 15]

• Seattle criteria: In the first 20 days after HCT, two or more of the following: bilirubin > 2 mg/dL (> 34 µmol/L); hepatomegaly or pain in the right upper quadrant; weight gain (> 2% basal weight).

Risk	Lower < Higher
Transplant	
Transplant type	syngeneic or autologous < allogeneic
Donor's type	sibling < another relative < unrelated
HLA compatibility	HLA match < any mismatch
Stem cell origin	peripheral blood < bone marrow
T-cell depletion	with TCD < without TCD
Diagnosis	non malignant disease < malignant disease
Status of the disease	remission < relapse
Transplant number	first < second
Hepatotoxic drugs	progestogens, ketoconazole, CsA, methotrexate, amphotericin B, vancomycin, acyclovir, IVIg
Conditioning	
Intensity	Cy alone < Cy + TBI < CVB
TBI	fractionated TBI < single dose TBI less than 12 Gy < more than 12 Gy low dose rate < high dose rate
Busulfan	IV Bu < adjusted oral Bu < non adjusted oral Bu
Timing	interval Cy – TBI 36 hours < 12 hours
Order of drugs	Cy/Bu < BuCy
Patient	
Age / Sex	younger < older / men < women
Karnofsky index	100 - 90 < lower than 90
ASAT/ALAT before HSCT	normal < high
Previous hepatic irradiation	no < yes
Previous mylotarg	no < yes
Pulmonary function	normal DLCO < reduced DLCO
Status of the liver	normal < fibrosis < cirrhosis or infiltration
CMV serological status	negative < positive
Fever in conditioning	absent < present
Antimicrobial in previous days	no < yes
Genetic predisposition	GSTM1 positive < GSTM1 null genotype

Table 1. Risk factors for VOD. The most important risk factors are indicated in bold type.

• Baltimore criteria: In the first 21 days after HCT, the presence of bilirubin > 2 mg/dL (> 34 µmol/L) plus two or more of the following: painful hepatomegaly, ascites or weight gain (> 5% basal weight).

For both sets of criteria, other possible causes of these clinical features should be excluded (see differential diagnosis).[16] It is also necessary to remember that some cases of VOD can appear late after HCT and that in children bilirubin can be normal: for this reason, paediatricians prefer to use the Seattle criteria.[14]

Other studies that can complement the diagnosis are described below.

Haemodynamic study of the liver carried out via the jugular or femoral veins. Despite its usefulness, it is only indicated to confirm the diagnosis before implementing a therapeutic measure that is potentially hazardous for the patient. A hepatic venous gradient pressure (HVGP) of ≥ 10 mmHg in a patient without previous liver disease allows a precise differential diagnosis with a high degree of specificity, but a normal HVGP does not exclude a diagnosis of this syndrome.[17]

Liver biopsy. The thrombocytopaenia usually present in the early phase after HCT precludes a transparietal liver biopsy. Consequently, hepatic tissue can only be obtained by means of a transvenous biopsy in the course of a haemodynamic study. In addition to the classical histological changes seen with VOD (concentric non-thrombotic narrowing of the lumen of small intrahepatic veins), other less specific abnormalities can be observed (e.g., eccentric narrowing of the venular lumen, phlebosclerosis, sinusoidal fibrosis or hepatocyte necrosis). Because of the patchy nature of VOD, a normal biopsy does not exclude a diagnosis of this syndrome.[12, 17]

Ultrasonography. Several abnormalities can be observed, including gallbladder wall thickening, ascites, hepatomegaly and attenuated or reversed portal flow, but these are all non-specific.[18]

Biological markers. Although patients with VOD show increased serum

levels of plasminogen activator inhibitor-1 (PAI-1) (the marker with the highest specificity and sensitivity for VOD), vWF, thrombomodulin, E-selectin, s-ICAM, aminopropeptides of type III collagen and hyaluronic acid, these are all of little utility in daily clinical practice.[4, 15, 19]

Differential Diagnosis

To accept a diagnosis of VOD, all of the following possible causes should be excluded:[5]

- Infection, including sepsis (cholangitis lenta), fungal infection and viral hepatitis.
- Immune dysfunction, hepatic graft versus host disease (GvHD) or autoimmune hepatitis.
- Drug toxicity, cyclosporine, azoles, methotrexate, progestogens and co-trimoxazole.
- Reduction of venous outflow because of constrictive pericarditis, congestive heart failure, fluid overload or renal failure
- Other causes, including pancreatic or chylous ascites and infiltration of the liver.

Incidence

This has been reported to range from 3% to 54% in the largest series. This variability is a consequence of the presence or absence of the well-known risk factors for this complication (see below). In the only prospective multicentre study published, the incidence of VOD was 8% in cases of allogeneic (allo)-HCT and 3% in cases of autologous HCT.[20] A recent and broad review of the literature suggested that the incidence of VOD has increased over the past 20 years.[21] However, in a large single-centre study focused on adults undergoing myeloablative allo-HCT, the cumulative incidence of VOD decreased from 14% to 8% (applying the Baltimore criteria) over the period of the study. This reduction was observed mainly in recipients of unrelated-donor HCT and was probably the consequence of better management of these patients and better donor selection. This study also showed that the incidence of VOD among patients receiving reduced intensity conditioning (RIC)-HCT was < 2% with HLA-identical sibling donors and increased to 8% when the donor was unrelated, suggesting that the beneficial effects of RIC could be counterbalanced by the negative effects of alloreactivity.[22]

Risk Factors

Risk factors for VOD can be transplant-, conditioning- or patient related. (Table 1).[5, 22, 23]

Transplantation. A large prospective European study showed that the incidence of VOD after allo-HCT is up to threefold higher than that seen in the autologous setting.[20] In addition, a higher incidence of VOD is observed among allo-HCT performed with an unrelated donor, a mismatched donor or a T-cell-replete transplant. These observations suggest a role of alloreactivity in VOD pathogenesis. Recipients of a second HCT, those with active malignant disease and those receiving hepatotoxic drugs also have a higher risk of VOD.

Conditioning. Several studies have demonstrated a higher incidence of VOD in patients receiving Bu and Cy compared with those receiving Cy and TBI.[24] The risk of VOD can be reduced by adjusting the Bu dose in base of blood levels,[25] administering Bu intravenously or administering Cy first (Cy/Bu regimen). Similarly, a lower dose rate for TBI, a lower total dose or fractionated doses also reduce the risk of VOD. VOD appears to be uncommon (< 2%) after RIC, and exceptional after non-myeloablative conditioning.[22]

Patient. The most relevant risk factor is an increased level of ASAT/ALAT before HCT, which increases the risk of VOD 3–4-fold, especially when the elevation is caused by an hepatitis C virus infection.[22, 23, 26] In fact, impaired liver function at the time of a planned transplant can be a contraindication for standard HCT. Thus, patients with active hepatitis, hepatic fibrosis or cirrhosis must be evaluated carefully[27] and, if the transplant is mandatory, an RIC-HCT must be considered. Exposure during the four months prior to HCT to gemtuzumab ozogamicin (Mylotarg), an anti–CD33 monoclonal antibody linked to the toxin calicheamycin that affects endothelial and stellate cells that also express CD33, markedly increases the risk of VOD (up to 68%).[28]

Prognosis

In most cases (70–80%) the clinical course of VOD is self-limiting, but the remaining cases have a very high mortality rate. It is rare for a patient with VOD to die of liver failure, with renal and cardiopulmonary failure being the main causes of death.[3-5] For years the severity of VOD cases was classified retrospectively using the Seattle criteria: mild VOD (complete resolution without treatment), moderate VOD (resolution requiring treatment) or severe VOD (liver damage does not resolve before death or day 100).[11] However, this classification was not useful to clinicians who needed to predict which cases of VOD would be severe, to allow implementation of aggressive therapeutic measures. Bearman and colleagues used a Cox regression model to generate risk curves predictive of severe VOD based on total serum bilirubin and percentage of weight gain at various time points. Unfortunately, this model can only be used in patients treated with specific conditioning regimens (CyTBI, BuCy or CBV).[29] Another clinical feature that is associated with a worse outcome of VOD is the development of ascites, which is present in more than 50% of patients with severe disease, and which reflects a significant increase in portal hypertension. Similarly, an HVPG over 15 mmHg is usually associated with a particularly poor prognosis.[17] In current clinical practice, VOD is considered severe when the patient develops MOF, including pulmonary failure (SO_2 < 90% in room air and/or ventilator dependence), renal failure (doubling of baseline creatinine and/or dialysis dependence) and CNS abnormalities (confusion, encephalopathy and coma).[21, 22, 30]

Prophylaxis

There are several measures that can be adopted in high-risk patients to

Author (Journal, year)	Patients (n)	CR rate (%)	Day +100 Survival (%)
Richardson *et al*. (Blood, 1998)	19	42	32
Chopra *et al*. (BJH, 2000)	28	36	36
Richardson *et al*. (Blood, 2002)	88	36	35
Corbacioglu *et al*. (BMT, 2004)	22	50	36
Bulley *et al*. (Ped Blood Cancer, 2007)	14	60	79
Sucak *et al*. (Transplant Proc, 2007)	6	50	50
Richardson *et al*. (BB&MT, 2010)	149	46	42

Table 2. Clinical experience with defibrotide in treatment of severe VOD with MOF

minimise the risk of VOD. Thus, by delaying HCT in patients with acute hepatitis, avoiding cytoreductive regimes that include Cy or Bu, adjusting the doses of Bu, increasing the interval between cytotoxic drugs and TBI, reducing the total dose and dose rate of TBI, shielding the liver during TBI, fractionating TBI, administering Bu after the other agents or using RIC-HCT, it is possible to reduce the risk of developing VOD.

The first randomised trial of a continuous infusion of low doses of heparin did not reduce the incidence of VOD,[31] but two subsequent randomised trials[32, 33] showed that heparin treatment did reduce the incidence of VOD. In a large European prospective survey, the incidence of VOD was similar in patients who received prophylactic heparin and those who did not.[20] In another randomised study, patients receiving low molecular weight heparin had a lower incidence of VOD.[34]

In a non-randomised study,[34] prostaglandin-E1 (PGE1) proved useful in the prevention of VOD after HCT. Bearman *et al*.,[36] however, could not reproduce these results in patients at high risk of developing VOD, and observed important toxicities, particularly in patients receiving cyclosporine. Several randomised placebo-controlled trials have reported a statistically significant benefit for the prophylactic administration of ursodeoxycholic acid in patients at high risk of VOD.[37] However, a further phase III study did not demonstrate any difference in the incidence of VOD, although patients receiving ursodeoxycholic acid obtained significant benefit in terms of reduced hepatic complications and severe GVHD and increased overall survival.[38] After the introduction of defibrotide (DF) for VOD treatment, several studies have used this drug for VOD prevention in small and non-controlled studies, with apparent ineffectiveness. However, a recent EBMT-sponsored randomised trial showed that in children, the prophylactic use of DF significantly reduced not only the incidence of VOD but also the incidence of GvHD.[14]

Treatment

Maintenance of an adequate fluid and sodium balance is the only therapeutic measure to adopt in most cases of VOD. The use of diuretics may reduce the fluid and sodium retention in patients with excessive extravascular volume, but risks generating intravascular volume depletion and increasing the risk of renal failure, especially in patients receiving other nephrotoxic drugs. The goal should be to maintain intravascular volume and renal perfusion while avoiding extravascular fluid accumulation. The administration of red cells, colloids and albumin can help to maintain the intravascular volume.[5] When fluid accumulation and renal failure cannot be controlled, haemodialysis or haemofiltration may be required. If ascites becomes uncomfortable or limits breathing, paracentesis may be considered. The use of a peritoneovenous shunt can improve this symptomatology, but there is a high risk of coagulopathy, septicaemia and a massive volume shift into the intravascular space.[7] Some VOD cases have been resolved with a transjugular intrahepatic portosystemic shunt (TIPS), but in others this procedure has worsened the disease.[39]

There are few therapeutic approaches directed towards the improvement of venular occlusion. Agents including antithrombin III, prostaglandin, corticosteroids, glutamine/vitamin E, activated protein C and N-acetylcysteine have been reported as effective in isolated cases. Recombinant tissue plasminogen activator (rt-PA) can resolve some VOD cases (< 30%), but is not recommended in patients with MOF, haemorrhage or severe hypertension.[40]

The introduction of DF has been the only clear advance in VOD treatment. DF is a polydisperse oligonucleotide with a molecular weight of 23 kD, which has anti-thrombotic, anti-ischaemic, anti-inflammatory, anti-adhesive and thrombolytic properties without significant systemic anti-coagulant activity.[27, 41] The use of DF offers a complete remission rate and survival rate at day +100 ranging between 36–60% and 32%–79%, respectively (Table 2), outstanding results if we consider that classically, severe VOD implied a 70–80% mortality rate. DF must be administered as soon as possible once VOD is suspected (delay in starting the treatment has a negative impact on survival) at a dose of 6.25 mg/kg every 6 hours over 14–21 days depending on the evolution of the disease.[30]

Conclusions

The advances achieved over the years in the management of patients receiving myeloablative HCT, and the introduction of RIC-HCT, have given the false impression that the incidence of VOD is decreasing. However, the incidence remains around 8% for myeloablative HCT and 2% for RIC-HCT. Unfortunately, at least 25% of these cases will

correspond to severe VOD with MOF, with an expected mortality of 70–80%. Despite our increasing knowledge of the pathogenesis of this disease, the only relevant advance in prevention and treatment of these life-threatening forms of VOD has been the introduction of DF.

References

1. Valla D, Benhamou JP. Disorders of the hepatic veins and venules. In: Mcintyre N, Benhamou JP, Bircher J, Rizzetto M, Rodés J, eds. Oxford Textbook of Clinical Hepatology, vol. 2. Oxford: Oxford University Press, 1991: 1004–1011.
2. Jacobs P, Miller JL, Uys CJ, *et al*. Fatal veno-occlusive disease of the liver after chemotherapy, whole body irradiation and bone marrow transplantation for refractory acute leukaemia. S Afr Med J. 1979; 55: 5–10.
3. Shulman HM, Hinterberger W. Hepatic veno-occlusive disease—liver toxicity syndrome after bone marrow transplantation. Bone Marrow Transplant. 1982; 10: 197–214.
4. Bearman SJ. The syndrome of hepatic veno-occlusive disease after marrow transplantation. Blood. 1995; 85: 3005–3020.
5. Carreras E. Veno-occlusive disease of the liver after hemopoietic cell transplantation. Eur J Haematol. 2000; 64: 281–291.
6. DeLeve LD, Shulman HM, McDonald GB. Toxic injury to hepatic sinusoids: sinusoidal obstruction syndrome (veno-occlusive disease). Semin Liver Dis. 2002; 22: 27–42.
7. Kumar S, DeLeve LD, Kamath PS, *et al*. Hepatic veno-occlusive disease (sinusoidal obstruction syndrome) after hematopoietic stem cell transplantation. Mayo Clin Proc. 2003; 78: 589–598.
8. Shulman HM, Gown AM, Nugent DJ. Hepatic veno-occlusive disease after bone marrow transplantation. Immunohistochemical identification of the material within occluded central venules. Am J Pathol. 1987; 127: 549–558.
9. Palomo M, Diaz-Ricart M, Carbo C, *et al*. The release of soluble factors contributing to endothelial activation and damage after hematopoietic stem cell transplantation is not limited to the allogeneic setting and involves several pathogenic mechanisms. Biol Blood Marrow Transplant. 2009; 15: 537–546.
10. Palomo M, Diaz-Ricart M, Carbo C, *et al*. Endothelial dysfunction after hematopoietic stem cell transplantation: role of the conditioning regimen and the type of transplantation. Biol Blood Marrow Transplant. 2010; 16: 985–993.
11. McDonald GB, Hinds MS, Fisher LD, *et al*. Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. Ann Intern Med. 1993; 118: 255–267.
12. Shulman HM, Fisher LB, Schoch HG, *et al*. Veno-occlusive disease of the liver after marrow transplantation: histological correlates of clinical signs and symptoms. Hepatology. 1984; 19: 1171–1180.
13. Carreras E, Rosiñol L, Terol MJ, *et al*. Veno-occlusive disease of the liver after high-dose cytoreductive therapy with busulfan and melphalan for autologous blood stem cell transplantation in multiple myeloma patients. Biol Blood Marrow Transplant. 2007; 13: 1448–1454.
14. Corbacioglu S, Cesaro S, Faraci M, *et al*. Defibrotide for prophylaxis of hepatic veno-occlusive disease in paediatric haemopoietic stem-cell transplantation: an open-label, phase 3, randomised controlled trial. Lancet. 2012; 379: 1301–1309.
15. Jones RJ, Lee KS, Beschorner WE, *et al*. Venoocclusive disease of the liver following bone marrow transplantation. Transplantation. 1987; 44: 778–783.
16. Carreras E, Grañena A, Navasa M, *et al*. On the reliability of clinical criteria for the diagnosis of hepatic veno-occlusive disease. Ann Hematol. 1993; 66: 77–80.
17. Carreras E, Grañena A, Navasa M, *et al*. Transjugular liver biopsy in BMT. Bone Marrow Transplant. 1993; 11: 21–26.
18. Nicolau C, Brú C, Carreras E, *et al*. Sonographic diagnosis and hemodynamic correlation in veno-occlusive disease of the liver. J Ultrasound Med. 1993; 12: 437–440.
19. Cutler C, Kim HT, Ayanian S, *et al*. Prediction of veno-occlusive disease using biomarkers of endothelial injury. Biol Blood Marrow Transplant. 2010; 16: 1180–1185.
20. Carreras E, Bertz H, Arcese W, *et al*. Incidence and outcome of hepatic veno-occlusive disease (VOD) after blood and marrow transplantation (BMT): a prospective cohort study of the European group for Blood and Marrow Transplantation (EBMT). Blood. 1998; 92: 3599–3604.
21. Coppell JA, Richardson PG, Soiffer R, *et al*. Hepatic veno-occlusive disease following stem cell transplantation: incidence, clinical course, and outcome. Biol Blood Marrow Transplant. 2010; 16: 157–168.
22. Carreras E, Díaz-Beyá M, Rosiñol L, *et al*. The incidence of veno-occlusive disease following allogeneic hematopoietic stem cell transplantation has diminished and the outcome improved over the last decade. Biol Blood Marrow Transplant. 2011; 17: 1713–1720.
23. Rozman C, Carreras E, Qian C, *et al*. Risk factors for hepatic veno-occlusive disease following HLA-identical sibling marrow transplants for leukemia. Bone Marrow Transplant. 1996; 17: 75–80.
24. Ringden O, Ruutu T, Remberger M, *et al*. A randomized trial comparing busulfan with total body irradiation as conditioning in allogeneic marrow transplant recipients with leukemia: a report from the Nordic Bone Marrow Transplantation Group. Blood. 1994; 83: 2723–2730.
25. Schuler U, Schroer S, Kuhnle A, *et al*. Busulfan pharmacokinetics in bone marrow transplant patients: is drug monitoring warranted? Bone Marrow Transplant. 1994; 14: 759–765.
26. Strasser SI, Myerson D, Spurgeon CL, *et al*. Hepatitis C virus infection after bone marrow transplantation: a cohort study with 10-year follow-up. Hepatology. 1999; 29: 1893–1899.
27. Palomo M, Diaz-Ricart M, Rovira M, *et al*. Defibrotide prevents the activation of macrovascular and microvascular endothelia caused by soluble factors released to blood by autologous hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2011; 17: 497–506.
28. Wadleigh M, Richardson P, Zahrieh D, *et al*. Prior gemtuzumab ozogamicin exposure significantly increases the risk of veno–occlusive disease in patients who undergo myeloablative allogeneic stem cell transplantation. Blood. 2003; 102: 1578–1582.
29. Bearman SI, Anderson GL, Mori M, *et al*. Venoocclusive disease of the liver: development of a model for predicting fatal outcome after marrow transplantation. J Clin Oncol. 1993; 11: 1729–1736.
30. Richardson PG, Soiffer RJ, Antin JH, *et al*. Defibrotide for the treatment of severe hepatic veno-occlusive disease and multiorgan failure after stem cell transplantation: a multicenter, randomized, dose-finding trial. Biol Blood Marrow Transplant. 2010; 16: 1005–1017.
31. Marsa-Vila L, Gorin NC, Laporte JP, *et al*. Prophylactic heparin does not prevent liver veno-occlusive disease following autologous bone marrow transplantation. Eur J Haematol. 1991; 47: 346–354.
32. Attal M, Huguet F, Rubie H, *et al*. Prevention of hepatic veno-occlusive disease after bone marrow transplantation by continuous infusion of low-dose heparin: a prospective, randomized trial. Blood. 1992; 79: 2834–2840.
33. Rosenthal J, Sender L, Seccola R, *et al*. Phase II trial of heparin prophylaxis for veno-occlusive disease of the liver in children undergoing bone marrow transplantation. Bone Marrow Transplant. 1996; 18: 185–191.
34. Or R, Nagler A, Shpilberg O, *et al*. Low molecular weight heparin for the prevention of veno-occlusive disease of the liver in bone marrow transplantation patients. Transplantation. 1996; 61: 1067–1071.
35. Gluckman E, Jolivet I, Scrobohaci MI, *et al*. Use of prostaglandin E1 for prevention of liver veno-occlusive disease in leukaemic patients treated by allogeneic bone marrow transplantation. Br J Haematol. 1990; 74: 277–281.
36. Bearman SI, Shen DD, Hinds MS, *et al*. A phase I/II study of prostaglandin E1 for the prevention of hepatic venocclusive disease after bone marrow transplantation. Br J Haematol. 1993; 84: 724–730.
37. Essell JH, Schroeder MT, Harman GS, *et al*. Ursodiol prophylaxis against hepatic complications of allogeneic bone marrow transplantation. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 1998; 128 (12 Pt 1): 975–981.
38. Ruutu T, Eriksson B, Remes K, *et al*. Ursodeoxycholic acid for the prevention of hepatic complications in allogeneic stem cell transplantation. Blood. 2002; 100: 1977–1983.
39. Azoulay D, Castaing D, Lemoine A, *et al*. Transjugular intrahepatic portosystemic shunt (TIPS) for severe veno-occlusive disease of the liver following bone marrow transplantation. Bone Marrow Transplant. 2000; 25: 987–992.
40. Bearman SI, Lee JL, Barón AE, *et al*. Treatment of hepatic venocclusive disease with recombinant human ti1sue plasminogen activator and heparin in 42 marrow transplant patients. Blood. 1997; 89: 1501–1506.
41. Kornblum N, Ayyanar K, Benimetskaya L, *et al*. Defibrotide, a polydisperse mixture of single-stranded phosphodiester oligonucleotides with lifesaving activity in severe hepatic veno-occlusive disease: clinical outcomes and potential mechanisms of action. Oligonucleotides. 2006; 16: 105–114.

Upcoming Congresses and Meetings

Pennsylvania Society of Oncology and Hematology (PSOH) Annual Scientific Meeting

20 September 2013

King of Prussia, USA

The Pennsylvania Society of Oncology and Hematology is an organisation of physicians and healthcare professionals devoted to the improvement of haematologic and oncologic care of patients. This year, the scientific meeting will feature keynote presentations on melanoma and multiple myeloma, as well as focusing on the effect of Pennsylvanian legislation on medical practice.

ESH - iCMLf International Conference on CML - Biology and Therapy

26 – 29 September 2013

Estoril, Portugal

The European School of Haematology (ESH) is a non-profit institution for continuing education, founded to promote and facilitate access to state-of-the-art and cutting-edge knowledge in haematology and related disciplines, at the European level. ESH also develops tools for continuing education produced in collaboration with international experts in the field. This year's international conference will focus on the biology and therapy of CML.

UK Photopheresis Society (UKPS) Conference

27 September 2013

London, UK

The UK Photopheresis Society's aim is to provide a forum for clinicians who are either involved in the management or referral of patients who require this immunomodulatory therapy. It is also intended to act as an information resource for clinicians, nurses and patients, who may be receiving or considering Photopheresis therapy. There is active international research into Photopheresis and its mechanism of action, and it is an additional aim of the Society to promote and discuss an increased understanding of the current scientific evidence base. As this service is provided on a regional or supraregional basis within the UK, the Society considers one of its key roles is to disseminate best evidenced based practice and act as a resource for clinicians and commissioners involved in decisions regarding the application of this therapy.

The European Multidisciplinary Cancer Congress

27 September - 01 October 2013

Amsterdam, The Netherlands

The European Multidisciplinary Cancer Congress will bring together the ECCO, ESMO and ESTRO Congresses in partnership with ECCO, ESMO, ESTRO, ESSO, EACR, EONS and SIOPE. These Congresses are the premier European cancer meetings and the largest European platform at which to present the latest, groundbreaking data in the field of oncology. The congress will feature sessions on leukaemia and lymphoma amongst others.

Eurothrombosis 2013

03 - 05 October 2013

Uppsala, Sweden

Eurothrombosis 2013 is the official scientific meeting of the European Society of Cardiology's Working Group of Thrombosis. This year's meeting will have a translational focus, with lectures and symposia ranging from pre-clinical thrombosis research to clinical trials. In addition to addressing the most common clinical situations related to thrombosis, the programme will also cover novel fields such as the obesity epidemic and thrombosis. Attendees will enjoy an exciting programme in a lively university environment.

ESH International Conference on Multiple Myeloma

04 – 06 October 2013

Dublin, Ireland

The 2013 ESH International Conference on Multiple Myeloma will be held in a world-class faculty and will offer a comprehensive overview of the most up-to-date treatment strategies for multiple myeloma. Over the past decade, the understanding of multiple myeloma pathophysiology has made major advances. Patient survival has improved significantly thanks to novel agents such as immunomodulatory drugs and proteasome inhibitors, and this will be explored at the congress. Speakers will also present the most efficient treatment options for elderly and relapsing patients.

The 2nd International Congress on Controversies in Stem Cell Transplantation and Cellular Therapies (COSTEM)

10 - 13 October 2013

Berlin, Germany

The international congress on COSTEM acts as a unique platform where the most compelling and controversial topics facing clinicians in all fields of stem cell and cellular therapies are presented through dynamic debate forums and international expert presentations. This interactive congress provides both experts and clinicians with an opportunity to share and compare experiences, in order to outline the right treatment for patients.

6th Trends in Medical Mycology

11 - 14 October 2013

Copenhagen, Denmark

The 6th Trends in Medical Mycology (TIMM) is jointly organised by the European Confederation of Medical Mycology (ECMM) and the Infectious Diseases Group of the European Organisation for Research and Treatment of Cancer (IDG-EORTC). TIMM has become an essential Congress in the field of fungal infections. The outstanding program will appeal to infectious disease specialists, haematologists, oncologists, transplant physicians, microbiologists, immunologists, dermatologists and paediatricians.

Clinical Leaders of Thrombosis (CLOT) Conference 2013

18 October 2013

Birmingham, England

This multidisciplinary conference aims to develop the service providers responsible for delivering high-quality care in order to shape anticoagulation and DVT services for the future. Sessions are delivered by leading healthcare professionals in the field, and discussion and debate are encouraged in order for attendees to gain the most from the conference. One of the most important areas this year will be the reversal of the new oral anticoagulants, and case study workshops will be a key feature of the programme.

39th Annual American Society for Histocompatibility and Immunogenetics (ASHI) Meeting

17 - 21 November 2013

Chicago, USA

This meeting is designed to provide delegates with a comprehensive review of all of the variables that govern the pre- and post transplant management of solid organ and haematopoietic stem cell transplant recipients. A range of sessions will take place, including roundtable discussions, symposia and interactive workshops on topics such as Current Trends in Haematopoietic Stem Cell Transplantation and New Horizons in Complement.

55th American Society of Hematology (ASH) Annual Meeting and Exposition

07 - 10 December 2013

New Orleans, Louisianna

ASH invites you to New Orleans for their 55th Annual Meeting and Exposition. ASH aims to offer invaluable benefits for all attendees, and the meeting hopes to provide the opportunity for individuals to grow professionally. Haematology is a constantly changing field, and ASH's Education and Scientific Programme can help you stay up-to-date on the latest research, therapies, and tools that you need to succeed. The meeting offers many networking events which will allow you to connect with colleagues and interact with leaders in the field to learn and share your ideas.

ESH Clinical Updates in Haematology on Lymphoid Neoplasms and Myeloma

05 - 06 February 2014

Paris, France

This ESH meeting will feature a clinically and practically orientated programme which reflects the most recent research, discoveries and treatments in Lymphoid Neoplasms and Myeloma. Additionally, the meeting will feature updates on patient management from world class experts, as well as in-depth presentations given by leading experts and interactive discussion sessions, as well as handouts comprised of the lecture summaries, slides and recent, selected references.

10th European Congress on Hematologic Malignancies from Clinical Science to Clinical Practice

07 – 09 March 2014

Vienna, Austria

The 10th European Congress on Hematologic Malignancies from Clinical Science to Clinical Practice will provide important clinical updates in the management of patients. New findings, techniques and recent efforts in research and discovery have altered patient care to a more individualised approach, and the congress will reflect this in its scientific programme. Furthermore, all slide presentations will be available to view on electronic devices, and will be available to download after the congress.

40th Annual Meeting of the European Group for Blood and Marrow Transplantation (EBMT)

30 March - 2 April 2014

Milan, Italy

The EBMT is devoted to the promotion of all aspects associated with the transplantation of haematopoietic stem cells from all donor sources and types, including basic and clinical research, education, standarisation and quality control. The EBMT Annual Meeting is one of the most important annual events for research and education in the field of bone marrow transplantation in Europe. The Meeting now attracts over 4,000 participants and includes symposia, educational sessions and workshops covering key issues relating to bone marrow and stem cell transplantation.

54th Annual Scientific Meeting of the British Society for Haematology

28 – 30 April 2014

Birmingham, UK

The BSH is now one of the most active haematology societies in the UK, which aims to advance the practise and study of haematology and facilitate contact between interested parties. The annual meeting now attracts around 1,000 delegates and plays host to over 30 top quality international speakers who are all leaders in their field. The meeting links up with the European and African division of the International Society of Haematology (ISH) due to their increasing international interests.

19th Congress of the EHA

12 - 15 June 2014

Milan, Italy

The EHA aims to promote excellence in clinical practice, research and education, and the 19th Congress of the EHA compliments these goals with a variety of session types and a diverse range of topics covered. In addition to the education and scientific programmes, which will cover established clinical practise, recent advances and different viewpoints, the committee is developing selected sessions for various target groups, and is combining these into tracks.

The Cambridge Research Centre
Coppergate House
16 Brune Street
London
E1 7NJ