TREATMENT STRATEGIES
BLOOD & MARROW TRANSPLANTATION

Volume 1 Issue 1

- Review of EBMT 2014 - Milan
- Allogeneic Transplantation
- Autologous Transplantation
- Clinical-grade T Cells
- Chronic Myeloid Leukemia
- Hematopoietic Stem Cell Transplantation
- Hodgkin Lymphoma
- Myelofibrosis
- Paroxysmal Nocturnal Hemoglobinuria

Excellence in Science
EBMT
European Group for Blood and Marrow Transplantation

Includes a Review of the 40th Annual Meeting of the EBMT

40th ANNUAL MEETING

DENVER, COLORADO

MONDAY, OCTOBER 20 – FRIDAY, OCTOBER 24, 2014

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TREATMENT STRATEGIES - BLOOD & MARROW TRANSPLANTATION

TREATMENT STRATEGIES - BLOOD & MARROW TRANSPLANTATION - May 2014

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

Managing Director **Nigel Lloyd**
nigel@cambridgeresearchcentre.co.uk
Publishing Director **Sara Taheri**
sara@cambridgeresearchcentre.co.uk
Chief Sub-editor **Libby Cooper**
libby@cambridgeresearchcentre.co.uk
Sales and Advertising **Steve Bishop**
Filming **Martin Janes**
video@cambridgeresearchcentre.co.uk
Credit Control Manager **Emma Jones**
emma@cambridgeresearchcentre.co.uk
Accounts **Vipul Patel**

Published by The Cambridge Research Centre
info@cambridgeresearchcentre.co.uk
www.cambridgeresearchcentre.co.uk
T: +44 (0) 20 8265 9098

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Welcome...

I am delighted to welcome you to the inaugural edition of *Treatment Strategies - Blood & Marrow Transplantation*. In this edition we bring you a range of informative articles, papers and reports, as well as an in-depth review of 40th Annual Meeting of the European Society for Blood & Marrow Transplantation (EBMT), which was held in Milan this March. Our EBMT meeting review will provide you with the breaking news, research highlights and the best of the symposia, as well as a number of poster synopses. We really feel that this review will provide you with the not to be missed highlights of the congress.

This edition also features a number of interesting and informative papers on subjects such as allogeneic transplantation, autologous transplantation, clinical-grade T cells, chronic myeloid leukemia, hematopoietic stem cell transplantation, hodgkin lymphoma, myelofibrosis and paroxysmal nocturnal hemoglobinuria. With these papers we aim to bring you new insights into the latest treatment strategies for a number of haematological conditions, and we hope that you enjoy the carefully chosen content.

So far, 2014 is proving to be a fantastic year for The Cambridge Research Centre, with some exciting changes including Treatment Strategies TV, where you can find footage from the most important scientific conferences, meetings and congresses, as well as interviews, symposia proceedings, roundtable events and much more. We also launched our range of interactive eBooks on iBooks, which is a great new way to read and download our content to your devices. Have you liked our new Facebook page? Here you can find all of the latest news about new projects and upcoming releases, and the Treatment Strategies' team are also all active on Twitter and LinkedIn.

We hope that you enjoy this edition of *Treatment Strategies – Blood & Marrow Transplantation*, and please do share your thoughts with us on this issue as well as what you would like to see in our next edition, which will feature a review of EBMT 2015 - See you in Istanbul.

Nigel Lloyd, Managing Director

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

THALASSAEMIA INTERNATIONAL FEDERATION

In official relations with the World Health Organization

Thalassaemia International Federation (TIF) is a non-governmental, patient-oriented association, established in 1986 to safeguard the rights of patients with haemoglobin disorders, thalassaemia, and sickle cell disease globally and to ensure their access to quality medical care. Today, TIF represents the voice of patients in 117 National Patients/Parents Associations, registered in 56 countries across the world and works in official relations with the World Health Organisation since 1996.

In addition, TIF collaborates with a wide range of official health, and policy oriented bodies and agencies across the world and a wide range of professional medical bodies and other disease-oriented organisations (see our website http://www.thalassaemia.org.cy/collaboration-partnerships-networks/collaboration-partnerships-networks.shtml). TIF's vision defines its mission which is the establishment of national strategies for the prevention and management of thalassaemia.

TIF's work is based on four pillars of activities:

1. Educational Programme
2. Collaboration, Partnerships, Networks
3. Advocacy & Policy Development
4. Communication & Awareness

TIF's Educational Programme is one that stands out and one for which TIF can claim having a truly measurable impact on a global scale. The Educational Programme encompasses three important elements:

1. The preparation and organisation of Educational Events at a national, regional, and international level (so far TIF has organised over 67 local, national, regional, international conferences and other events (workshops, seminars, delegation visits in 60 countries worldwide).

International Conferences on Thalassaemia and other Haemoglobinopathies are organised exclusively by TIF in the context of two parallel sessions, one for medical specialists and health professionals and another for patients and parents.

The latest International Conference has been carried out with great success in Abu Dhabi, United Arab Emirates, 20-23 October 2013. The next one is scheduled for 2017.

In this context, we are delighted to announce that TIF is organising the 4th Regional Pan-European Conference, which will take place in Athens, 7-8 November 2014.

Other regional conferences in Asia and the Middle East are scheduled to take place in 2015, and 2016 respectively.

2. The preparation, translation, publication and distribution of material to the medical community, the patients' community, and the community at large as a free of charge service (19 publications translated in 20 languages, distributed in over 60 countries).

3. The development of academic post-graduate courses including: a) an e-Msc course in Haemoglobinopathies for medical professionals and b) the Experts-Patients Programme for the patient community

TIF was the first body to publish:

1. Guidelines on the management of Thalassaemia and other haemoglobin disorders Guidelines for the Clinical Management of Thalassaemia (2005) Cappellini M-D, Cohen A, Eleftheriou A, Piga A, Porter J, Taher A, ISBN: 978-9963-623-70-9

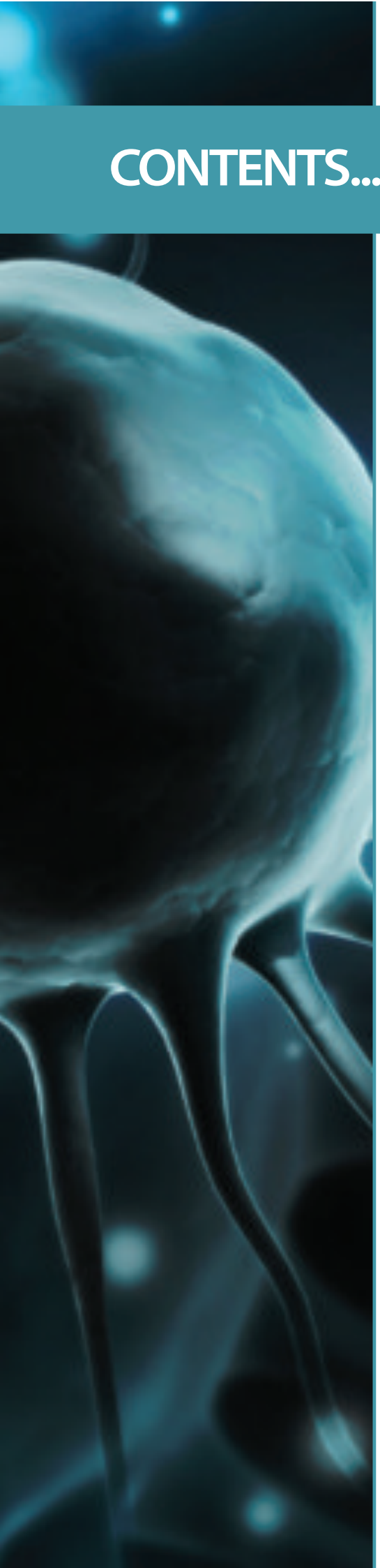
2. Guidelines on Prevention of Haemoglobin Disorders: Prevention of Thalassaemias and other Haemoglobin Disorders, Vol 1, 2nd Edition (2013) - Old J, Galanello R, Eleftheriou A, Traeger-Synodinou J, Petrou M, Angastiniotis M, ISBN 9963 623 39 5 Prevention of Thalassaemias and Other Haemoglobin Disorders, Vol. 2: Laboratory Protocols (2012)- Old J, Harteveld C L, Traeger-Synodinos J, Petrou M, Angastiniotis M, Galanello R, ISBN 978-9963-717-01-9

3. Guidelines for Haemoglobinopathy Nurses A Guide for the Haemoglobinopathy Nurse (2013) - Aimiuwu E, Thomas A, Roheemun N, Khairallah T, Nacouzi A N, Georgiou A, Papadopoulou C, ISBN 978-9963-717-02-6

4. Guidelines for the Management of the Non-Dependent Transfusion Thalassaemias Guidelines for the Clinical Management of Non-Transfusion Dependent Thalassaemias (2013)- Cappellini M D, Taher A, Musallam K, ISBN 978-9963-717-03-3

5. The first book for the patient and the parent about Thalassaemia About Thalassaemia (2007) Eleftheriou A, ISBN: 9963-623-40-9

6. The first cartoon book for children, also released as a cartoon animation. All About Thalassaemia (2010) Dr Eleftheriou A, ISBN 978-9963-623- 95-2



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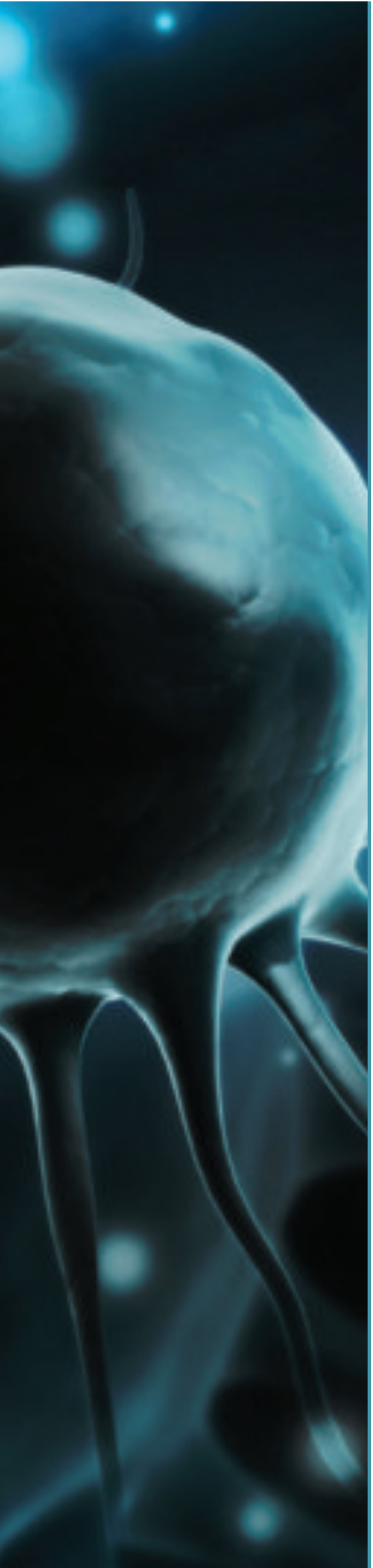

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Foreword

Michael Angastiniotis[1] and **Androulla Eleftheriou**[2]
1.Scientific Advisor; 2. Executive Director, Thalassaemia International Federation

The Thalassaemia International Federation (TIF) as a patient-driven, umbrella organisation, serving as a voice for national organisations in 57 countries, welcomes this new edition of Treatment Strategies, which deals with diseases of blood. Blood diseases are prominent in the spectrum of pathological conditions affecting the human race. They include pathology affecting all components of blood and cause a wide variety of diseases which are often life threatening. TIF is concerned with a group of hereditary red cell disorders, which are the commonest of the serious monogenic disorders affecting man. The severity, the chronicity and the difficult and expensive management as well as the wide geographical distribution make these disorders an important public health issue, especially in high prevalence areas. Migrations are currently introducing the conditions to low prevalence areas especially in Europe and North America, making for a global problem requiring health service planning.

For the beta thalassaemia syndromes alone it is estimated that there are over 200 million carriers across the world with around 60000 new affected births each year. Despite this, less than 300000 thousand patients are known to be receiving treatment across the world. Patient figures are lacking in most countries due the lack of registries making health planning even more difficult.

Michael Angastiniotis is a scientific advisor for the Thalassaemia International Federation. He has been a Member of the WHO Committee for the Control of Hereditory Anaemias since 1983, a member of the WHO Expert Advisory Panel on Human Genetics since 1990 and is a Member of the Board of Directors on the Cyprus Institute of Neurology and Genetics. Until retirement he was Director of the Paediatric Department of Archbishop Makarios III Hospital in Cyprus, as well as the Thalassaemia Center. He Graduated in Medicine from the University of Aberdeen in 1966 and received his graduate training in Paediatriwcs in Scottish hospitals and hospitals in Oxford, UK.

Androulla Eleftheriou obtained her graduate and postgraduate degrees from the University of London, in the fields of Biochemistry, Microbiology and Virology. She has been awarded a number of scholarships by the Cyprus government, the World Health Organization and the Fulbright Commission. Her postdoctoral fellowship was completed at the Centre for Disease Control in Atlanta. From 1990 until 2006, Dr Eleftheriou was Head of the Virus Reference Centre of the Cyprus Ministry of Health – a centre she was closely involved in establishing. She is currently the Director of the Cyprus WHO Thalassaemia Collaborating Centre. She has been working with TIF since 1993 as a Scientific Coordinator of TIF's educational programme, and since 2006 as a formal employee at the post of the Executive Director of the Federation. She is the author of a number of publications, in peer-reviewed journals, in those published by TIF, as well as a number prepared in collaboration with WHO and other international bodies on a wide range of scientific and patients' oriented topics and is the Chief Editor of *TIF Magazine*.

Much has been achieved over the years in terms both of limitation of new births and in treating the various syndromes so that survival has improved, complication rates are reduced and quality of life has been achieved such that many adult patients are now enjoying a career, marriage and family life. This is in contrast to many countries where the patient population is still in childhood due to lack of basic care.

The good results observed in some countries, is due to the adoption of scientific advancements. Examples of such advances include the better understanding of the molecular mechanisms which modify the clinical manifestations. In beta thalassaemia several modifiers have been identified which affect the balance of the globin chains of the haemoglobin molecule, either reducing the alpha globin chains (as in coinheritance of alpha thalassaemia) or in affecting foetal haemoglobin production due to modifiers in the Xmn 1 Gγ polymorphism or Bcl 11A and others. Such understanding of genetic modifiers has led to more informed decision-making as to whether and when to start transfusions. Research has focused on finding agents to also modify chain imbalance such as the butyrates to increase foetal haemoglobin. More recently agents affecting red cell maturation have been shown both in animal studies and phase 2 clinical studies to increase haemoglobin level and are now progressing to phase 3 trials. In addition new iron chelating agents are entering human trials.

So far none of these researches are aiming at a total cure of haemoglobin disorders. A cure can be achieved through haemopoietic stem cell transplantation but this is generally successful in young patients with an HLA compatible sibling donor, which is available to less than 20% of patients and to most the cost is prohibitive. Advances in using matched unrelated donors and cord blood transplantation may increase the number of patients for which transplantation is an option but more clinical trials are needed before these techniques are generally adopted.

Finally gene therapy trials are now underway. Despite one patient 'cured' much has yet to be done before patients can benefit from this approach. However haemoglobin disorders are approaching a crossroads as far as advances in treatment are concerned and support for research is a TIF concern.

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EBMT Meeting 2014

30 March - 2 April, Milan

40th Annual Meeting of the European Society for Blood and Marrow Transplantation

INSIDE...

The Meeting

Sara Taheri, Treatment Strategies, is delighted to bring to you our review of the 40th Annual Meeting of the EBMT. In this review we cover the breaking news, research, and some of the major symposia proceedings, as well as the most innovative products, treatments and strategies from the meeting. This comprehensive review also includes posters and papers that were presented at the event.

A non-profit organisation, the European Group for Blood and Marrow Transplantation (EBMT) was established in 1974. The EBMT enables scientists and physicians in the area of clinical bone marrow transplantation to share their experience and develop co-operative studies. The EBMT is devoted to the promotion of transplantation of haematopoietic stem cells from all donor sources and donor types including basic and clinical research, education, standardisation, quality control, and accreditation for transplant procedures.

Attendees were welcomed to the 40th Annual Meeting of the EBMT, 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, the 8th EBMT Patient & Family and the 1st Donor Day.

The EBMT is unique, in that it includes physicians, nurses, allied health professionals, data managers, quality managers and processing laboratory personnel in its society activities. Throughout the meeting several initiatives brought together all of the professionals who work together to care for and manage the treatment of transplant patient.

The 30th anniversary of the EBMT Nurses Group was celebrated and the program focused on the developments in patient care. The meeting focused on appreciating transplantation as a part of a complex therapeutic program, that needs contribution from different specialists and different professional expertise.

The advancements in hematopoietic stem cell transplantation were appreciated whilst new challenges such as new genome technologies, integration of targeted therapies into transplant strategy, the emerging role of cell therapies, the choice of donor were also recognised.

A new feature of the 2014 program included the "Cooperative Trials in Progress" sessions, where national and international cooperative groups focused on a specific disease and had the opportunity to present their studies in progress, and share preliminary results.

The meeting included the second Paediatric Day, and a day dedicated to the various aspects of cell processing and therapy,'Cell Therapy Day' organised in cooperation with ISCT (International Society for Cellular Therapy).

The 8th Patient and Family Day took place with the involvement of the Italian Marrow Donor Association (ADMO) as well as patient and volunteer organisations. This has been reformed to 'Patient, Family, and Donor Day' and formally included donors as association and as single volunteers into the organisation.

The meeting was hosted in Milan's, Milano Convention Centre (MiCo) the largest convention centre in Italy.

As always the EBMT acknowledged the pioneering advances in the field of Bone and Marrow Transplantation by presenting a number of awards and prizes for abstracts submitted for Oral and Poster presentations. This year 12 awards in total were presented to individuals for their outstanding contributions to science and to the EBMT.

The EBMT Physicians Group Awards and Prizes include the Jon J. van Rood Award for the Best Paper in the Immunology of Allogeneic Hematopoietic Transplantation.

The EBMT Immunobiology Working Party presented a prestigious (€ 10,000) award sponsored by Neovii Biotec GmbH for outstanding contributions in the field of hematopoietic transplantation immunology, immunogenetics, tolerance and graft-vs-leukaemia/tumour effects. The winner was selected by a distinguished international jury under the supervision of Prof. Jon J. van Rood and presented to Sanja Stevanovic.

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

Supported by Clinigen, the Basic Science Award of 2,500€ was presented as part of the Opening Session to Nicoletta Cieri, lead author of the abstract entitled: ' Tracking T Cell Dynamics In the First Month After Allogeneic HSCT Offers A Unique Opportunity to Unveil the Mechanism of Memory Stem T Cell Formation in Humans. The purpose of this award is to increase the level of basic science and in particular to encourage young investigators to submit their work. A Scientific Committee headed by the Chairs of the EBMT Developmental Committee selected the prize winning abstract from the top-scoring abstracts.

For his continuous and dedicated work in the development of ProMISe EBMT Registry, Professor Ronald Brand was awarded with the Outstanding Contribution to the EBMT award.

The EBMT offers Honorary Memberships to retired members whose passionate work has contributed enormously to the development and successful achievement of the EBMT Mission. Shaun McCann and Massimo Martelli were both welcomed as Honorary Members at this years meeting.

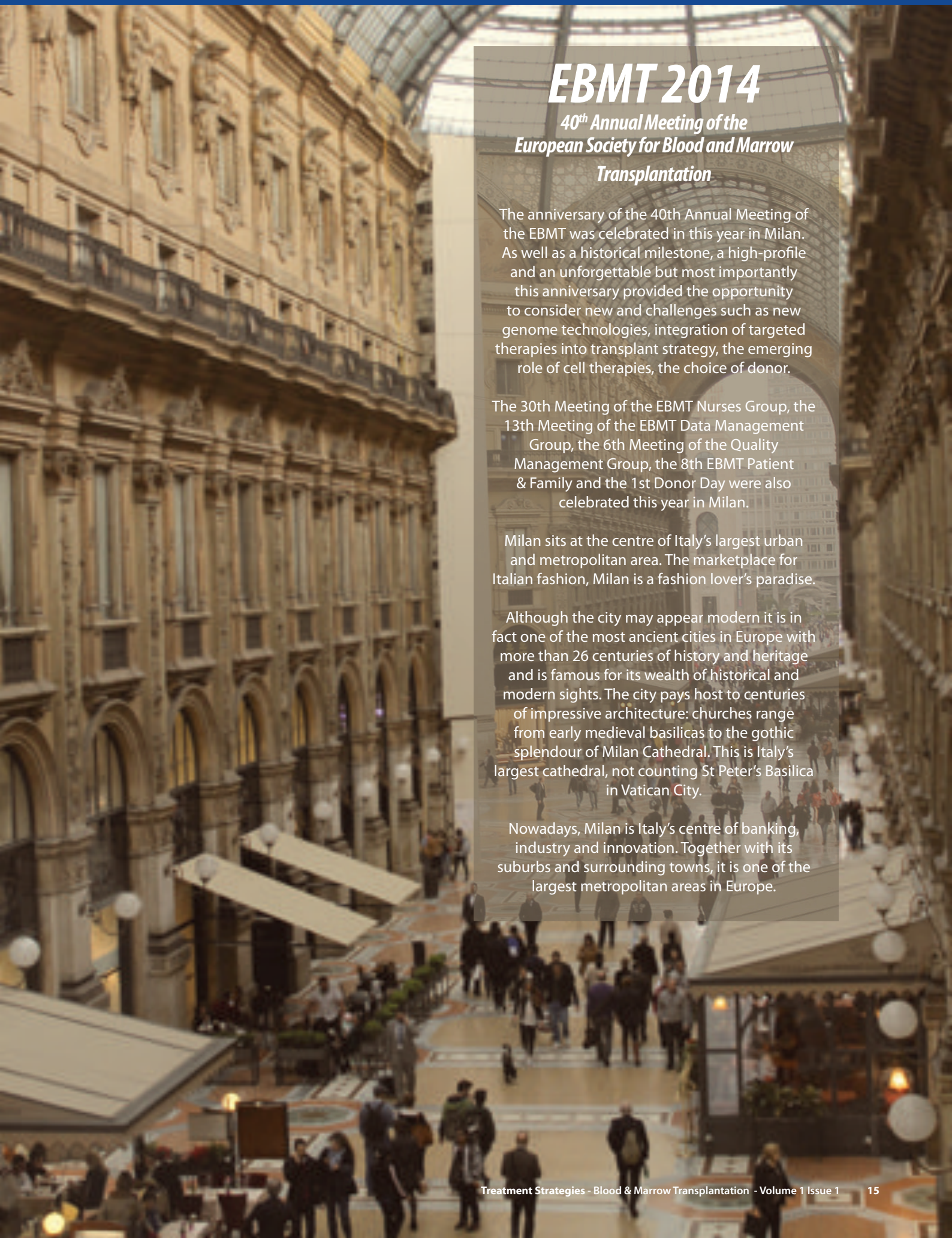
For her life-long achievements on the field of Bone and Marrow Transplantation Professor Rose Hamladji was awarded with the Clinical Achievement Award.

Jian-Jian Luan Award for Lymphoma Transplant Research award is in commemoration of Jian-Jian Luan, who worked for the EBMT Lymphoma Working Party (LWP) and tragically died in an alpine hiking accident. The award was presented during the LWP session with 1,000€ donated by the LWP to Anne-Claire Mamez.

The Nature Publishing Poster Awards for the best clinical and best science physician poster presentations were awarded by Mohammad Mohty at the closing ceremony. The Best Clinical Poster Award of £1,000 was awarded to Hyeoung-Joon Kim, for his poster entitled: 'Clinical Implications of TET2 Mutations with Normal Karyotype Acute Myeloid Leukaemia In Younger Patients.' The Best Science Poster Award of 1,000£ was awarded to Sarah Oelsner, lead author of the poster entitled: 'Genetically Modified Cytokine-Induced Killer (CIK) Cells for Targeted Cancer Therapy.

The 6th Distinguished Merit Award was given to Monica Fliedner for her long-standing dedication and strategic work emphasizing research, education and collaboration as fundamentals contributing to the successful development of the Nurses Group.

Laure Tardieu receiver The Best Poster Award went to Laure Tardieu for her team's poster: 'Evaluation of Discharge Instruction Booklet for Allograft Patient.'



EBMT 2014

40th Annual Meeting of the European Society for Blood and Marrow Transplantation

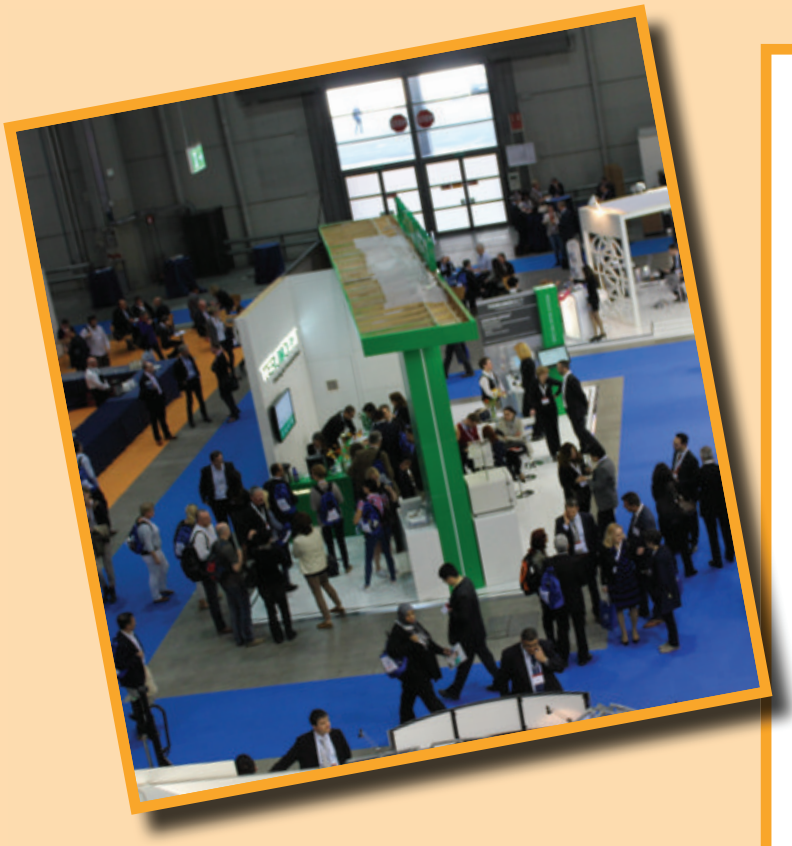
The anniversary of the 40th Annual Meeting of the EBMT was celebrated in this year in Milan. As well as a historical milestone, a high-profile and an unforgettable but most importantly this anniversary provided the opportunity to consider new and challenges such as new genome technologies, integration of targeted therapies into transplant strategy, the emerging role of cell therapies, the choice of donor.

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, the 8th EBMT Patient & Family and the 1st Donor Day were also celebrated this year in Milan.

Milan sits at the centre of Italy's largest urban and metropolitan area. The marketplace for Italian fashion, Milan is a fashion lover's paradise.

Although the city may appear modern it is in fact one of the most ancient cities in Europe with more than 26 centuries of history and heritage and is famous for its wealth of historical and modern sights. The city pays host to centuries of impressive architecture: churches range from early medieval basilicas to the gothic splendour of Milan Cathedral. This is Italy's largest cathedral, not counting St Peter's Basilica in Vatican City.

Nowadays, Milan is Italy's centre of banking, industry and innovation. Together with its suburbs and surrounding towns, it is one of the largest metropolitan areas in Europe.



Advancing Apheresis Award

Terumo BCT Launched the Advancing Apheresis Award on the 31 March 2014 at the 40th EBMT meeting in Milan.

A global leader in blood component and cellular technologies, Terumo BCT provides a unique combination of apheresis collections, manual and automated whole blood processing, and pathogen reduction coupled with leading technologies in therapeutic apheresis and cell processing.

With their on-going effort to encourage scientific evidence, research and understanding of therapeutic apheresis and cell collections, Terumo BCT have developed a grant program to recognise and support the efforts of clinicians, physicians and operators who are furthering the practice of apheresis and patient care around the world.

The Advancing Apheresis Award will be awarded to the winning site that has best displayed its on-going dedication to advancing apheresis.

This is an educational grant of $10,000 awarded to support and recognise outstanding efforts in the advancement of apheresis and patient care. With this award, Terumo BCT will support further efforts in research, education, training, and or improvements in patient care in the areas of therapeutic apheresis and cell collections.

Applications for the Advancing Apheresis Award will be accepted from 12 May 2014 and must be received by 5 September 2014, the award winner will be announced in October 2014.

Application evaluations will be based on:
- Educational leadership
- Patient care, comfort and impact
- Stated plan for use of the grant award

An international third-party committee, consisting of one recognised and independent key opinion leader from each global region will make all award decisions. Grant Committee members have been selected based on their leadership role in the practice of patient care in therapeutic apheresis and or cell collections, within their region. The Grant Committee will fairly select the winning site based on the scores achieved on a set of pre-determined criteria.

WWW.TERUMOBCT.COM/advancingapheresis

Salvage Therapy in Patients with DLBCL

Professor Anna Sureda, Spain, chaired the cti Life Sciences symposium entitled 'Is Salvage Therapy Possible in Patients with DLBCL (Diffuse large B-cell lymphoma) after Failure of Standard Second Line Regimens?' on Sunday 30th March.

The symposium highlighted clinical perspectives on various challenges faced in everyday practice of the treatment of relapsed and refractory patients.

The presentations assed the strengths and limitations of current therapies and in turn discussed the data that supports new approaches to treatment in this area.

Professor Sureda, opened with a welcome and introduction talk, summarising the purpose and content of the symposium. She discussed the fact that although valuable advances in understanding and managing DLBDL have been made thus far, to further improve the outcome for such patients significant challenges must be overcome.

Professor Christian Gisselbrecht, France, discussed the outcome of patients who failed R-DHAP or R-ICE in CORAL study.

This was followed by a presentation entitled 'Outcome of chemo-insensitive patients on PIX301 and late stage salvage with pixantrone - the German experience' by Professor Norbert Schmitz, Germany.

Before her concluding remarks, Professor Sureda discussed the role of allogenic stem cell transplantation in DLBCL, and potential registry study for EBMT in chemo-insensitive transplant eligible patients.

Immunotherapeutic Options following Haploidentical HSCT

On Sunday, 30 March 2014, Kiadis Pharma, a clinical stage biopharmaceutical company focused on the development of new therapies for late stage blood cancers and related disorders held a symposium highlighting the use of their lead product, 'ATIR' ™ in immunotherapy.

'ATIR™ as immunotherapy following haploidentical HSCT: No need for prophylactic immunosuppressants due to low risk of GvHD' was the title of this symposium. Dr Armand Keating, Toronto, Canada, started off the discussion with a general introduction.

In the subsequent presentation, 'Challenges of alternative donor transplantation', Dr Irwin Walker, Canada, went on to discuss the challenges as well as the opportunities to solve generic problems in transplantation.

Dr Stephan Mielke, Germany, went on to discuss the use of new strategies to improve immune reconstitution after haploidentical stem cell transplantation to reduce infectious complications in his presentation, 'Selective depletion to overcome challenges in allogeneic transplants'

He explained that in order to improve outcomes of both matched and mismatched transplants, *ex vivo* and *in vivo* selective depletion of alloreactive T cells using immunotoxins, suicide genes and photodynamic procedures have become the focus of todays translational transplant approaches.

Dr Denis-Claude Roy, Canada, presented results from a phase I clinical trial of haploidentical stem cell transplantation in which patients were administered with increasing doses of T cells treated with ATIR process. The results confirmed the clinical potential of this immunotherapeutic strategy and urged the development of a phase II trial to evaluate ATIR in a larger number of patients.

Dr Robert Preti, USA, then continued with a presentation entitled 'Change control during process development: How the application of Quality by Design (QbD) resulted in stable and robust ATIRTM manufacturing'.

Dr Preti reviewed the basic principles of QbD and how they were applied to the development program of ATIR, a promising cellular therapy in oncology.

Finally, ATIR™ was compared with other transplant strategies in the talk given by Dr John Barrett, USA.

Future Visions for Cellular Therapy

Sunday, 30 March 2014, Professor Franco Aversa, welcomed attendees to the EBMT Satellite Symposium supported by Miltenyi Biotec.

Professor Aversa chaired the symposium titled 'Cellular therapy: Facts, developments, future visions'.

Stefan Miltenyi, Germany, discussed novel therapies in his opening presentation: 'Enabling novel therapies'.

The topic then moved on to allogeneic HSCT with Nicolaus Kröger, from Germany, discussing the use of stem cell boost as a rescue therapy for patients with poor graft function after allogeneic HSCT.

Koen Van Besien, from the USA, gave an update on engraftment kinetics and long-term outcomes. This focused on cord blood transplantation supported by third party donor cells.

In two separate presentations, which followed, Franco Locatelli, from Italy, and Professor Aversa focused on *ex vivo* TCRα/β and CD19. In the first, Franco Locatelli presented results from a single-centre trial on *ex vivo* TCRα/β and CD19 depletion in haploidentical SCT for children with hematological disease.

Following this presentation, Professor Aversa discussed the *ex vivo* TCRα/β/CD19 T and B cell depletion in HSCT for treatment of adult patients with hematological disease.

Finally Professor Aversa concluded the symposium discussions and summed up cellular therapy and future visions.

New Treatment Options for Relapsed Refractory Lymphoma Patient

On Sunday 30 March, a multidisciplinary panel of experts took part in a Takeda-organised and sponsored satellite symposium to discuss how innovative targeted therapies can be used to offer new hope to patients; 'Managing the Relapsed Refractory Lymphoma Patient in the Era of Targeted Therapies'.

Following the failure of stem cell transplantation, patients with relapsed/refractory (R/R) Hodgkin lymphoma (HL) or systemic anaplastic large cell lymphoma (sALCL) faced a poor prognosis in previous year. Similarly, those unable or unwilling to undergo transplantation had few treatment alternatives available to them.

New treatment options resulting from the development of effective targeted therapies are gradually changing this for patients and their physicians.

This symposium began with a welcome and faculty introduction from the Chair, Mohamad Mohty, who then went on to discuss the Current state of the art: SCT in lymphoma.

During the symposium the multidisciplinary panel discussed how treatment for patients with R/R lymphomas is changing.

Targeted therapies and their impact on relapsed/refractory lymphoma treatment was the focus of Peter Dreger's talk.

Simonetta Viviani then talked about transplant-ineligible patients and posed the question if any progress has been made in their management?

In addition the speakers touched on the latest clinical data for brentuximab vedotin in post-transplant and transplant-naïve/ineligible patients with R/R HL or R/R sALCL.

Risk Factors for Fungal Infections

On 30 March 2014, Johan Maertens from the University Hospitals Leuven, Belgium, chaired the Gilead sponsored symposium entitled 'Considering the host – risk factors for fungal infections'.

Dr Maertens began the symposium by welcoming the delegates and providing a brief introduction at the EBMT meeting in Milan.

In this interactive and thought-provoking symposium the speakers reviewed primary and secondary immune deficiencies and genetic risk factors in the risk of fungal infections. Clinical cases where reviewed and discussed to provide additional information and achieve a deeper understanding of the risk for fungal infections.

Dr Emmanuel Roilides from the Hippokration Hospital, Greece, followed the welcome and presented a detailed look at primary immune deficiencies and fungal infections.

From the Necker Hospital, Imagine Institute, France, Anne Puel looked at Genetic risk factors for fungal infections.

In two separate presentations Dr Georg Maschmeyer from the Klinikum Ernst von Bergmann, Germany, looked at the issue of immune deficiency. Dr Maschmeyer focused on host factors and discussed secondary immune deficiencies and immunomodulatory treatments In the first of these presentations

With the aid of patient case examples, Dr Maschmeyer went on to further clarify immune deficiency in his second presentation.

TREOSULFAN - In Stem Cell Transplantation

The use of Treosulfan in stem cell Transplantation was discussed in a Medac-sponsored symposium on the 30 March, at the 40th Annual Meeting of the EBMT. The symposium was jointly chaired by Professor Fabio Ciceri from the Scientific Institute H. San Raffaele Hematology and Bone Marrow Transplantation, Italy, and Professor Franco Locatelli from the Department of Pediatric Hematology and Oncology IRCCS Ospedale Pediatrico Bambino Gesù, Italy.

The symposium was entitled 'TREOSULFAN- A Conditioning Agent on Its Way to an Established Alternative in Stem Cell Transplantation' and started with a welcome and introduction from Professor Fabio Ciceri.

TREOSULFAN as conditioning agent in haematopoietic stem cell transplantation was the focus of this symposium. The presentations considered the current status and the future perspectives of the clinical development of this agent.

In particular recent results of the use of an effective alkylating agent with obviously limited organ toxiticity as an alternative for conditioning therapy were discussed.

The presentations included:
- Treosulfan in Adult Patients with AML and MDS
- Treosulfan Based Conditioning pre-Allo-SCT for Lymphatic Malignancies
- 8 Years Italian Experience in Haploidentical HSCT
- Treosulfan Based Conditioning in the US for Patients Undergoing Umbilical Cord Blood Transplantation
- Treosulfan Based Conditioning in Children with Primary Immunodeficiency
- Myeloablation & Low Toxicity in SCT - A Contradiction?

The Next Level of Cell Manufacturing

Effectively any cell type can be processed and separated with the CliniMACS® System, which was on display at the Mitenyi Biotec booth during the EBMT meeting.

The CliniMACS Cell Separation System was first introduced in 1997 and has since become a proven technology for the development and establishment of cell-based therapies.

The new CliniMACS Prodigy now offers a flexible platform enabling the separation of virtually any cell type as well as customised separation protocols that meet specific sample requirements.

CliniMACS Prodigy® represents the next generation in automated cell processing. This integrates the MACS® Technology into a fully automated cell processing device that offers advanced solutions for streamlining cell processing workflows. This GMP-compliant device offers advanced integrated solutions to streamline cell-processing workflows: from cell fractionation through cell culture to formulation of the final product. With this flexible technology platform, fully automated cell processing for innovative and complex cell manufacturing protocols becomes a reality.

The device combines CliniMACS® Separation Technology with a wide range of sensor- controlled, cell processing capabilities. This provides a system that automates and standardizes complete cellular product manufacturing processes allowing easy access to complex procedures. More automation, fewer manual processing steps and reduced cleanroom requirements also allow a cost-effective benefit in cell product manufacturing with this CliniMACS Prodigy®.

With a high level of standardisation, the device has eradicated the need to skilfully handle different appliances and has reduced operator-to-operator variability and handling errors.

For more information please visit www.clinimacs-prodigy.com

Information Management Tool for Cryo-banks and Labs

CryoAbility™, a new information management tool for cryo-banks and controlled contamination Labs was showcased SIAD Healthcare.

This software enables bio-bank operators to manage any system and instrument used in the collection, preparation and storage of biological samples, in compliance with standards and procedures.

Features of the CryoAbility™ management system are as follows:

- Client- server architecture, with web-based interface
- Easy to use and navigate operator interface
- Bio-bank access and presence control
- Automation and safety systems monitoring and management
- Real-time event and alarm
- Creation of logs with the information collected
- Interaction with the samples database for relevant data cross-referencing
- Possibility for customization and implementation of additional modules

CryoAbility™ is synonymous with:
Liability towards operators– understanding the high level of responsibility associated with bio-banking, the system has capacity to assist and guide the actions of individuals

Traceability of all events – the system has the capability to detect, realize and save all events affecting the functioning of a bio-bank and the stored samples.

System and performance reliability – CryoAbility™ has been designed according to the GAMP (Good Automated Manufacturing Practice) standards and ensures the highest degree of reliability.

Tailor-made adaptability – with user-friendly features, information is provided clearly and concisely.

Capability of continuous growing – designed in accordance to cutting edge technologies, CryoAbility™ is suitable for expansion with the addition of new modules.

For more information visit www.siadhealthcare.com

The Bags of STEM CHOICE®

SIAD Healthcare also had on show STEM CHOICE, the new line of the brand [SAFE2]® at this year's EBMT meeting.

Developed to ensure the greatest safety for bio samples, [SAFE2]®, is the first double bag for cryopreservation up to -196°C. The inside bag contains the sample, while the outer bag protects them.

The freezing bags are made of biocompatible material, EVA (Ethyl Vinyl Acetate). With a new formulation that makes the product particularly flexible and highly resistant to the operating temperature range (-196 °C to +37 °C).

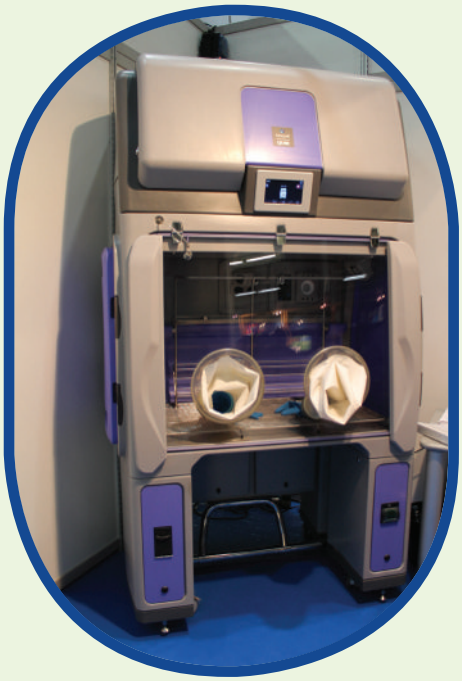
The Overwrap Bag provides a extra protections for samples by ensuring good cryoconservation and preventing the risk of cross-contamination.

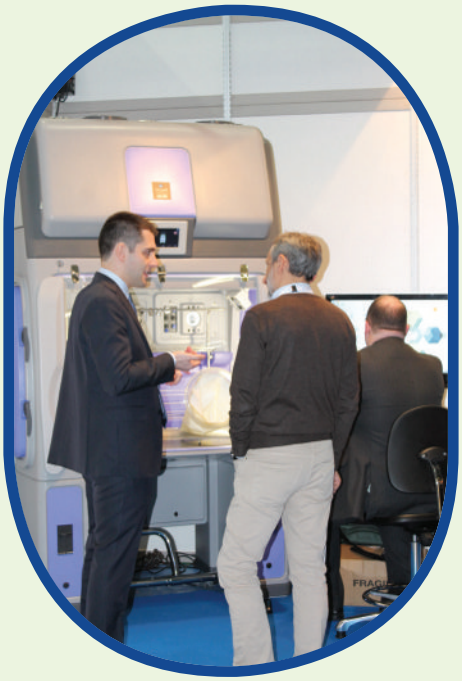
With the same performances of the inside bag the material also allows for further resistance with final welding.

In addition to this, it is possible to realise a double welding, obtaining a "pocket" for the test-segments.

For more information please visit http://www.siadhealthcare.com

Eradication of False Positives in Sterility Testing





Bioquell QUBE, an economical modular aseptic workstation system with built-in rapid bio-decontamination was on display at the Bioquell stand at the EBMT meeting.

The Bioquell QUBE has been designed to enable the sterility test process to be carried out in an aseptic working environment for the testing of small, medium or large batches of samples with reduced chances of false positives.

The combination of a uni-directional airflow, an ISO 5 and Grade A working environment, hydrogen peroxide vapour (HPV) and rapid bio-decontamination ensures the Bioquell QUBE is suitable for applications such as pharmacy compounding, sterility testing, and many others. Performance is assured by using Bioquell approved hydrogen peroxide solution. The Bioquell QUBE modules allow a variety of configurations that tailor the workspace to the needs of the specific process.

Key features and benefits include:
ISO 5 Grade A chamber with uni-directional airflow to meet the required regulatory standards for sample and operator protection.

User selectable for operating at -100 pa to +100pa depending on application requirements. 316 grade stainless steel floor with optional integrated sterility test pump

Ergonomically designed to maximise user comfort, the Bioquell QUBE has integrated foot switches for door operation, a foot rest and can be supplied with a height adjustable stool for optimum working conditions. Glove ports supplied with safe change gloves for comfort and an optional easy to use in-built glove leak tester

The Bioquell QUBE allows fast decontamination cycles for highest throughput and maximum responsiveness to support productivity in either sterility test or pharmaceutical compounding. All features are operated from an intuitive, icon-driven built in touch screen.

Hydrogen peroxide is supplied in 150ml RFID tagged bottles for traceability and documentation is available through a printer incorporated into the system legs. Also available with optimised chemical and biological indicators to ensure performance.

Learn more about the Bioquell QUBE here, **www.bioquell.com**

Web Software Solutions

A dedicated, specialised web software solution for tissue banks and cellular therapy, T.C.S. (Tissue, Cord Blood, Stem Cells) solution was presented by MAK-SYSTEM.

MAK-SYSTEM International Group is a leading medical information system solution provider who has been providing a broad portfolio of information management system to healthcare organisations worldwide since its inception in 1984.

Few healthcare operations are as complex as managing blood components and derivatives. Blood banks, hospital transfusion services, plasma collection centers, tissue banks and cellular-therapy laboratories require robust, reliable and secure software systems to track components and products through the complex supply chain.

Specialised in delivering innovative software solutions with state-of-the-art functionality MAK-SYSTEM provides a fully web based, off the shelf solution for this field. T.C.S. is a complete and effective solution for the management of all facets of tissue, cord blood and stem cells processing, from collection to transplantation.

T.C.S. meets the diverse operational requirements of the many tissue banks, cord blood banks and cellular therapy laboratories through the use of dictionaries, functional parameter switches and business rules.

T.C.S. is designed for use by diverse of organizations that operate in a variety of ways, ideal for new implementations and conversions of inherited systems.

Based on current IT standards T.C.S. provides higher capabilities of integration, maintainability and continuity of services compared to other competitor technologies.

In addition the functionalities are continuously updated to meet the needs of its international users.

Modular software, the T.C.S. has the following capabilities:

- Donor Management – Connection to the national donor lists or manually registering the donor
- Collection Management – The option to register the collection at the collection at destination or directly at the collection site
- Laboratory Management – The option to connect to Lab devices or to manually enter results
- Process Management or Transformation – The capacity for user to design and improve their own processes
- Medical Release – The ability to set rules for medical releases
- Inventory Management – The ability to localise and manage stock
- Shipment Management – Tracking of product date until transplantation
- Recipient Management – Monitoring the recipient's condition
- Reports / Statistics/ Labeling – Facilities to customize and generate reports and statistics

For more information visit www.mak-system.net

Measurement of CMV- Reactive Effector Cells

A test to determine the functionality of cell-mediated immunity (CMI) of CMV seropositive patients was showcased by Lophius Biosciences. The test enables a semi-quantitative evaluation of the CMV-specific immune competence of these patients.

The T-Track® CMV assay is based on the highly sensitive ELISpot technique (Enzyme Linked Immunosorbent Spot). This method enables a highly specific and sensitive detection of CMV-specific protein-reactive effector cells. In principle, the ELISpot method is a solid-phase ELISA.

Blood leukocytes (PBMC) are incubated with selected CMV proteins on a membrane saturated with IFN-γ-specific antibodies (capture antibody). The cytokine IFN-γ, secreted by reactive cells, is captured by the capture antibodies immobilized on the membrane. Cells are removed after 19 hours of stimulation. A second enzyme-conjugated IFN-γ-specific antibody (detection antibody) marks the bound IFN-γ. A soluble substrate is added to each well. By the enzymatic conversion of the soluble substrate to an insoluble precipitate, spots are generated on the membrane, representing footprints of antigen-reactive, IFN-γ-producing cells. The spots can be detected and counted either with a microscope or with an automatic imaging system (ELISpot reader).

This highly sensitive test method makes it possible to detect secreted cytokines as specific markers of the immune response on the level of individual cells.

For more information visit www.lophius.com

The Most Efficient Vapour Freezer

The Treatment Strategies team visited the MVE Chart booth at the EBMT meeting, where the latest cryogenic freezers, were being showcased.

Cryogenic freezers are greatly used in the biological storage of human tissue, cord blood, bone marrow, stem cell and other highly sensitive biotech and pharmaceutical applications.

With cryogenic freezing it is necessary to produce temperatures cold enough to liquefy gases, and cryopreservation often occurs through the use of liquid nitrogen.

To maintain sample viability over time, depending on the material being stored these can be stored in the extremely low temperatures produced by a cryogenic freezer.

In order to improve products that better fulfil the needs and requests of users Chart MVE's newest member is the redesigned MVE HEco series. The MVE HEco Series has been updated to provide a more aesthetically pleasing cryogenic freezer, along with technical improvements, which offer even greater functionality. Providing a 20% reduction in LN2 usage the new MVE HEco freezers are amongst the most efficient vapour freezer available.

These new highly efficient freezers incorporate hinged work platforms that fully enclose al electronics and plumbing to enhance safety and usability. The hinged work platforms offer a specified area to rest tools or samples before, during, and after transfer of samples. They also serve to protect the wiring and plumbing of the MVE HEco Series cryogenic freezers by keeping them fully enclosed so there is no chance of unintended contact.

The MVE HEco Series freezers provide maximum storage density as well as the industry's longest hold time and highest sample security.

The cryogenic freezers in the MVE 1500 Series -190°C provide storage for up to 42,000 1.2 / 2.0 ml vials. These freezers provide maximum storage density and provide the industry's longest hold time with comparable cryogenic freezers.

Global Leader in the Science of Extracorporeal Photopheresis, an Immune Cell Therapy

At the 40th annual meeting of the EBMT the Treatment Strategies team met with Therakos and discussed the photopheresis system, CELLEX®.

Therakos, Inc. has developed and marketed the only integrated systems for Extracorporeal Photopheresis (ECP) for over 26 years. Therakos is committed to advancing immunoscience and serving scientists, physicians and the patients who suffer from a variety of debilitating immune-based diseases. THERAKOS® Photopheresis systems are the world's only clinically validated, automated and integrated photopheresis systems CE marked to perform photopheresis. Proprietary system is designed to achieve photopheresis via a unique one-step process that is user and patient friendly, fast, efficient and safe.

THERAKOS® CELLEX® Photopheresis System

The CELLEX® System's features make the technology easy to use:

- Ability to treat in double- or single needle mode and to effortlessly change from one to the other
- Versatile, user-friendly touch-screen
- Automatic calculation algorithm of photoactivation time and the required dose of methoxsalen

The CELLEX® System's double – or single-needle mode enables:

- Treatment to be completed in approximately 1.5 hours
- Optimal fluid management

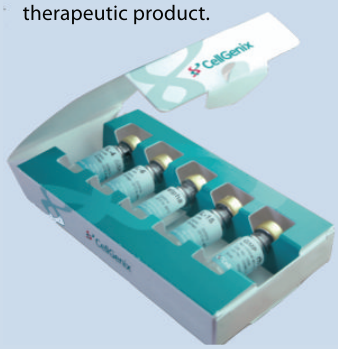
For more information visit www.therakos.co.uk

High Quality GMP Reagents for Clinical Cell Culture

An internationally leading manufacturer and supplier of GMP cell processing reagents, CellGenix had on display their range of High Quality GMP Reagents For Clinical Cell Culture.

Recent advancement in cell culture technology has driven developments in both stem cell research and regenerative medicine. CellGenix operates a state-of-the-art GMP facility for production of cell-processing reagents that include cytokines and serum-free media for this expanding market.

Cytokines, growth factors and media are commonly used in the processing of cells for therapeutic applications. The quality of these products is crucial for the quality of the finished therapeutic product.



Ancillary reagents are biological land chemical substances that are used in the manufacture of the therapeutics based on or derived from cell culture. They are not intended to part of the final product.

Cellgenix continuously expands its portfolio of serum-free media, cytokines and other cell supplements according to the needs of the growing cell therapy field.

Cellgenix offer a diverse set of high quality products for various cell types developed for different indications and therapeutic approaches which include:

- Dendritic Cells
- Hematopoietic Stem and Progenitor Cells T- Cells
- Chondrocytes
- Embryonic Stem Cells and Induced Pluripotent Stem Cells
- Mesenchymal Stromal Cells
- Natural Killer Cells

For more information visits;
www.cellgenix.com

Leaders in Transfusion Medicine

With the evolution of transfusion medicine towards new cell therapies, Macopharma has diversified into biotherapies and is developing a wide range of products for cell and tissue therapy, regenerative medicine and transplantation. With a prominent booth at this years annual EBMT meeting visitors had the opportunity to discover the various products from the Photophoresis and Cord Blood ranges.

Macopharma has become one of the leaders in the field of Transfusion medicine and their products are used in more than 70 countries, they are one of the largest suppliers of blood bags and in-line systems for leucodepletion worldwide.

The Biotherapy range of products provided by Macopharma presents a solution for every step of the cellular therapy process from collection, processing and expansion of stem cells, up to the treatment and transplantation of cells or organs to patients.

Cord Blood: A complete range of devices and bags for collection, processing, freezing and expansion of cord blood Hematopoietic stem cells to achieve an optimal collection volume and maximise cell recovery.

Bio Banking: Macopharma has developed EVA (Ethyl Vinyl Acetate) freezing bags suitable for cell cryopreservation. These bags are designed to tolerate long-term storage at temperatures down to -196°C, facilitating the optimal storage of cells and tissues.

Cell culture: Innovative closed systems and media for expansion & preservation of stem cells in GMP conditions for clinical application.

Photopheresis: Extracorporeal Photochemotherapy (ECP) has been recognised as a therapeutic option to treat various disorders, such as:

- Cutaneous T-cell lymphoma (Sezary syndrome) . GVHD (acute and chronic)
- Organ transplant rejection
- Autoimmune disorders

Macopharma has developed a range of products to treat patients with the Macopharma ECP technique: THERAFLEX-ECP.

Transplant: In partnership with academic research teams in France, Macopharma has developed organ preservation solutions and medical devices designed to optimise organ collection, preservation and transplantation.

Please visit www.macopharma.com

MHC Multimer & ELISPOT Proficiency Panels

Immudex had on display information on the worldwide MHC Multimer and ELISPOT Pro-ficiency Panels, which they are conducting in collaboration with the CIC (the US Cancer Immuno-therapy Consortium of the CRI) and CIMT (the European Association for Cancer Immunotherapy).

In order to achieve efficient translation of immunological knowledge into clinical therapies it is crucial for T cell assays to be both reliable and reproducible.

Since 2005 the CIC and CIMT have held a series of proficiency panel studies to improve the intra- and inter-lab reproducibility of the Multimer and ELISpot (Enzyme Linked Immunosorbent Spot) immunoassays suitable for clinical trial monitoring.

Proficiency panels are held once a year allows the testing of a lab's ability in performing an immune monitoring assay. It also allows for anonymized comparison of the lab's performance with peers in the field. Participants whose results differ significantly from their peers' results are offered help to identify changes to the protocol that might improve the participant's performance.

Any lab or person may participate, whether working in basic or clinical research, and in any field, e.g. Cancer Vaccines or Cell Therapy, Auto-immune diseases e.g. MS and Diabetes, Infectious diseases e.g. HIV, TB, Malaria, Measles, Hepatitis, CMV, or any other area where reliable immune monitoring is important.

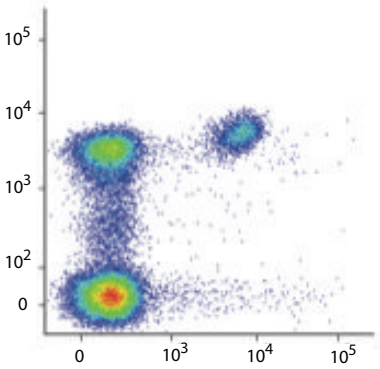
This practice has led to the documentation of a series of 'Harmonization Guidelines' that define critical parameters for performing reliable and reproducible immunoassays.

By participating in proficiency panel participants can:

- Validate the proficiency of their lab
- Build credibility
- Accelerate research and development
- Benchmarking
- Training of new personnel
- Ethical responsibility

For registration and more information visit:
www.proficiencypanel.com

ELISpot Assay



MHC Multimer Assay

Abbott Molecular and Real-time PCR

The Treatment Strategies' team visited the Abbott Molecular stand at EBMT Milan and were given an insight into their AlleleSEQR Chimerism Assay (RUO).

The AlleleSEQR Chimerism assays are real-time quantitative polymerase chain reaction (PCR) assays based on TaqMan® chemistry. Real-time PCR has been shown in various studies to quantitate target nucleic acid samples over an 11-log dynamic range in optimised conditions (100 billion-fold differences in starting copy number; Nolan, *et al.*, 2006). Comparison of cycle thresholds (CTs) from different samples can be used to determine the relative amounts of DNA in two different samples (Livak and Schmittgen, 2001).

This highly sensitive PCR method is the result of the very large dynamic range of the real-time amplification method and is limited essentially by the input copy number of total genomic DNA that can be added to the PCR reaction.

For more information visit www.abbott.com

FIRST APPROVED LIFE-SAVING TREATMENT FOR SEVERE HEPATIC VENO-OCCLUSIVE DISEASE

Jazz Pharmaceuticals plc and Gentium S.p.A., a Jazz Pharmaceuticals company have announced the commencement of the European commercial launch of Defitelio®q (defibrotide), the first licensed product for the treatment of severe hepatic veno-occlusive disease (severe VOD or sVOD) in patients over one month of age undergoing haematopoietic stem cell transplantation (HSCT) therapy.[1] The companies have launched Defitelio in Germany and Austria and expect to continue the launch in 27 additional European countries on a rolling basis during 2014 and 2015.

Severe VOD, one of the most serious early complications in HSCT therapy, is associated with multi-organ failure and is fatal in over 80% of patients.[2,3] HSCTs are performed with curative intent in patients with haematological malignancies, selected solid tumours and some non-malignant disorders, such as serious haemoglobinopathies.[4,5]

"The commercial availability of Defitelio as the first medicine licensed for the treatment of sVOD in Europe is an important step forward for patients with this life-threatening condition," said Bruce C. Cozadd, chairman and CEO at Jazz Pharmaceuticals plc. "Additionally, this European launch represents a key milestone for the combined Jazz Pharmaceuticals and Gentium team following the acquisition of Gentium by Jazz Pharmaceuticals earlier this year, and reinforces our commitment to bringing important therapies to patients who have significant unmet medical needs in the areas of haematology and oncology."

"Severe VOD is a complex and unpredictable disease, and its impact on patients, physicians and resources is substantial. Early and effective intervention is crucial in saving lives and limiting the potentially significant burden of this disease, and physicians have been eagerly awaiting the commercial availability of Defitelio in Europe," said Professor Mohamad Mohty, President-Elect of the EBMT and Professor of Haematology, Saint-Antoine Hospital and University Pierre & Marie Curie, Paris.

The efficacy of Defitelio to treat sVOD in HSCT patients is supported by data from a pivotal, multi-centre Phase 3 trial that evaluated Defitelio for the treatment of sVOD compared with a historical control group of patients who had received standard supportive care.[1] In this trial, Defitelio was shown to provide a significant increase in survival rates for patients with sVOD in HSCT. The results demonstrated a 52% increase in survival at 100 days after transplantation for patients treated with Defitelio compared to patients in the historical control group (38.2% in the Defitelio group vs. 25.0% in the historical control group; p=0.0341).[1] In the clinical trial, 23.5% of patients treated with Defitelio achieved complete response at 100 days after transplantation versus 9.4% of patients in the historical control group (p=0.013).[1]

The efficacy data from this pivotal trial are supported with data from a Phase 2 dose-finding study, as well as data from the International Compassionate Use Programme and an interim analysis (subset of patients with sVOD) of an ongoing, open-label treatment investigational new drug (IND) study being conducted in the United States (U.S.).[1] Additionally, data derived from an independent registry in the U.S. supported the European approval of Defitelio for use in patients with sVOD.[1]

Treatment with Defitelio has generally been well tolerated in all age groups.[1,6] In the Phase 3 pivotal trial, the overall incidence of adverse events was similar in the Defitelio treatment group and in the control group.[7] The most frequent adverse events observed during pre-marketing use were haemorrhage, hypotension and coagulopathy.[1] Please consult the Defitelio SmPC for the full list of all side effects reported with Defitelio.

References
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3. Coppell JA et al. Biol Blood Marrow Transplant 2010;16:157–168
4. Tsakiris DA & Tichelli A. Best Pract Res Clin Haematol 2009;22:137–145
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Xuri™ Cell Expansion System W25

GE Healthcare Life Sciences launched Xuri™, a new technology family designed to support and advance the field of cell therapy in November 2013.

The Xuri Cell Expansion System W25 that has been specifically designed for cell therapy manufacturing applications was showcased at the EBMT meeting.

This functionally closed system is based on the proven WAVE™ rocking technology, which provides mixing and aeration to the culture, resulting in high cell densities, whilst minimizing the risk of contamination. The system is enhanced by sensors to monitor your cell expansion, and is operated via UNICORN™ control software.

Xuri Cell Expansion System W25 offers the following benefits:

• Minimized risk of cell contamination

• Reach high cell densities with confidence

• Advanced control of cell expansion through process monitoring and remote operation

• Suitability for use in a regulated environment

The system delivers reliable and accurate performance across research, process development, and manufacturing environments.

System Benefits

Minimised risk of cell contamination

Xuri Cell Expansion System W25 is a functionally closed system, which minimizes the risk of contamination between different patient samples or with adventitious agents. The cells are grown in a single-use Cellbag™ bioreactor.

High cell densities and perfusion culture

The system is designed for both batch and perfusion culture. Perfusion culture in a single Cellbag bioreactor results in high cell density, which is often needed for cell therapy purposes. This means that an entire dose of cells for a patient can be obtained in one functionally closed bioreactor system without the need to open the vessels to change the cell culture medium. This also eliminates the difficulties of combining culture vessels as is necessary with manual systems. High cell density, together with the small instrument footprint maximizes use of limited manufacturing space.

Advanced control with process monitoring and remote operation

Xuri Cell Expansion System W25 is equipped with sensors and automated controllers for key culture parameters— rocking speed, dissolved oxygen (DO), pH, and perfusion rate—which enable cell specific optimisation of the cell expansion culture environment and production of high- quality and high-density cells.

Once a culture is underway, data is logged and recorded in the base unit as well as in the software. The system can be monitored and controlled remotely and it can send e-mail alerts if user-defined limits are exceeded. These features provide the monitoring and tracking necessary in a regulated environment .

Designed for use in a regulated environment

Xuri Cell Expansion System W25 is designed to meet the demands and standards required in a regulated environment. Documentation accompanying the system includes material certificates, system specifications, installation and operational qualification (IQ/OQ) protocols, and a detailed user manual. UNICORN software is suitable for use in a manner that complies with 21 CFR Part 11 and Good Automated Manufacturing Practice (GAMP) 5.

Xuri Cell Expansion System W25 is designed for cell therapy manufacturing applications

Poster Synopsis

Implementation of Nutritional Screening and a Treatment Specific Care Pathway for Adult Transplant Patients

Zoe Hull

Specialist Paediatric Dietitian, Bristol Royal Hospital for Children, Bristol, UK

Introduction

Haematopoietic stem cell transplant patients receiving intensive conditioning regimens that manifest severe mucositis and gastrointestinal toxicities are at risk of malnutrition both during and after transplantation.[1] Malnutrition and weight loss have been adversely linked with prognosis, increased infections and reduced survival.[2] Optimal nutrition support therefore becomes essential early in the course of treatment.[3] Many patients now receive 'reduced intensity conditioning', which does not predispose the patient to the same degree of mucositis and so patients can often maintain oral diet. Initial assessment and ongoing monitoring of nutritional status can help to establish the degree of nutritional risk and decide an appropriate nutrition support treatment pathway for high nutritional risk patients.

Implementing a Screening Tool

The Malnutrition Universal Screening Tool 'MUST' has been shown to be effective at identifying malnutrition risk amongst universal hospital inpatients.[4] National nutrition support guidance advises that all inpatients must be screen upon admission and all outpatients screened at their initial visit and thereafter upon clinical concern.[4] To promote a multidisciplinary approach to monitoring nutritional risk throughout the patient's transplant journey, an individualised nutritional screening care pathway using 'MUST' was developed for Bristol Bone Marrow Transplant Unit. A similar screening tool and care plan was developed for the day care unit to continue monitoring the patient's nutritional risk once they are discharged from the unit.

Development of a Treatment Specific Multidisciplinary Care Pathway

Each patient is screened using 'MUST'. A low, moderate or high risk care plan is then implemented by nursing staff. All patients identified as low to moderate risk commence on an oral nutrition support care plan and are rescreened weekly. Patients identified as high risk are referred to the Dietitian and advised nasogastric tube placement on the day of transplantation. This includes patients receiving high intensity conditioning, who are more likely to experience significant mucositis and a prolonged period (> 7 days) of minimal oral intake. Nutritional support in the form of enteral feeding is preferable to total parenteral nutrition while the gastrointestinal tract still functions[4]. The care pathway has helped guide staff to intervene more quickly in patients who likely to need artificial nutrition support.

Following discharge, all patients are screened weekly up to 100 days post-transplant, and thereafter upon clinical concern in clinic. An outpatient nutrition screening tool and care plan pathway was adapted from one in use at Bristol Oncology Centre. This has helped nursing staff to identify patients at risk of malnutrition, for example those with chronic graft versus host disease, and provide interim advice until the Dietitian can see the patient and assess.

Education and Resources

Nutrition education boards were designed for the ward and the day unit to raise awareness of the importance of nutritional screening amongst medical and nursing staff. Alongside this an education programme was rolled out for nursing staff, incorporating scenarios and patient case studies. Oral nutrition advice sheets were developed for nursing staff to offer oral nutrition support advice for low to moderate risk patients. Dietary advice booklets for the management of symptoms that can hamper oral intake were produced for the units.

Conclusions

Nutritional screening using 'MUST' and a treatment-specific care pathway can be implemented in a specialist HSCT inpatient and outpatient unit. This allows optimal monitoring and transition of nutritional care for patients during and after transplantation. Treatment-specific nutrition care pathways can help staff to pre-empt conditioning side-effects that may affect a patient's ability to maintain their nutritional status and intervene early on. Dietetic and nursing resources can be directed towards patients with the highest risk of malnutrition.

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Zoe Hull is the clinical lead specialist Dietitian for Oncology and Bone Marrow Transplant at Bristol Children's Hospital. The service treats both adults and children. Her professional interests lie in the dietary management of gastrointestinal complications in transplant patients. She was the lead Dietitian for paediatric gastroenterology over the past 7 years in addition to transplant. Previous publications have also focused on the importance of a multidisciplinary team approach to nutritional care in transplant patients.

Poster Synopsis

Cryotherapy Reduces the Severity and Duration of Oral Mucositis Following High Dose Melphalan in Multiple Myeloma Patients

C.L. Ottevanger,[1] M.C.M van der Lans[1] and E. Witkamp[2]

1. Department of Hematology ErasmusMc Cancer Institute Rotterdam, The Netherlands

2. Department of Medical Oncology and Public Health, ErasmusMc Cancer Institute Rotterdam, The Netherlands

Background

Oral mucositis (OM) is a toxic inflammatory reaction of the mucous membrane in the mouth where oedema, ulceration and/ or pain can occur. The ulcers increase the chance of bleeds and systemic infections, especially in patients with a compromised immune system (Dutch guideline OM 2007). The majority of multiple myeloma (MM) patients who receive high dose mephalan (HDM) as conditioning for an autologous stem cell transplant suffer OM as a result.[2]

Cryotherapy, in which the area around the mouth where the HDM is administered is cooled by the patient sucking on wafers of ice, could reduce the severity and duration of OM caused by HDM. The most common hypothesis to explain this effect is found in vasoconstriction caused by the cooling. This could reduce the local delivery of the chemotherapy in the mucous membrane of the mouth.

Materials & Methods

We conducted a literature search in Pubmed and Cochrane into all relevant English articles from 2004 wherein the affect of cryotherapy during HDM administration was investigated and described. The search terms used where "oral mucositis", Mephalan, "autologous transplantation" and cryotherapy.

Data from the randomised studies where the results concerned the effect on severity and duration of OM, tolerability of the intervention and the influence of using cryotherapy on the use of intravenous opiates and total parenteral nutrition (TPN) were reviewed. The reliability of the results (p-values, nurse training, use of validated measuring tools) and the size of the groups investigated where also considered.

Results

The literature search resulted in one Randomised Clinical Trial and three useable explorative investigations for answering the study research question. Also four systematic reviews were found and two letters to the editor.

Most of the research involved small patient numbers, average N=50 and the same measuring tools were not used in all the studies to determine the severity of OM.

In named studies there was a significant difference in cryotherapy versus no therapy found, variability from 3-14% grade 2-3 OM with cryotherapy versus 40 – 86% without cryotherapy.

Discussion

Because it differs how people can stand the coldness, which effects the quality of cooling, its predictable that outcomes will be different too. Nurses have an important role in well informing the patients about the intervention and coach them to keep on taking the ice as long as needed.

Conclusion

Cryotherapy is simple for nurses to implement, cost effective and easily tolerated by the patients (Vorkurka 2011). Despite the small size of the investigated cohorts and the fact that the implementation of a larger study is desirable, we recommend the administration of cryotherapy.

Leonie Ottevanger is a clinical nurse of the department of hematology at Erasmus MC Cancer Institute in Rotterdam, The Netherlands. She is interested in evidence based nursing and contributes to quality of care, with small literature research and implementing relevant outcomes.

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Role of Allogeneic Transplantation in Multiple Myeloma

Gösta Gahrton
Professor Emeritus, Medicine, Karolinska Institutet, Stockholm, Sweden

Introduction

Allogeneic hematopoietic stem cell transplantation (allo) is a curative method for several hematologic malignancies including acute leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia and myelodysplastic syndrome. However, irrespective of the transplantation method used, i.e. myeloablative or reduced intensity conditioning (RIC) (non-myeloablative) or variation of graft-versus-host disease (GVHD) prevention methods, the transplant-related mortality (TRM) is significant. Furthermore, the progress in drug treatment has made allo obsolete in some malignancies, particularly in up front treatment of chronic myelocytic leukemia where thyrosine kínase inhibitors provide excellent results.

Allo has been attempted in multiple myeloma since the mid 1980s.[1-3] Myeloablative conditioning was associated with very high TRM, amounting to 40 - 50 percent.[4,5] However, progress in supportive care and reducing the intensity in the conditioning has diminished the TRM to 10-15 percent.[6,7] In parallel with this improvement of the transplant procedure, new effective drugs have appeared for the treatment of multiple myeloma, being the reason for the debate about allo as an indication for the treatment of this disorder.[8-11] In this review, I will discuss the pros and cons with allo in multiple myeloma, mainly based on prospective trials using RICallo in tandem with autologous transplantation (auto/RICallo).

Gösta Gahrton, MD, PhD, Professor emeritus in Medicine at the Karolinska Institutet, Stockholm, is a leading haematologist worldwide. He was head of the Department of Medicine Karolinska University Hospital, Huddinge, from 1985 to 1998 and has been chairman of the Nobel committee at the Karolinska Institutet, 0 and of the World Marrow Donor Association (WMDA). He is honorary member of many international societies, among them the EBMT, and has received several international awards, among them the Robert A Kyle Life Time Achievement Award in 2013. He is the editor of four books, including "Myeloma and related disorders" (Arnold, London 2004). His research area is haematological malignancies with a focus on translational research and clinical application of stem cell transplantation, particularly in multiple myeloma. He has published more than 400 scientific articles.

Upfront Allogeneic Transplantation

Response

A complete remission (CR) or near complete remission (nCR) rate of between 40 and 60 percent can be obtained with allo.[12-18] The high response rate and CR rate obtained with myeloablative conditioning[4,18] can also be obtained with tandem auto/RICallo.[12-17] It appears to be more advantageous to perform the RIC/allo in a planned fashion after auto, in order to reduce the tumor burden before the RIC allo, than performing the RICallo upfront after the induction regimen.[19] As with auto the response is significantly associated with better progression-free survival (PFS) and overall survival (OS), i.e. the best outcome is obtained if CR is obtained.[5,20] In three out of the six prospective studies comparing tandem auto/RICallo to auto/auto or auto, the CR rate was significantly higher with the auto/RIC allo procedure (Table 1),[13-17] while in the remaining three studies there was no significant difference between the groups. The CR + VGPR was between 35 and 62 percent in the auto/RICallo groups, and between 11 and 51 percent in the auto/auto groups in these studies.

Previous studies have shown that a molecular remission among those patients that obtained hematologic CR was more frequently obtained with allo than with auto.[21] Also, obtaining a molecular remission translated into a longer PFS in the study by Corradini *et al*.[22] Progression was not seen in any of those patients that had obtained molecular remission until five years from the start of treatment. Thus, if a complete hematological remission was obtained with allogeneic transplantation, it could be predicted that there was a higher chance of having a better quality of this remission translating into a better outcome. This was supported by a recent study by the EBMT showing that CR obtained after auto/RICallo sustained for a longer period of time than when obtained after auto/auto.[20]

Non-relapsed Mortality (NRM) (or Transplant-related Mortality (TRM))

Allo using myeloablative conditioning is hampered by a very high TRM[4]. Initially, this amounted to 40-50 percent, however with

Reference	No. Pts Auto/RICAllo vs Auto Intention to Treat	CR Rate (%) Auto/RICAllo	Auto (Auto/Auto)	p value
IFM Garban *et al.* Moreau *et al.* Blood 2006, 2008	Intension to treat 65/219	62 (CR+VGPR)	51 (CR+VGPR)	NS
Italian Group Bruno at NEJM 2007 Giaconne Blood 2011	80 vs. 82	55	26	0.004
PETHEMA Rosinol *et al.* Blood 2008	25 vs. 85 (only high risk)	40	11	0.001
HOVON 50/54 Lokhorst *et al.* Blood 2012	122 vs. 138	43	37	0.67
BMT-CTN 0102 (USA) Krishnan *et al.* Lancet Oncol 2012	189 vs. 436 (standard risk B2micro< 4 mg/dl + 13qdel absent))	35 at day 57	26 at day 57	0.07
BMT-CTN 0102 (USA) Krishnan *et al.* Lancet Oncol 2012	37 vs. 48 (only high risk β2micro≥ 4 mg/dl + 13qdel)	41 (at day 57)	37 (at day 57)	NR
EBMT Björkstrand *et al.* JCO 2011 Gahrton *et al.* Blood 2013	108 vs. 249	50	41	0.02

+

Table 1. Auto/RIC allotransplantation versus autologous transplantation (or maintenance). CR rate.

improved supportive care, somewhat lower TRM may be obtained. A significant reduction in TRM was seen when the Seattle group introduced so-called reduced intensity or non-myeloablative conditioning.[6] In their experimental original trials in dogs, a dose of 200 cGy total body irradiation gave the best results.[23] However, due to a higher relapse rate than with myeloablative conditioning RICallo has been combined with previous autologous transplantation (auto) in order to diminish the burden of tumor cells before the allo.[6, 24] The tandem auto/RICallo transplantation modality has given a TRM between 12 and 17 percent in six studies.[12-17, 25] However, when prospectively compared to a TRM of only 2-4 % using single or tandem auto , the transplant-related mortality is still significant in the RICallo setting. Taking into account the risk of acute graft-versus-host disease (aGVHD) and chronic graft-versus-host disease (cGVHD), both morbidity and TRM are significant problems in allo for multiple myeloma.

Progression-free Survival

Progression-free survival (PFS) is superior if CR is reached irrespective if it occurs after auto[20, 26-28] or allo.[5, 20] However, according to EBMT[29] or International Myeloma Working Group (IMWG) criteria,[30] a CR (see above) obtained with allo is superior to the CR obtained with auto.[20] In the six prospective studies comparing auto/RICallo to auto/auto, PFS was superior after the auto/RICallo procedure in two[12-14] out of the six studies, while there was a tendency for superior PFS in two of the remaining four, one including only high risk patients that had not responded to previous treatment[17] (p= 0.08) and the other including only those patients that received the transplant as allocated[16] (not intention to treat p=0.07))(Table 2). However due to the initial higher treatment-related mortality with auto/RICallo, there was no significant difference in PFS at three years in the EBMT study corroborating with the results of the other studies at this time with the exception of the Italian study.

The actual PFS curves for auto/RICallo were relatively similar as were PFS at comparable time points in five of the studies (exempting the study by Garban *et al.*) i.e. the 3 year PFS in standard risk patients in BMT-CTN was 43% and the same as in all EBMT patients - 43%; the 6 year PFS in the donor group of HOVON54 was 28% and in the group that received the RICallo 35% as compared to the 5 year PFS on an intention to treat basis of 33% in the EBMT trial.

Overall Survival

Two out of the six prospective studies showed a significantly better OS with the auto/RICallo procedure (Table 3). In the four remaining studies, there was no significant difference. However, it has to be pointed out that in three of them long-term survival analyses were not performed.

Conclusions - Upfront Auto-RIC Transplantation

Although two of the six prospective studies clearly showed an advantage long term for the auto/RICallo procedure both concerning PFS and OS it has to be pointed out that in these studies induction treatment did not contain any of the new drugs. Both the EBMT and the Italian Group study used VAD (vincristine –adriamycine-dexamethasone) or VAD-like regimens upfront and the treatment of progression is so far not investigated. There are differences in the type of conditioning that was used. For example, the Italian group used only irradiation 200 cGy, while the EBMT study used 200 cGy + fludarabin. The HOVON study and the BMT-CTN study used only TBI 2 Gy, while IFM used fludarabin, busulfan + ATG, and the PETHEMA Group used fludarabin + melphalan 140 mg. The IFM included only patients with a high beta-2-microglobuline and chromosome 13q-, while the PETHEMA Group only included patients that had not reached CR or nCR with the first transplant. In the EBMT study, patients under 70 years of age were included and with a relatively equal distribution between 13q- and non13q-patients.

The treatment of progression in these studies varied greatly. New drugs were used frequently but there was no systematic study of their impact. In the EBMT study, the survival following progression was significantly superior in the auto/RICallo group[12]. This may be due partly to the use of donor-lymphocyte transfusions (DLI) but could also be associated with a better response in the auto/RIC allo group to some of the immune modulators that were frequently used. However, this has not been systematically studied.

Thus, although long-term results appear advantageous in some studies with the auto/RICallo procedure in the era of numerous new available effective drugs allo alone or in combination with auto cannot be recommended upfront in low or standard-risk patients. In high-risk patients such treatment should preferably be performed within clinical trials and novel drugs included both before and after the transplant.[31]

Allogeneic Transplantation in Progression or Relapse Patients

Most of the allogeneic transplants reported to the EBMT registry are performed following progression/relapse after a previous auto. In the NMAM2000 study, PFS was the primary endpoint. Therefore, treatment at progression was optional. Out of 205 patients in the auto or auto/auto group that had progressed, 11 patients received an allo following progression and 9 out of 64 patients in the auto/RICallo group received a second allo. Several retrospective and small phase II studies of patients receiving allo at progression or relapse have been published[32-37] but prospective comparative studies are lacking. With myeloablative transplantation, TRM has

Reference	No. Pts Auto/RICAllo vs Auto Intention to Treat	PFS/EFS Months (median) or Percent at Months		
		Auto/RICAllo	Auto (Auto/Auto)	p value
IFM Garban *et al.* Moreau *et al.* Blood 2006, 2008	Intension to treat 65/219	19	22	0.58
Italian Group Bruno at NEJM 2007 Giaccone Blood 2011	80 vs. 82	35	29	0.005
PETHEMA Rosinol *et al.* Blood 2008	25 vs. 85 (only high risk)	Median not reached	31	0.08
HOVON 50/54 Lokhorst *et al.* Blood 2012	122 don vs. 138 no don 99 allo vs 112 no allo	28% - 6 years 35% - 6 years	22% - 6 years 21% - 6 years	0.19 0.07
BMT-CTN 0102 (USA) Krishnan *et al.* Lancet Oncol 2012	189 vs. 436 (standard risk)	43% - 3 years	46% - 3 years	0.67
BMT-CTN 0102 (USA) Krishnan *et al.* Lancet Oncol 2012	37 vs. 48 High risk	40% - 3 years	33% - 3 years	0.74
EBMT Björkstrand *et al.* JCO 2011 Gahrton *et al.* Blood 2013	108 vs. 249	43% - 3 years 33% - 5 years 22% - 8 years	39% - 3 years 18% - 5 years 12% - 8 years	0.012

Table 2. Auto/RIC allo vs Autotransplantation (or maintenance). PFS/EFS.

Reference	No. Pts Auto/RICAllo vs Auto Intention to Treat	Overall Survival Months (median) or Percent at Months		
		Auto/RICAllo	Auto or Auto/Auto	p value
IFM Garban *et al.* Moreau *et al.* Blood 2006, 2008	Intension to treat 65/219	34	48	0.07
Italian Group Bruno at NEJM 2007 Giaccone Blood 2011	80 vs. 82	Not reached	51	0.001
PETHEMA Rosinol *et al.* Blood 2008	25 vs. 85 (only high risk)	Not reached	58	0.9
HOVON 50/54 Lokhorst *et al.* Blood 2012	122 don vs. 138 no don 100 allo vs 114 no allo	55% - 6 years 61% - 6 years	55% - 6 years 51% - 6 years	ns 0.38
BMT-CTN 0102 (USA) Krishnan *et al.* Lancet Oncol 2012	189 vs. 436 (standard risk B2micro< 4 mg/dl + 13qdel absent))	77% - 3 years	80% - 3 years	0.19
BMT-CTN 0102 (USA) Krishnan *et al.* Lancet Oncol 2012	37 vs. 48 (only high risk β2micro≥ 4 mg/dl + 13qdel)	59% - 3 years	67% - 3 years	0.46
EBMT Björkstrand *et al.* JCO 2011 Gahrton *et al.* Blood 2013	108 vs. 249	71% - 3 years 64% - 5 years 49% - 8 years	68% - 3 years 57% - 5 years 36% - 8 years	P = ns P= 0.022 P=0.020

Table 3. Auto/RIC allotransplantation versus autologous transplantation (or maintenance). Overall survival.

been high,[4, 5, 38] and even with RIC TRM is significant, amounting to 17% – 33%.[32, 33, 36, 39-41] In a large retrospective EBMT study comprising 320 patients (28% were progressive and 76% had received prior transplants) that had received RIC and 196 that had received MAC (21% progressive and 11% had received prior transplants) the non-relapse mortality (NRM) was 24% and 37% respectively.[42] However in the 28% progressive RIC patients the NRM was about twice as high as in responding patients (HR 1.96; 95% CI 1.12 – 3.14). Thus the TRM (or NRM) is in general considerably higher even with RIC when used at the time of progression as compared to upfront use after response to induction therapy.

More recent studies have shown encouraging PFS and OS using RIC despite significant NRM. In the study by Patriarca *et al.*[43] relapsed patients after auto that had an available matched donor received a RICallo transplant combined with novel drugs, while those who did not have a donor received only novel drug combinations. The 2-year PFS was 42% in the donor group and 18% in the no-donor group ($P < .0001$). The 2-year OS) was 54% in the donor group and 53% in the no-donor group ($P = .329$). No donor was a significant adverse prognostic factor for PFS and cGVHD had a protective effect on OS in the RICallo group.

In a recent long term follow-up study of 42 consecutive patients with advanced stage myeloma - 14 had relapsed or had progressive disease at transplantation – the ten- year PFS and OS were 43% and 45% respectively, with no obvious difference between high-risk responsive or progressive patients at transplant.[44] In another study[41] of 56 consecutive patients – 26 treated with allo as consolidation after auto and 30 treated at progression after a first auto PFS was 57% and 21% respectively at 5 years from treatment. OS was 82% and 38% respectively. Patients with cGVHD had the best results supporting a graft versus myeloma effect. Recently, the CIBMTR made a retrospective study of patients reported between 1995 and 2008 and who had relapsed or progressed after first auto[45]. A comparison was made between 137 patients who received a second auto transplant with those receiving an allotransplant (n=152) with non-myeloablative conditioning or RIC (NST/RIC). The NRM at one year was 13 percent in the NST/RIC group compared to 2 percent in the auto group. Three-year PFS and OS for the NST/RIC group was 6 percent and 20 percent compared to 12 percent and 46 percent in the group for auto. However, the study was hampered by unbalanced groups – response status before the second transplant was 39% CR/PR in the auto group and <1% CR/PR in the NST/RIC group. Furthermore, response status at transplant was missing in 78% in the NST/RIC patients, thus making the comparison of little value. Frequently allo is performed late in the course of the disease as a last rescue attempt. Then the patient is often in poor condition and the risk of performing allo is high.

Conclusion - Allotransplantation in Progressive and Relapsed Patients

Allotransplantation in the auto/RICallo setting may be an option in early relapse and progression. However late in the course of the disease when the patient is in a poor condition allotransplantation is usually unsuccessful due to high TRM. Improvements in supportive care and transplantation in specialised units with experience in allotransplantation have prospects for better outcome. Due to high relapse and progression rate with RICallo in this patient category trials using a somewhat higher nonmyeloablaive conditioning dosage are warranted.

Acknowledgements

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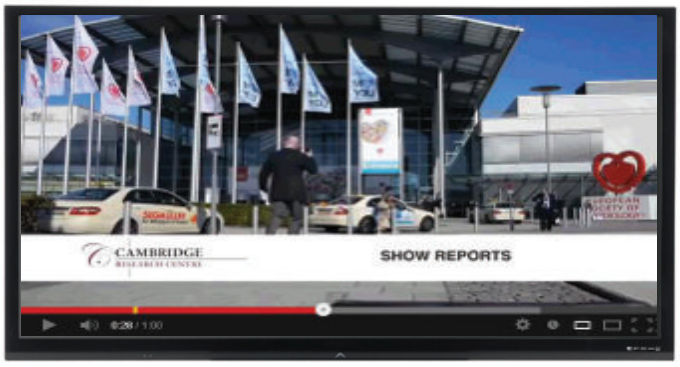

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Consequences of Cure: Survivorship after Allogeneic Hematopoietic Cell Transplantation

Nandita Khera
Assistant Professor of Medicine, Division of Hematology/Oncology, Mayo Clinic College of Medicine, Arizona, USA

Introduction

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative modality of treatment for multiple hematological disorders. Various advances in the field have improved the overall survival of patients undergoing this complex medical procedure.[1] Improved survival along with the increasing number of transplants being performed worldwide has increased the number of HCT survivors, with an estimated number of survivors at 250,000 by the year 2015.[2,3] This review will provide an overview of the late complications faced by these survivors, including issues such as employment/ financial challenges, fertility problems and caregiver stress. We will also summarise guidelines for screening and preventive care of allogeneic HCT survivors and the current state of science for delivery of long term follow-up care.

Late Complications

The multitude of medical and non-medical problems after allogeneic HCT has prompted HCT survivorship to be termed as 'A call to arms'.[4] Chronic graft vs. host disease (GVHD) is a unique late complication itself as well as predisposes to a host of other problems after an allogeneic HCT, with an impact both on longevity as well as quality of life of patients.[5-7] It occurs in 30 to 70% of HCT patients with a median time of onset between 4 to 6 months after HCT, though 5 to 10% cases can be diagnosed after one year of HCT.[8] Median duration of immunosuppressive therapy for treatment of chronic GVHD is 23 months with 15% of patients requiring therapy beyond 7 yrs.[9] Detailed discussion about the pathogenesis, risk factors and management of chronic GVHD is beyond the scope of this article.

Nandita Khera is an Assistant Professor of Medicine in the Mayo Clinic College of Medicine. She joined Mayo Clinic Arizona in 2011 and has served as a Senior Associate Consultant in the Division of Hematology/ Oncology since that time. She completed her medical school in New Delhi, India; an Internal Medicine residency at Western Pennsylvania Hospital; and a fellowship in Hematology-Oncology at University of Washington. She has conducted several studies of outcomes and late effects after hematopoietic cell transplantation. She is an active member of the chronic GVHD Consortium and BMT-Clinical Trials Network: Late Effects State of the Science committee.

Other late effects range from organ dysfunction to secondary cancers, problems in growth/ development, sexuality and psychosocial challenges. (Table 1). Use of cytotoxic agents such as busulfan, total body irradiation (TBI), GVHD and immunosuppressive treatments, treatments prior to HCT interact with host factors such as age, concomitant conditions, genetic predisposition and lifestyle to predispose survivors to these late effects. (Figure 1).

Late Mortality/ Morbidity

Various large studies have shown that the HCT survivor population remains vulnerable as compared to the general population even 10 to 20 years after the HCT.[10,11,12,13] While relapse remains the major cause of mortality in this population especially early after the HCT, late complications like chronic GVHD, secondary cancers, delayed infections and cardiovascular disease emerge as important causes of death with increasing time from HCT.[14,15] (Figure 2).

These late effects impact both mortality and morbidity in this patient population. While multiple investigators have described individual complications, only a few have assessed the overall burden of these late effects which tends to increase with time after HCT.[12,13,16-19] Presence of severe/life-threatening conditions, chronic GVHD and self-reported poor health was shown to be associated with increased risk for somatic distress in the survivors.[20] Having greater than three late effects was also associated with lower physical functioning/ Karnofsky score, lower likelihood of returning to work and a higher likelihood of limitation in usual activities.[12]

Other Issues in HCT Survivors

Employment/ Financial burden

Return to work has been considered as a measure of physical recovery after illness and subsequent treatment. Approximately 50-80% of survivors have returned to working at five years after HCT. Being a female, having chronic GVHD, experiencing ≥3 late effects and physical dysfunction were associated with slower return.[12,21,22]

Financial toxicity of cancer treatments has been highlighted

ORGAN SPECIFIC COMPLICATIONS	
Cardiovascular	Cardiomyopathy Congestive heart failure Arrhythmias Valvular anomaly Coronary artery disease Cerebrovascular disease Peripheral arterial disease
Pulmonary	diopathic pneumonia syndrome Bronchiolitis obliterans syndrome Cryptogenic organizing pneumonia Sino-pulmonary infections
Renal and Genitourinary	Chronic kidney disease Bladder dysfunction Urinary tract infections
Liver	Graft vs. host disease Hepatitis B and C Iron overload
Endocrine/Metabolic	Hypothyroidism Hypoadrenalism Hypogonadism Diabetes Mellitus Dyslipidemia
Musculoskeletal	Osteopenia/osteoporosis Avascular necrosis Myopathy Fascitis/scleroderma Polymyositis
Ocular	Cataracts Sicca syndrome Microvascular retinopathy
Oral	Sicca syndrome Caries
Nervous System	Leukoencephalopathy Neuropsychological and cognitive deficits Calcineurin neurotoxicity Peripheral neuropathy
Immune System	Infections
SECONDARY CANCERS	
	Solid tumours Hematologic malignancies Post-transplant lymphoproliferative disorder
GROWTH AND DEVELOPMENT COMPLICATIONS	
	Impaired skeletal maturation Cognitive dysfunction Impaired sexual maturation Accelerated ageing
SEXUALITY/ FERTILITY COMPLICATIONS	
	Infertility Sexual dysfunction
PSYCHOSOCIAL COMPLICATIONS	
	Depression Anxiety Fatigue Increased rate of suicidal/accidental deaths Financial Burden

Table 1. Late Complications.

recently. Despite being insured, these patients bear a high financial burden which may have an adverse impact on the quality of life of survivors.[23] In a recent study, we found that 47% allogeneic HCT patients experienced adverse financial consequences such as decrease in household income by >50%, selling/mortgaging home or withdrawing money from retirement accounts.[24] 3% had to declare bankruptcy as a result of medical expenditure.

Sexuality/ Fertility Problems

Sexual well-being is an important determinant of mental and physical quality of life. Sexual dysfunction remains a major problem for HCT survivors.[25,26] Studies in this area indicate the need for optimum communication between patients and health care providers about these concerns and treatment and appropriate referrals to help address these concerns.

In patients in reproductive age group, infertility is another late effect that has the potential of adversely affecting the quality of life. Urgency of HCT, lack of awareness in the physicians, inadequate access to reproductive specialists, and financial constraints may be the barriers for fertility preservation in HCT patients. A task force from the CIBMTR late effects working committee recently presented options for fertility preservation that are specific to HCT recipients.[27] Patients should be advised to delay pregnancy for at least 2 years after HCT, as this is the period of highest risk of relapse after transplantation.

Caregiver/Spousal Issues

There is a paucity of information about emotional and physical challenges of caring for a HCT recipient. The prolonged trajectory of recovery after HCT puts additional strain on the caregiver and family thus predisposing them to increased distress, fatigue, depressive symptoms and cognitive dysfunction.[28] In an online survey conducted by BMT Infonet, spouses were more concerned about financial problems, psychological health of the family, and caregiver burnout than the survivors themselves.[29] As a part of the ongoing efforts to improve overall survivorship care, multiple agencies and organisations now offer information, support and assistance to the caregivers.

Post HCT Care

Many of the late effects cannot be prevented, but with early diagnosis and management, their impact can be minimised. Post HCT phase provides a 'teachable moment' for the patient to focus on improving their health having survived a life-threatening illness and a risky treatment. It provides the physicians an opportunity to incorporate the principles of health promotion as well as all three phases of prevention in the management of these patients. A healthy lifestyle with well-balanced

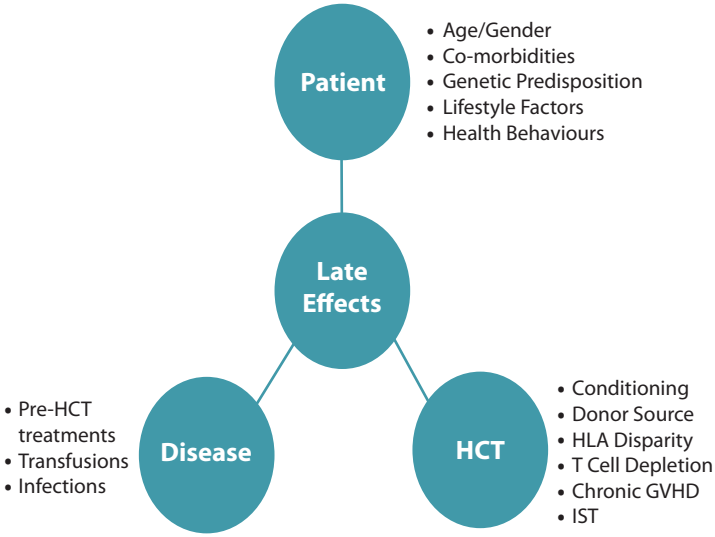

Figure 1. Risk Factors for late complications. Abbreviations: HCT, Hematopoietic cell transplantation; HLA, human leukocyte antigen; GVHD, Graft vs. host disease; IST, Immunosuppressive therapy.

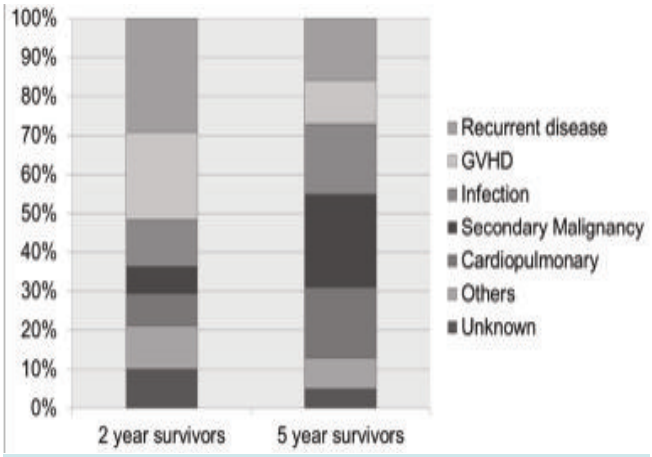

Figure 2. Causes of death in 2 year and 5 year HCT survivors. Adapted from Bhatia *et al.*[14] for 2 year survivors and Syrjala *et al.*[15] for 5 year survivors.

diet, exercise regimen, smoking avoidance, preventing prolonged sun exposure and minimising infection risk is recommended for all HCT recipients. Savani *et al.* have summarised the practical strategies to manage some of the late complications.[30] Since the average rates of organ dysfunction, second neoplasms, metabolic and musculoskeletal complications are higher in HCT survivors than in general population, specific recommendations for screening and preventive practices for survivors of HCT were recently updated by an international panel of transplant experts.[31] (Table 2 and 3). In addition, patients should be encouraged to follow up with their primary care physicians to get age and gender appropriate screening as per US Preventive Services Task Force guidelines.[32]

Immunisations

Delayed immune reconstitution increases the risk of delayed

Test or Study	6 months	1 year	Annual	Comments
CARDIOVASCULAR				
Cardiovascular risk-factor assessment	#	X	X	
Consider Cardiology Evaluation (including echocardiogram, EKG)		X		For high risk patients: anthracycline exposure > 300mg/m2 +TBI, h/o mediastinal XRT, pre-existing cardiac and vascular abnormalities
PULMONARY				
Routine clinical pulmonary assessment	X	X	X	
Assessment of tobacco use and counselling against smoking	X	X	X	
Pulmonary function tests		X	#	Consider PFTs every three months for patients with chronic GVHD
Chest X-ray	#	#	#	
RENAL				
Blood Pressure/ urine protein screening/ BUN/ creatinine	X	X	X	
LIVER				
Liver function testing	X	X	X	
Serum ferritin		#	#	Frequent and close monitoring with elevated levels if continued RBC transfusions, abnormal liver function tests, HCV infection
ENDOCRINE/ METABOLIC				
Thyroid Function Studies	#	X	X	
Gonadal function assessment (FSH/LH/ Estradiol for post-pubertal females)		X	#	
FSH/ LH/ Testosterone for males		#	#	
MUSCULOSKELETAL				
Bone Mineral Density		X	#	Consider scan at an earlier date with prolonged corticosteroid or calcineurin inhibitor exposure. Subsequent testing determined by prior defects or to assess response to therapy or with continued steroid treatment
OCULAR/ ORAL				
Oral/ Ocular clinical symptom evaluation	X	X	X	
Ophthalmology Evaluation		X	X	
Dental Evaluation	#	X	X	More frequent oral evaluations for patients at high risk for intra-oral malignancy
NERVOUS SYSTEM				
Clinical evaluation for neurologic and cognitive dysfunction	#	X	X	
Cognitive development milestones in pediatric patients		X	X	
SECONDARY CANCERS				
Screening Mammogram for female patients within 8 years of TBI/chest irradiation or by 25 years of age, whichever is first, but no later than 40 years		X	X	
Testicular/breast clinical exam/ gynecologic assessment		X	X	More frequent gynecologic assessments in patients with chronic GVHD and post TBI
US Preventive task force guidelines			X	
FERTILITY/ SEXUALITY COMPLICATIONS				
Sexual function assessment	X	X	X	
PSYCHOSOCIAL COMPLICATIONS				
Psychological evaluation	X	X	X	
Assessment of caregiver psychological adjustment	X	X	X	

Table 2. Screening Recommendations. Source: 31. Majhail *et al*. BBMT 2012. # Reassessment recommended for abnormal testing previously or for new sins/ symptoms. X Recommended for all patients. Abbreviations: EKG: Electrocardiogram; TBI: total body irradiation; XRT: Radiation treatment; PFT, Pulmonary function test; BUN, Blood Urea Nitrogen; HCV, hepatitis C virus; FSH, Follicle stimulating hormone; LH, luteinizing hormone; GVHD, Graft vs. host disease.

infections in patients after an allogeneic HCT. Risk may be higher in patients with chronic GVHD, those who have received T cell depleted transplants or have received extensive lymphotoxic therapy prior to HCT. Initiating immunisations may potentially decrease the incidence of vaccine preventable diseases even in patients with chronic GVHD. Inactivated vaccines can be started as early as 6 months after the HCT. Guidelines for immunisations are available.[33]

Delivery of Long-term Follow Up Care

Optimum delivery of survivorship care is needed to help address the issues of this growing segment of medically complicated population with multiple barriers to care. (Table 4). The Institute of Medicine in the 2005 report 'From Cancer Patient to Cancer Survivor' has proposed essential elements of survivorship care.[34] Various models have been suggested for survivorship care to incorporate all these elements. The shared care model based on

All patients	Additional Recommendations for Patients with Chronic GVHD
CARDIOVASCULAR	
Education and counseling for 'heart healthy lifestyle'	
Appropriate treatment of DM, HTN, hyperlipidemia	
Triglycerides> 500 mg/dL should be treated to prevent pancreatitis- use omega 3 fatty acid	
PULMONARY	
Counselling against active and passive smoking	Consultation with transplant center if suspicion for bronchiolitis obliterans or cryptogenic organising pneumonia
RENAL/ GENITOURINARY	
Optimum control of blood pressure	
Avoid nephrotoxins in patients with progressive renal dysfunction	
ENDOCRINE	
Refer to an endocrinologist for men who may need testosterone replacement therapy	Slow terminal tapering of steroids if prolonged usage, consider stress dose steroids during acute illness
MUSCULOSKELETAL	
Elemental calcium intake of 1000 to 1500 mg/day in divided doses as well as vitamin D at 1000 IU/day	Frequent clinical evaluation for myopathy by manual muscle tests or by assessing ability to go from sitting to standing position (especially if on steroids)
Bisphosphonates for established osteoporosis and for osteopenia specially if risk for progressive bone loss	Consider bisphosphonates in patients with chronic GVHD and osteopenia who may need prolonged steroids
	Physical therapy consult in patients with chronic GVHD with sclerosis/ fasciitis to assess baseline function and suggest range of motion and strengthening exercises
ORAL	
Routine/ non-urgent dental care should be delayed for at least after the first year of transplant	
Counselling against smoking, chewing tobacco	
IMMUNE SYSTEM	
PCP prophylaxis for at least initial 6 months after transplant	PCP and encapsulated bacterial prophylaxis for the duration of immunosuppression
Acyclovir for at least 1 year post-transplant to patients at risk for VZV disease	Acyclovir for the duration of immunosuppression
American Heart Association prophylactic antibiotic recommendations for oral procedures	
IV Immunoglobulin beyond 3 months of transplant may be given to patients transplanted for primary immunodeficiency, persistent low IgG levels (<400), patients with myeloma, CLL or other low grade lymphomas or those with recurrent sino-pulmonary infections.	
SECONDARY CANCERS	
Avoidance of high risk behaviors e.g. smoking, excessive unprotected skin UV exposure	

Table 3. Recommendations for prevention and management of late complications in Allogeneic Hematopoietic Cell transplantation patients. Source: 31. Majhail *et al.* BBMT 2012. Abbreviations: GVHD, Graft vs. host disease; DM, Diabetes Mellitus; HTN, hypertension; PCP, Pneumocystis Carinii; VZV, Varicella zoster virus; CLL, Chronic Lymphocytic leukemia; UV, Ultraviolet.

Patient	Provider/Healthcare system
Lack of knowledge about late effects	Workforce shortage
Socioeconomic barriers for access to care	Lack of knowledge/ discordant management preferences
Lack of ability to navigate complex systems	Paucity of randomised trials testing diagnostic and treatment approaches
	Inadequate reimbursement for complex care
	Fragmented post-transplant care (between transplant center, hematologist/ oncologist and primary care physician)

Table 4: Barriers in delivery of survivorship care for HCT survivors.

coordination between transplant center, hematologists and primary care physician may provide the optimum care for the patient.[35] Some transplant centers have developed, or are in the process of developing different variants of a dedicated survivorship clinic for HCT survivors to reflect this concept. While the ongoing care can be provided by a hematologist/ oncologist, a comprehensive evaluation of HCT survivors should be done at least annually at the transplant center since such assessment can help detect a high frequency of medical problems.[36]

Another strategy to address some of these challenges is development of a survivorship care plan. An ongoing national study funded by Patient-Centered Outcomes Research Institute is developing individualised care plans using Center for International Blood and Marrow Transplantation (CIBMTR) framework and will evaluate its impact on patient knowledge and health behaviors. Investigators are also studying the use of internet based programs for enhancing survivorship outcomes after HCT by providing tailored educational opportunities.[37]

Areas of Research

Our knowledge about HCT survivorship has increased considerably through the large descriptive studies about late effects in the last decade. However, there is still a need to better evaluate the impact of the advances in techniques of HCT in the last decade e.g. the impact of reduced intensity/ non-myeloablative transplants, alternative donor sources and newer GVHD prophylaxis regimens on the occurrence and spectrum of the late effects. It is also not clear as to what impact the comorbidities or more extensive pre-HCT treatments may have on the survivor's health status and on the risk for occurrence and severity of late effects. There needs to be a paradigm shift in survivorship research with studies evaluating overall burden rather than specific late effects in isolation, elucidating the mechanistic aspects of these late effects and incorporating all domains (physiologic, psychosocial and economic) while trying to design interventions to address these late effects.

Conclusions

Increasing attention is being focused on developing approaches to meet the needs of the growing HCT survivor population. Steps to improve survivorship care further will involve not just clinical, educational, and research efforts but also the need to engage the policy makers to ensure system changes in reimbursement patterns, federal research funding, and access to affordable multidisciplinary care.

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What Can We Expect From Autologous Transplantation in Relapsed Diffuse Large B Cell Lymphoma? Lessons from the CORAL Study

Christian Gisselbrecht[1] and **Nicolas Mounier**[2]

1. Professor of Hematology, Paris Diderot VII University, Institut d'hématologie, Hôpital Saint Louis, Paris, France; 2. Professor of Medical Oncology at Nice Sophia-Antipolis University, France

The use of rituximab as first-line treatment in patients with diffuse large B cell lymphoma (DLBCL) has improved the 5-year event-free survival (EFS) by 15% to 20% depending on the patients' age and prognostic factors[1, 2] and improved overall survival (OS) as well. Fewer relapses have been seen among patients with 0-2 International Prognostic Index (IPI) factors (10-20%); however, 30 to 50% of relapses still occur in patients with more than 2 IPI factors treated by the standard R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) regimen.[3, 4] Without stem cell transplantation, the outcome of relapsing patients remains poor. Over the age of 60 years,[5] the median OS after progression was 0.7 months for patients treated with R-CHOP. In younger or fit elderly patients, the initial approach to the management of relapsed DLBCL has been to determine whether the patients are candidates for high-dose therapy (HDT) and autologous stem cell transplant (ASCT).[6] While patients need to be responsive to chemotherapy after salvage regimens, several important issues must be answered to optimise the therapeutic response: first, the type of salvage regimen; second, the indication for rituximab use when R-CHOP is the accepted standard of care as first-line therapy; and third, the biological parameters integrating the patients' risk factors.

Christian Gisselbrecht, MD, is a professor of hematology at Paris Diderot University at the Hôpital Saint Louis in Paris. He is co-founder of the GELA, now LYSA(Lymphoma Study Association) in 1987 and president of the group until 2002. Dr Gisselbrecht has been lead investigator of several clinico-pathologic studies, and stem cell transplantation with a focus on relapses or poor prognosis lymphoma and T cell lymphoma. He has published over 300 peer-reviewed papers and several book chapters.

Nicolas Mounier, MD, PhD, is Professor of Medical Oncology at Nice Sophia-Antipolis University, France. He is the Head of Onco-Hematology Department in Nice Teaching Hospital l'Archet. Its research activity focuses on NHL treatment. In particular, the indication of Stem-Cell Transplantation in the targeted drug area. One permitting tool to tailor an individualized treatment strategy is the identification of predictive biological profiles.

Selecting a Salvage Regimen

For relapsed patients, until now, no comparative studies have been performed to evaluate the efficacy of different salvage regimens with rituximab.[7] The present CORAL (COllaborative trial in Relapsed Aggressive Lymphoma) study was conducted in a collaborative effort among 12 countries. We wanted to compare two established salvage regimens followed by ASCT.[8] We were also interested in determining which parameters influenced the effectiveness of either regimen and whether the widespread use of rituximab as part of the first-line therapy influenced the outcome of patients with relapsed DLBCL.[9]

Patients with refractory or relapsed CD20+ DLBCL were randomised between two widely used regimens, ICE (ifosfamide, carboplatin, etoposide)[10] and DHAP (dexamethasone, cytarabine, cisplatin),[11] each combined with rituximab. In responsive patients, peripheral progenitor cells were collected after chemotherapy and reinfused after a high-dose chemotherapy conditioning regimen (BEAM). We also investigated the impact of post-transplant administration of rituximab through a randomised comparison of patients who were only observed and those who received rituximab every 2 months for one year.

Altogether, 481 patients were randomised into the R-ICE arm (243 patients) and the R-DHAP arm (234 patients). A total of 255 patients (53%) who achieved complete (142 cases), partial (92 cases) or stable response (7 cases) after the third cycle of salvage treatment received consolidation therapy with ASCT, and 242 patients received maintenance rituximab (122 cases) or were subjected to observation only (120 cases).

The overall response rate (ORR: CR+ Cru+ PR) after salvage chemotherapy and prior to transplantation was 63 % for the R-ICE group and 64 % for the R-DHAP group.[12]

Several factors significantly affected the overall response rate

(p<0.0001): refractory disease/relapse in <12 months, a secondary IPI (IPI at relapse) of 2-3 and prior exposure to rituximab. However, the treatment arm did not affect the overall response rate. For patients with prior exposure to rituximab and early progression < 12 months, the ORR was only 46 percent.

After a median follow-up of 44 months for the 469 evaluable patients who participated since the onset of the study, no differences in survival were detected. The OS was 43% (95% CI 36-50) for the R-ICE group and 51% (95% CI 44-58) for the R-DHAP group (p=0.3) (figure 1). The EFS was 26% (95% CI 20-32) for the R-ICE group and 34% (95% CI 36-50) for the R-DHAP group (p=0.2).

Are Biological Factors Important?

An Diffuse large B-cell lymphoma is a well-defined entity and the most common form of adult non-Hodgkin's lymphoma.[13] The complexity and heterogeneity of this disease have been demonstrated over the past 10 years through the analyses of gene expression profiling, leading to a molecular classification of DLBCL into distinct subtypes: germinal centre B cell-like (GCB) and activated B cell-like (non-GCB). Improvements in patients' outcome may possibly be achieved through a greater understanding of the genetic abnormalities specifically associated with poorer prognosis. The prognostic value of the cell of origin (COO) in patients with relapsed/refractory diffuse large B cell lymphoma was studied in a subset of 394 patients who were part of the CORAL trial. Histological specimens were available for a total of 249 patients at diagnosis (n=189 cases) and/or at relapse (n=147 cases).[14] The cases were analysed by immunochemistry for the expression of CD10, BCL6, MUM1, FOXP1, and BCL2 and by FISH for the breakpoints BCL2, BCL6 and c-MYC. Based on Hans's algorithm,[15] 49% of the cases were classified as GCB and 51% as non-GCB.

When treatment interactions were tested, the R-DHAP arm was significantly associated with a better PFS in the GCB-like group of patients with DLBCL. In multivariate analysis, independent prognostic significance was found for Hans's phenotype treatment

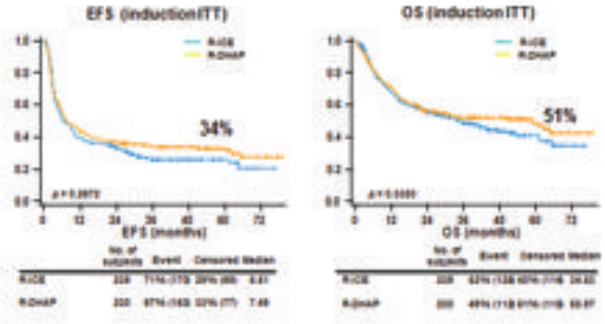

Figure 1. CORAL Trial: Event Free Survival and Overall Survival by Second Line Treatment

interaction for the GCB and non-GCB subtypes (p=0.04), prior exposure to rituximab (p=0.0052), and secondary IPI (p=0.039).

Among the tumour samples exhibiting interpretable FISH signals, the gene rearrangements BCL2/18q21, BCL6/3q27, and c-MYC/8q24 were identified in 31%, 18% and 13% of the cases, respectively. The BCL2 and c-MYC rearrangements were strongly associated with the GCB subtype of DLBCL. The presence of c-MYC gene rearrangement was the only parameter significantly correlated with a poorer PFS (p= 0.02) and OS (p=0.04). No significant additive effect of the so-called 'double hit' BCL2 and c-MYC mutations was identified.

Outcome After ASCT

A total of 245 patients received BEAM and ASCT, of which 242 patients were evaluable. These patients were randomised into two groups prior to ASCT: 142 (58%) patients who exhibited CR (complete remission) or CRu (CR uncertain), and 92 patients (38%) who exhibited PR (partial remission). Rituximab was administered at a planned dose, and 78 patients (67%) received 6 cycles of treatment. At the end of the maintenance period, the CR rates were 57% and 50% for the rituximab and observational groups, respectively, including all deaths.

Considering only patients transplanted, the four-year EFS was 52 % (95 % CI 42-61) in the rituximab group and 53 % (95 % CI 44-62) in the observational group in the maintenance arm after ASCT (p=0.7). No difference in PFS (p=0.8) and OS was observed between the rituximab group and the observational group. No significant difference in PFS and OS was observed between the patients who achieved complete and partial remission prior to ASCT.

Four-year EFS, PFS and OS of patients post-ASCT were affected by prior treatment with rituximab, early relapse of their disease, and secondary IPI. However, the Cox model (Table 1) revealed that only secondary aaIPI 2-3 remained statistically significant (p<0.001) for EFS, PFS and OS; prior treatment with rituximab no longer reached statistical significance. Female patients performed significantly better than their male counterparts, a finding that was related to the superior survival of women in the rituximab group. In multivariate analyses of PFS, the gender (p=0.01) and IPI (p=0.0004) variables remained statistically significant; in contrast, the treatment arm, early relapse, prior rituximab exposure, and PR were not statistically significant. However, in a subset analysis of gender between the rituximab and observational groups, the three-year EFS was 43 percent (95% CI 31-54) in males and 69% (95% CI 53-81) (p=0.1) in females.

We also stratified our biological profiles for transplanted patients (n=107) into 4 groups: MYC+/BCL2+, MYC+/BCL2-, MYC-/BCL2+ and MYC-/BCL2-. The five-year OS was not significantly reduced in any of these groups: 40%, 52%, 50%, and 50%, respectively (p=0.6). This finding suggested that in

patients with DLBCL, concurrent expression of the MYC and BCL2 proteins was not associated with a worse clinical outcome after ASCT. Similar confirmatory results were obtained by FISH analysis.

Perspectives

The present study demonstrated a similar response rate of 63% for the two initial regimens of chemotherapy with a 4-year follow-up, but only 37% of patients attained complete remission. Only 53% of patients were able to undergo ASCT. Toxicities were quite similar between the treatment regimens; however, a larger number of platelet transfusions were required and renal toxicity was observed in the R-DHAP group. No difference in survival was observed between the two treatment regimens, and the treatment arm for R-ICE was not significantly different (p=0.07, HR 1.5). However, a trend for an improved outcome was noted with the R-DHAP treatment arm. In fact, the subset of patients with the GCB profile analysed by immunohistochemistry had a better outcome under the R-DHAP regimen, but no difference was observed for the non-GCB subtype. Recently, a similar study named LY 12[16] reported the comparison of the R-DHAP and RGDP (gemcitabine, dexamethasone, cisplatin) treatment regimens in relapsed DLBCL and transformed indolent lymphoma. No differences between the two treatment arms were observed. These findings underlined the need to study the effect of new treatments according to DLBCL subtypes.

This study identified a patient population with late relapse who benefited from the addition of rituximab to their salvage regimen and exhibited an 80% response rate and a 4-year EFS of 40 to 50 percent. However, one group of patients with a poor prognosis was identified whose prior treatment with rituximab was predictive, in cases of early relapse, of a response rate of 40% and a 4-year EFS of only 20 percent. As all patients now receive rituximab as first-line treatment, new therapies should pay attention to patients with high-risk IPI or patients with the non-GCB subtype. There is an unmet need for this population of patients, and new drugs could be evaluated in these patients more quickly. Increasing the response rate to salvage treatment is the major surrogate endpoint towards improvement of the survival of patients with relapsed DLBCL. However, despite the large amount of data from genome-wide analyses in the last 10 years, only a few identified therapeutic targets have progressed to phase III trials.

The use of rituximab post-transplantation was expected to reduce the relapse rate; however, no difference between these two treatment groups was identified. Similar results have been reported in the LY12 study. This first randomised study does not concur with the promising results described in a phase 2 study,[17] but it is consistent with the SWOG study[18] that reported no impact of maintenance therapy in patients who were previously exposed to rituximab. One striking result of the present study was the significant difference in survival between female and male patients who received maintenance therapy with rituximab. The secondary aaIPI score was the only significant variable associated with gender in multivariate analyses. A higher clearance rate of rituximab in males, which resulted in lower therapeutic levels of rituximab, has been reported previously.[19]

The role of rituximab in patients with DLBCL requires further analysis of the contribution of gender in large randomised studies with or without rituximab or other randomised studies of maintenance therapy after ASCT in patients with relapsing DLBCL.

Table 1. CORAL Maintenance:
Prognostic Factors for Maintenance Post-ASCT - Multivariate
Cox Model

	EFS		PFS		OS	
Parameter	*p-valve*	Hazard Ratio (95% CI)	*p-valve*	Hazard Ratio (95% CI)	*p-valve*	Hazard Ratio (95% CI)
Prior Treatment with Rituximab: no	0.1971	0.748	0.3509	0.808	0.2874	0.760
Failure from Diagnosis < 12 Months	0.4658	1.179	0.4536	1.188	0.5665	1.159
S Age-adjusted IPI 2-3	0.0030	1.846	0.0007	2.028	0.0004	2.252
Response after Complete Induction: PR	0.2050	1.295	0.4286	1.180	0.4638	1.186
Arm R-ICE	0.0853	1.417	0.0676	1.457	0.0716	1.511
Arm of Second Randomisation: Rituximab	0.9208	1.020	0.6104	1.111	0.4822	1.175

In conclusion, the CORAL study population was representative of patients with DLBCL who will require treatment in the future. The patients' outcome even after ASCT requires further improvement. New drugs designed to increase the response rate of salvage regimens and novel consolidation approaches, including allogeneic transplantation, should be explored.[20] Novel targeted therapy designed upon an improved understanding of the biology of DLBCL,[14] including studies of patient tumour specimens, is anticipated to play a key role.

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Automating the Manufacture of Clinically Appealing Designer T Cells

Pappanaicken Kumaresan,[1] Matthew Figliola,[1] Priti Tewari,[1] Judy S.E. Moyes,[1] Helen M. Huls[1] and Laurence J.N. Cooper[1,2]

1. Division of Pediatrics, UT MD Anderson Cancer Center, Houston, Texas, USA; 2. The University of Texas Graduate School of Biomedical Sciences, UT MD Anderson Cancer Center, Houston, Texas, USA

Introduction

There are numerous approaches to numerically expand clinical-grade T cells with the method of choice depending on the desired characteristics of the end products. The therapeutic success of adoptive T-cell therapy is generally considered to be due to infusing sufficient numbers, lack of replicative senescence, and presence of T cells with desired specificity.[1] The length of time T cells are in culture, especially if they are propagated under non-physiological conditions, may erode quality of the product despite increases in quantity. Thus, it is appealing to generate T cells that are minimally manipulated and processed for infusion within hours or a few days of collection. This has immediate appeal as it avoids the expense and risk of contamination associated with prolonged tissue culture, and reduces labour associated with producing a given product. Furthermore, expedited production may improve the therapeutic potential of the manufactured T cells as they avoid terminal differentiation that is associated with loss of sustaining *in vivo* persistence. Approaches to producing T cells with desired activity in compliance with current good manufacturing practice (cGMP) are typically based on the *ex vivo* application of reagents that identify antigen-specific T cells. These include the binding of fluorescence-labelled or paramagnetically-labelled probes that dock to T-cell receptors (TCRs), thus identifying T cells with desired specificity. These T cells subsequently undergo fluorescence-activated cell sorting (FACS) or magnetic selection to generate a homogeneously tagged product that can be immediately infused or propagated after meeting release criteria.[2,3] The success of this approach can be measured in terms of the time to identify antigen-specific T cells and the specificity of the harvested product. The appeal of this approach is limited (i) when there is low frequency of antigen-specific T cells in the donor below the limit of detection with probes, (ii) by the need to generate GMP-grade reagents that identify just a subset of TCRs restricted to commonly expressed human leukocyte antigens (HLA) and (iii) by the skills needed to operate the sorting/selection devices in compliance with cGMP. Nevertheless, clinical trials have successfully reported on the adoptive transfer of T cells with specificity for viral antigens that have undergone FACS or magnetic separation to derive a minimally manipulated product that can be immediately infused (Table 1).

Laurence Cooper is a tenured Professor at The University of Texas (UT) MD Anderson Cancer Center (MDACC), with joint appointments in the Departments of Pediatrics and Immunology. He is Section Chief of Cell Therapy at the Children's Cancer Hospital (CCH) at MDACC. He earned his medical and doctorate degrees from Case Western Reserve University and completed his fellowship in Pediatric Hematology/Oncology at the Fred Hutchinson Cancer Research Center. He has recently launched nine trials under INDs infusing T cells and NK cells and undertook the first gene therapy trials using Sleeping Beauty transposon/transposase system.

A Closed System for Automated and GMP-compliant Cell Manufacturing

The successful manufacture of biological products for cellular therapies requires standardised and defined GMP-compliant processes. Typically, the production of a specific cell type, such as a T cell, is based on complex multi-step procedures, which when handled manually are prone to unwanted variation. It is thus appealing to automate the process. To address the specific requirements for manufacturing of cell-therapy biologics the automated cell-processing system will ideally standardise the entire manufacturing process from sample preparation to final formulation of the cellular product in a closed single-use system. This is likely to reduce the risk of contamination, avoid multiple manual steps and operator fatigue, accelerate processing and save time as well as costs.

One such automated processing system is the new CliniMACS Prodigy System that provides for a high level of standardisation as it has the capacity to automatically execute a series of user-defined cell manufacturing processes, including (i) fractionation of the starting material, (ii) cell washing, (iii) magnetic labelling and separation based on MicroBead technology,[4] (iv) cell transfection as well as culture, (v) volume reduction and (vi) final product formulation. These processing steps are all accomplished in an enclosed environment consisting of single-use tubing sets with multiple input and output lines for sterile connections, and an integrated centrifugation and cell cultivation chamber. A suite of dedicated tubing sets with or without magnetic separation columns are available to allow for the manufacture of specific cellular products.

Virus	Target Antigen	Method of Isolation	Average Number of T Cells Infused (per recipient)	References
CMV	CMV lysates	Cell culture	10^7 cells/m^2	26
	CMV pp65	Cell culture	5 x 10^5 cells/kg	27
	CMV pp65	Streptamer-based CliniMACS	2.2×10^5 cells/kg	28
	HLA_A2: NLVPVATM	Pentamer-based CliniMACS	7.7×10^4 cells/kg	29
EBV	HLA_A1: TDLGQNLLY	Pentamer-based CliniMACS	1.8×10^4 cells/kg	29

Table 1. Clinical trials infusing antigen-specific T cells after cell culture or magnetic sorting.

This second-generation CliniMACS Prodigy System (Figure 1) is based on the CliniMACS Cell Separation principle that was developed for efficient T-cell depletion from stem cell grafts. Details of the complete instrumentation and functionality of the CliniMACS Prodigy System have been published.[5]

Applications for an Automated Manufacturing System to Generate Cell-based Therapies

The CliniMACS Prodigy System provides a foundation for manufacturing clinical-grade cells for a wide range of applications, which includes tissue regeneration, graft manipulation and NK-cell based immunotherapy, in addition to administration of antigen-specific T cells.

Role of CliniMACS Device in Tissue Regeneration

Currently, clinical studies are underway to investigate whether autologous bone marrow–derived CD133$^+$ haematopoietic stem cells (HSC) may improve tissue regeneration when injected into the myocardium as an adjunct to coronary artery bypass graft (CABG) treatment.[6,7] Magnetic selection has been used to isolate autologous CD133$^+$ stem cells collected from bone marrow before CABG surgery in compliance with cGMP conditions. Infusion into damaged myocardium has been well tolerated and resulted in some improvement.[8]

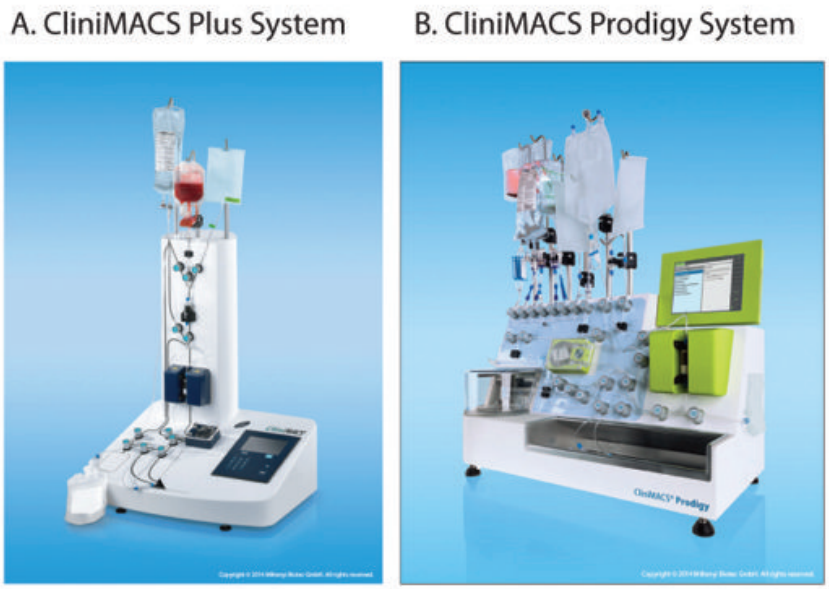

Figure 1. CliniMACS devices. (A) CliniMACS Plus Cell Separation System is a first-generation, semi-automated cell separation system based on MACS® Technology for the development of GMP-compliant manufacturing processes for cellular products. It enables the operator to perform clinical-scale magnetic enrichment of target cells or depletion of unwanted cells in a closed and sterile system. It employs user-friendly software that enables automatic collection of separated cells in different collection bags. (B) The second generation CliniMACS Prodigy System is a closed system enabling GMP-compliant manufacturing and reduction in requirements for cleanrooms, thereby helping facilities achieve GMP compliance without the need for dedicated and expensive manufacturing sites. Several standardised and validated magnetic separation processes can be chosen from the instrument's touchscreen-based menu. Processes have already been developed for the isolation of HSC, specific depletion of alloreactive T cells, enrichment of antigen-specific T cells, and the isolation of monocytes for generation of dendritic cells. In addition, the Prodigy provides a flexible programming option to allow for full integration and automation of complex steps within cell manufacturing workflows.

Role of CliniMACS Device for Graft Manipulation

High-dose chemotherapy accompanied by haematopoietic stem-cell transplantation (HSCT) can be curative for some patients with subsets of haematological malignancies. The HSC can be manipulated ex vivo prior to infusion to improve therapeutic potential. For example, patient-derived HSC infused to restore haematopoiesis may inadvertently contain contaminating tumour cells, which can be removed using magnetic selection when isolating CD34$^+$ cells.[9] In another setting, excessive donor-derived T cells may contribute to clinically intolerable GVHD when infused with allogeneic HSC, especially when there is mismatch in HLA between donor and recipient. Magnetic separation can be used to isolate CD34$^+$ HSC to passively deplete contaminating T cells or to actively deplete CD3$^+$ T cells prior to infusion of the allograft for recipients of allogeneic HSCT.[10-12] Another experimental approach being developed to help prevent and possibly treat GVHD that lends itself to automation is based on the infusion of regulatory T cells (Tregs), which may be isolated by a series of magnetic depletion and enrichment steps to generate CD4$^+$CD25$^+$ Tregs.[13-15]

Virus	Target Antigen	Method of Isolation	Average Number of T Cells Infused (per kg recipient weight)	References
CMV	CMV pp65 protein	CliniMACS	~21.3×10^3	30
	Peptide pool from CMV pp65	CliniMACS	~ 2.8×10^3	20
EBV	Multimer peptide mix	CliniMACS	5.83×10^4	31
Adenovirus	ADV lysate	CliniMACS	12×10^3	32

Table 2. Clinical trials infusing antigen-specific "captured" T cells secreting IFN-γ .

Role of CliniMACS Device for Production of NK Cells

Natural killer (NK) cells isolated, activated and propagated *ex vivo* retain an ability to recognise tumour cells. Typically, infusion of large numbers of NK cells is thought to be required to achieve a therapeutic effect. This has been accomplished by the recursive manual addition of γ-irradiated designer K-562-derived artificial antigen-presenting cells (aAPC) in the presence of soluble recombinant interleukin (IL)-2.[16] Some of this manufacturing procedure is immediately amenable to automation, such as removal of erythrocytes and granulocytes from peripheral blood mononuclear cells (PBMC), which can be achieved using the Prodigy's automated Ficoll-based density gradient centrifugation capabilities. Since the device also contains a bioreactor, it may be possible to co-culture NK cells with aAPC in the presence of soluble cytokine(s) to achieve sufficient cell numbers.[17,18] Contaminating allogeneic T cells that may cause GVHD may be removed using paramagnetic beads conjugated to monoclonal antibodies (mAb) recognising CD3 or αβTCR.[19]

Role of CliniMACS Device for Production of Viral-specific T Cells

Adoptive cell therapy with viral-specific T cells has potential for the prevention and treatment of opportunistic infections in immunosuppressed patients, as occurring after allogeneic HSCT.[20] The cytokine-capture system (CCS) uses magnetic enrichment of antigen-stimulated T cells with IFN-γ tethered to the surface independent of HLA. The first-generation CliniMACS device with CCS has been employed to isolate and infuse cytomegalovirus (CMV) pp65–specific T cells.[21] Despite the need for skilled operators attending to this technology, it has been successfully performed in compliance with cGMP (Table 2). A practical limitation to capturing these desired T cells is the cumbersome and labour-intensive procedure associated with using the first-generation CliniMACS device. The 2nd generation CliniMACS Prodigy System resolves this issue using automation to accomplish the tasks that required assistance by the operator(s) of the 1st generation device. Indeed, as we and others are now demonstrating (https://isctmalachite-mgmt.site-ym.com/store/ViewProduct.aspx?id=2226513), the Prodigy device may be used in combination with CCS to isolate virus-specific T cells with the automation reducing the need for staff to be physically present during the manufacturing process.

Registration of the CliniMACS Prodigy System for Use in the United States

There is a wealth of practical experience and regulatory review governing the clinical application of the 1st generation CliniMACS device. Recently, the U.S. Food and Drug Administration (FDA) approved the CliniMACS CD34 Reagent System as a Humanitarian Use Device for the prevention of graft-versus-host-disease (GVHD) in patients with acute myeloid leukaemia (AML) undergoing allogeneic HSCT.[22] This provides the foundation for the transition of the Prodigy system to generate clinical-grade products. Documents to seek federal regulatory approval for the device and tubing sets will be needed before the Prodigy device can be used to generate T cells for human application. The timeline for submission of these documents is the summer of 2014. This will enable end users to seek institutional and federal regulatory approvals for infusion of IFN-γ-captured T cells (Figure 2).

Translational Developments for CliniMACS Prodigy System

Currently, the Prodigy processes T cells collected from steady-state apheresis. It remains to be determined if the device can process whole blood, which is desirable to save cost as well as inconvenience to the donor. Furthermore, whole blood might be collected using standard operating procedures available at blood banking centres worldwide. Approximately 8 x 10^9 total nucleated cells (TNC) can be harvested after a standard 2-hour steady-state apheresis of a healthy volunteer donor. This is in excess of what is currently loaded (10^9 TNC) into the Prodigy device and it is not known whether the system can be adapted to accommodate increased numbers of TNC. Thus, for donors who have only a few circulating T cells capable of specifically secreting IFN-γ, it may be possible to obtain additional cells from an apheresis product that is split before loading onto the Prodigy system to enable additional round(s) of capture. It is anticipated that some donors may be harvested at distance from processing on the Prodigy device. However, it is not yet known the length of time an apheresis collection remains viable for subsequent processing. At present, it is estimated that the apheresis material will need to be loaded into the Prodigy device within 24 hours of donation. Once stability of apheresis (or whole blood) is determined investigators can determine whether

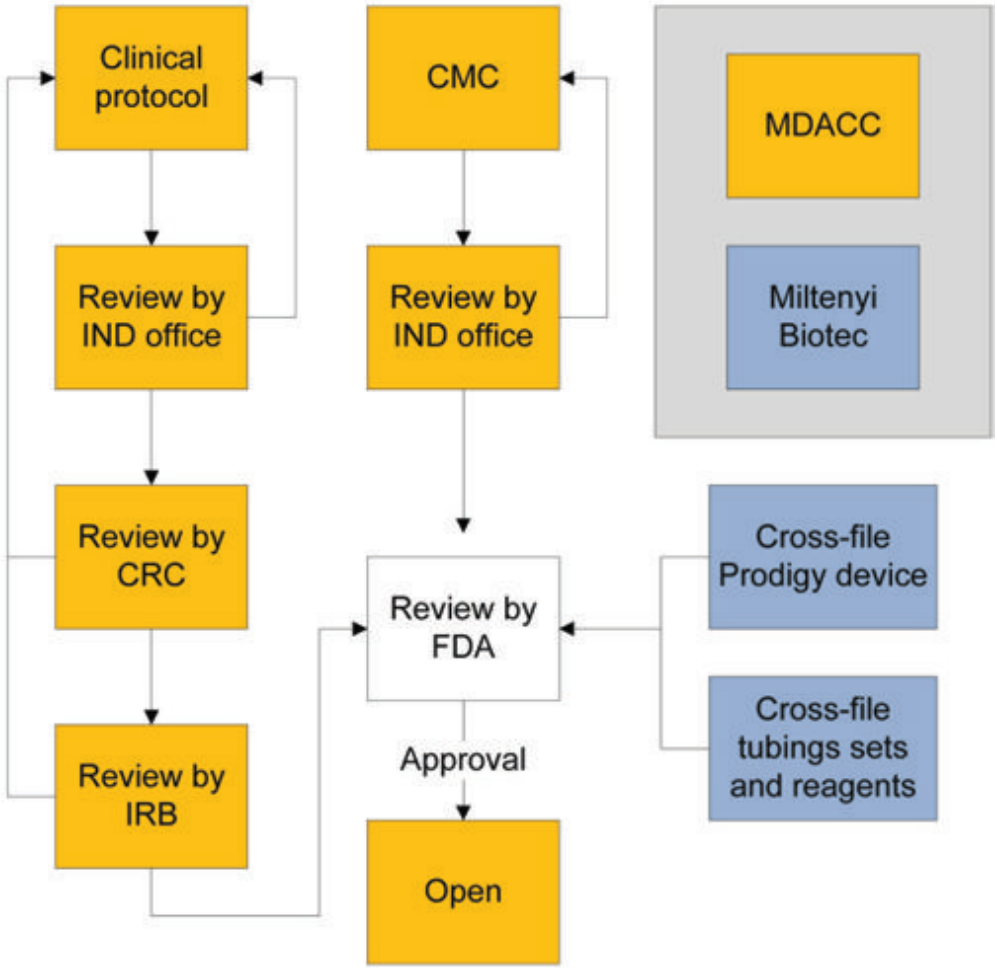

Figure 2. Schematic of the institutional and federal regulatory approvals needed at MD Anderson Cancer Center (MDACC) and Food and Drug Administration (FDA) to initiate clinical trials infusing CMV-specific T cells harvested by the CliniMACS Prodigy System. Abbreviations: IND: Investigational New Drug; CRC: Clinical Research Committee; CMC: Chemistry, Manufacturing and Control; IRB: Institutional Review Board.

one can split the product for recursive processing and/or repeated infusions and whether it is feasible to harvest from geographically distant sites.

Clinical Trials for CliniMACS Prodigy System

The initial human application of the Prodigy system in the field of immunotherapy will be the infusion of CMV-specific T cells in combination with CCS. In these trials, donor-derived pp65-specific T cells will be adoptively transferred into recipients of allogeneic HSCT at risk of CMV disease or who have end-organ damage from this viral infection. However, further anticipated process developments for the Prodigy System will enable the administration of populations of T cells with multiple specificities to sequentially or simultaneously target a panel of infectious targets including funghi. In addition, the system can be adpated to generate tumour-specific T cells, such as targeting Wilms' tumour gene 1 (WT1) using the same approach as generating viral-specific T cells.[23] Some clinical applications, such as infusion of allogeneic antigen-specific T cells after HSCT, are not possible due to the donor of HSC being unavailable or anonymous. However, potential recipients may benefit from infusion of "captured" T cells from 3rd party donors that recognise antigen through an HLA molecule shared between the recipient and the donor. These "off-the-shelf" allogeneic T cells may be pre-manufactured and cryopreserved so that they can be infused on demand. Precedence for this has been reported with 3rd party EBV-specific T cells and multi-virus specific T cells having been infused.[24, 25] If more than one donor is available, then the pre-screening of T cells (obtained by simple venipuncture from these individuals) for sufficient antigen-specific secretion of IFN-γ will determine the most suitable donor. This pre-screening will reduce costs and inconvenience associated with apheresis and decrease wastage of the single-use Prodigy tubing sets and clinical-grade reagents.

Conclusion

The CliniMACS Prodigy System appears to be a versatile instrument that can be used to enrich or deplete a range of immune cells or stem cells for a range of applications. MD Anderson Cancer Center is currently undertaking pre-clinical studies to generate CMV-specific T cells for

infusion after allogeneic HSCT. This is but one application for a device with ability to automatically execute a multitude of cell manufacturing processes in a closed system with single-use tubing sets and clinical-grade reagents. As clinical experience is gained with this device, users and medical centres may be motivated to undertake the production of clinical-grade cells in blood banks outside traditional GMP facilities, which will broaden application and reduce costs.

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Conflict of Interest

Dr. Cooper founded and owns InCellerate, Inc. He has patents with Sangamo BioSciences with artificial nucleases. He consults with Targazyme, Inc. (formerly American Stem cells, Inc.), GE Healthcare, Ferring Pharmaceuticals, Inc., and Bristol-Myers Squibb. He receives honoraria from Miltenyi Biotec.

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Importance of Tolerability Profile in Chronic Myeloid Leukemia

Adriano Salaroli, Giuliana Alimena and **Massimo Breccia**
Department of Cellular Biotechnologies and Hematology, Sapienza University, Rome, Italy

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterised by the presence of Philadelphia chromosome, which results from a chromosome reciprocal translocation between the long arms of chromosome 9 and chromosome 22 that fuses the breakpoint cluster region (BCR) and c-abl oncogene 1 (ABL1) genes. The result is a novel fusion gene, BCR-ABL, which encodes for a constitutively activated tyrosine kinase.[1] In the last decade CML outcome has dramatically changed since the introduction of tyrosine kinase inhibitors (TKIs).[2]

Before TKIs were introduced as the frontline therapy, CML was a progressive and fatal disease. Prior to the introduction of Imatinib (Gleevec, Novartis), Interferon Alfa (IFNa) and Cytarabine (Ara-C) were the standard therapies for patients who were not eligible for an allogenic haematopoietic stem cell transplantation. As shown by O'Brien *et al*. with the IRIS study,[2] outcome and tolerability were clearly superior in the imatinib group compared to the combination therapy group. Since imatinib was introduced in 2001, other TKIs have become available, initially for imatinib-resistant patients and then also as a first-line therapy. The second generation of TKIs is represented by Dasatinib (Sprycel, Bristol Myer Squibb, a strong multi-target second generation TKI)[3] and Nilotinib (a highly potent and selective TKI)[4] that were approved respectively in 2006 and 2007 as second-line therapy and in 2010 as front-line therapy. They have shown higher outcomes compared to imatinib, leading to quicker and deeper results. The third generation of TKIs is represented by Bosutinib (Bosulif, Pfizer, a dual SRC and ABL1 inhibitor)[5] and Ponatinib (Iclusig, ARIAD, pan-BCR/ABL inhibitor able to block native and mutated BCR/ABL including T315I mutation)[6] that have become available since 2012 for resistant CML. Although the outcome of patients affected by CML constitutes an exception in the onco-haematology, TKIs have not yet "cured" the disease. Their efficacy against the quiescent stem cell pool is an unresolved issue[7] and patients currently still require a life-long therapy with them, stressing the importance of a correct choice of the TKI and of a proper management of adverse events (AEs) in order to ensure an adequate adherence to the therapy. AEs can be classified in haematological or non-haematological side effects, short-term or long-term side effects and the so-called "off-target" complications.

Adriano Salaroli graduated with honours in Medicine and Surgery at Sapienza University of Rome, Italy. He was elective student at the Haematological Department in St. George's Hospital, London in 2010 and awarded a research grant at Sapienza University of Rome for the analysis and processing of clinical and biological features of patients treated with hypometilating agents in 2011. Presently, he is a specialist trainee in haematology at the Department of Human Biotechnologies and Hematology at Sapienza University and Policlinico Umberto I, Rome. He was an invited speaker at the 2014 European Hematology Academy on Chronic Myeloid Leukemia and has participated in numerous research programmes, resulting in national and international papers and abstracts.

Corresponding Author: Dr Adriano Salaroli, MD, Department of Human Biotechnologies and Hematology, Sapienza University, Rome, Via Benevento 6, 00161, Italy. E-mail: adriano.salaroli@gmail.com.

Imatinib

Imatinib was the first TKI to be successfully and largely used in clinical practice. As shown by the IRIS study,[2] tolerability is better with imatinib compared with the combination therapy (IFN plus Ara-C). In the imatinib arm (551 patients), AEs were generally grade 1 (mild) or grade 2 (moderate) and there were only rare occurrences of grade 3 and 4 events.

At a median follow-up of 19 months the most common haematological side effect was neutropaenia (60.8% of patients, 14.3% grades 3-4). All-grades thrombocytopaenia affected 56.6% of patients (7.8% grades 3-4). The commonest non-haematological side effect was superficial edema. All grades of edema were recorded in 55.5% of patients, and in 0.9% of them was a grade 3 and 4 edema, generally not requiring any intervention. Sometimes, it is manageable with a reduction of salt intake, topical ointments or diuretics. Nausea was present in 43.7% of patients (0.7% grades 3-4). Muscle cramps and musculoskeletal pain were present respectively in 38.3% and 36.5% of patients (1.3% and 2.7% of patients were grades 3 or 4). Other common AEs were rash, diarrhoea, headache, joint pain and myalgia. Noteworthy, all grades of fatigue were present in 34.5% of patients, 24.4% of which were grades 3 or 4.

As shown by Hochhaus *et al*.[8] at 6-year follow-up, tolerability profile

was really similar to that reported at 19 months and at 5-year follow-up.[9] After 3 years of treatment, grades 3 or 4 haematological AEs were quite rare, with only 1% of patients who experienced anaemia, 1% thrombocytopaenia and 1% neutropaenia. As Druker *et al.*[9] stated: "newly occurring or worsening grade 3 or 4 haematologic or biochemical adverse events were infrequent after both 2 and 4 years of therapy".

After a median follow-up of 60 months, edema (60%), nausea (50%), muscle cramps (49%), diarrhoea (45%) and fatigue (39%) were the most commonly reported long term non haematological side effect.[8] Between year 5 and 6 from the beginning of the IRIS study,[8] the most frequently reported serious adverse events (SAEs), were fever (n = 6 patients), vomiting (N = 4 patients), abdominal pain (n = 3) and nausea (n = 3). With a median of 6 years follow-up, only 5% of patients have discontinued imatinib therapy owing to AEs. An independent long-term study, the Imatinib Long Term Side Effects (ILTE),[10] evaluated long term tolerability of 832 patients on imatinib treatment. Inclusion criterion was complete cytogenetic response (CCyR) for at least 2 years in patients treated with standard dose imatinib. Long-term toxicity appeared to be modest, with 2.3% (n = 19) of patients that discontinued the treatment. Edema (11.5%), muscle cramps (10.7%) and gastrointestinal disturbances (8.9%) were the most common. The study suggests that patients treated with imatinib, although they generally did not experience SAEs, suffered from AEs that can reduce quality of life (QoL).

There are also other AEs that, though rare, have to be mentioned, such as imatinib cardiotoxicity. Atallah *et al.*[11] reviewed all SAEs related to cardiac events occurring in patients involved in clinical trials with imatinib, treated at M.D. Anderson Cancer Center (MDACC). Twenty-two patients out of 1,276 receiving imatinib 300-800mg/day were identified as having symptoms possibly or probably related to congestive heart failure (CHF). Eight of them were possibly or probably related to imatinib, where the incidence of CHF increased with the increasing of the patient's age. The incidence of CHF is similar to those expected in the general population, and other studies suggest no cardiotoxicity with imatinib.[12] Despite this, we would suggest a careful approach to those patients with a significant cardiac history. Finally, in the TIDEL 1 study,[13] we observed the effect of higher dose of imatinib: 103 patients received 600mg/day with dose escalation to 800mg/day in case of suboptimal responders. Especially in the first 6 months, haematological and non-haematological AEs were more frequent compared to imatinib standard dose.

Although imatinib is an effective treatment, it has demonstrated short-term tolerability issues, which can affect continuation of treatment. Most AEs (like superficial edema, gastrointestinal disturbances and muscle cramps) occur within the first 2 years. Long-term tolerability of standard-dose imatinib has demonstrated a low rate of SAEs, but even persistent low grade AEs can affect QoL and consequently patients' adherence to the therapy.[14] Since patients required life-long therapy, even just a few AEs can negatively affect their QoL.[10] Minimisation of factors affecting patients' adherence, such as AEs, should be a priority for all physicians in order to achieve optimal responses.[15]

Second Generation TKIs: Nilotinib and Dasatinib.

Nilotinib is a potent and selective inhibitor of BCR-ABL tyrosine kinase approved for the use after imatinib failure and in newly diagnosed CML.[16] In the ENESTnd trial,[17] Nilotinib was compared to Imatinib in newly diagnosed chronic phase of CML. After 24 months of follow-up, headache and rash were the only grade 3 or 4 non-haematological AEs reported. Headache was reported in 8 patients [3%] with nilotinib 300mg twice daily, four [1%] with nilotinib 400mg twice daily, and two [<1%] with Imatinib. Rashes were observed in 2 patients [<1%], seven [3%] and five [2%], respectively. At 2 years, all grades of non-haematological AEs were rare: all symptomatic QTc changes, pancreatitis, hepatotoxicity, effusions, substantial bleeding and ischaemic heart disease were below 5% of patients. Haematological AEs showed that a grade 3 or 4 neutropaenia was more common with imatinib than with both doses of nilotinib, with a rate of 12% with nilotinib 300mg twice daily, 11% with nilotinib 400mg twice daily and 21% with imatinib. Hepatic biochemical laboratory abnormalities deserve a special mention: all grades of aspartate aminotransferase, alanine aminostranferase and total bilirubin increases were found to be higher in the nilotinib group, especially in the 400mg BID group.[4] Fasting glucose level is modified by nilotinib treatment. As first-line therapy in the ENESTnd trial at 12 months follow-up, all grades of glucose increase were shown by the 36% of patients treated with nilotinib 300mg BID (6% grade 3 and 4), 41% with nilotinib 400mg BID (4% grade 3 and 4) and 20% with imatinib 400mg (0% grade 3 and 4).[4] As second-line therapy at 24-month follow-up,[18] all grades of glucose increase with nilotinib 400mg BID were observed in 70% of patients, with 12% of grade 3 and 4. Note that per ENESTnd exclusion/inclusion criteria patients with uncontrolled diabetes were excluded from the study. One of the so-called "off-target" complications is peripheral arterial occlusive disorder (PAOD) that has been described in patients treated with nilotinib. In the ENESTnd trial at 2-year follow-up 6 cases of patients were described that developed PAOD, 3 (1%) in each of the nilotinib groups.[18] In a retrospective study of 179 patients receiving nilotinib, 11 (6.2%) developed severe and previously unrecognised PAOD:[19] these patients required invasive therapy, such as angioplasty, stent implantation and/or amputation. But 10 out 11 of these patients had cardiovascular risk factors present before treatment (7 of them being affected by hypertension, 4 a history of nicotine abuse, 3 diabetes mellitus, 5 dyslipidemia, 3 were obese, 3 were male, 7

were older than 60 years). Mean time from initiation of nilotinib to the first PAOD was 105.1 weeks. These data seem to suggest that nilotinib may aggravate a pre-existing arteriosclerotic condition.

Dasatinib is a highly potent BCR-ABL inhibitor approved for both newly diagnosed CML patients and resistant or intolerant to a previous TKI. The majority of AEs with dasatinib occur during the first 2 years of treatment,[20] with very few of them presenting between the second and fifth year since the start of the therapy. Non-haematological AEs that occurred in >10% of patients, such as fluid retention, edema, myalgia, vomiting and rash were lower with dasatinib compared to imatinib. Instead, all grades of pleural effusions (PEs) were more common with dasatinib, affecting 14% (2% grade 3 and 4) of patients compared to 0% of patients treated with imatinib. The majority of PEs were grade 1 or 2 (13.6%) with a median duration of 50 days. They were usually reversible and easily managed by dose interruption, diuretics and corticosteroids, without relapses in 88% of patients.[20] Grade 3 and 4 haematological AEs showed that rates of anaemia and neutropaenia were similar between the dasatinib and the imatinib group, instead the rate of thrombocytopaenia was higher for patients on dasatinib compared to imatinib (19% with dasatinib, 11% with imatinib). Overall, most AEs occurred within the first year of treatment with minimal increase between years 1 and 2. Only 15% of patients discontinued dasatinib due to AEs at 2 years with a further 3% discontinuing between years 2 and 5.[21] A recent issue is pulmonary arterial hypertension (PAH), an "off-target" complication that has been described in a few patients. No dasatinib-treated patients developed PAH in the DASISION trial at 2-year follow-up.[20] Based on the Bristol Myer Squibb (BMS) global pharmacovigilance database and as reported by the US Food and Drug Administration (FDA) there have been 12 cases (0.04%) of confirmed PAH reported from a total of 32,882 patients treated with dasatinib between June 2006 and June 2011.[22]

Third Generation TKIs: Bosutinib and Ponatinib.

Bosutinib is a dual SRC and ABL1 inhibitor with high clinical potential in second- and third-line treatment. It has a unique toxicity and tolerability profile, with non-haematologic AEs different from that of others TKIs.[23] Treatment-emergent adverse events were primarily haematological, gastrointestinal and cutaneous. Bosutinib safety and tolerability was assessed in 570 patients enrolled in a phase 1/2 multicentre study in Ph+ leukaemias following resistance/intolerance to imatinib or other TKIs.[23-24] Toxicities were generally mild to moderate severity and easily managed with concomitant medication and/or dose reduction/interruption. SAEs occurred in 44% of patients (mainly pneumonia, pleural effusion and thrombocytopaenia) and they were more common in the advanced leukaemia group of patients (accelerate/blast cml phase and acute lymphoblastic leukaemia). Diarrhoea was the most common AE, occurring in 82% of all patients and presenting after a median time of 2.0 days. It is predominantly of grade 1/2, with only 8% of grade 3/4 diarrhoea. Its incidence decreased over time and it is transient, with a median duration of 1 to 2 days. It is easily manageable with concurrent medication, dose reduction or temporary interruption, with 97% of successful re-challenge.

All grades nausea and vomiting presented respectively in 47% and 39%, with only 1% and 3% of grade 3/4 nausea and vomiting. Pleural effusion occurred in 10% of patients, 4% experiencing a serious pleural effusion. Interestingly, 39% of them had a previous history of pleural effusion.

As suggested by these data, bosutinib is well tolerated despite the high incidence of gastrointestinal events. At the moment for the first-line indication, a positive risk-benefit balance could not be determined because of its gastrointestinal side effects compared to imatinib and its failure to demonstrate superior efficacy,[25] but many patients were not evaluated for cytogentic response (the primary end-point of BELA trial) because of early interruption of bosutinib due to AEs.

Ponatinib is a potent oral pan-TKI inhibitor, very effective against Ph+ leukaemias, both heavily pre-treated and carriers of the T315I mutations.[26-27] In the Phase 2 PACE Trial,[26] ponatinib showed promising results in chronic-phase CML, accelerated phase CML and blast crisis/Ph+ acute lymphoblastic leukaemia. The rising use of sequential multiple TKIs (with a mild increase in mutation frequency)[28] and the genetic instability associated with advanced diseases are giving ponatinib an increasing role since finding the growing number of patients that can benefit from it.

In the PACE Trial, the most common haematologic AEs were thrombocytopaenia (in 41% CP-CML of patients, with 32% of grade 3-4), neutropaenia (in 16% CP-CML of patients, with 14% of grade 3-4) and anaemia (in 10% CP-CML of patients, with 6% of grade 3-4). Haematologic SAEs were thrombocytopaenia (in 2%), anaemia (in 1%), neutropaenia (in 1%) and febrile neutropaenia.

The most common non-haematologic AEs were papular and erythematous rash (in 44% CP-CML of patients, with 4% of grade 3-4), dry skin (in 41% CP-CML of patients, with 2% of grade 3-4) and abdominal pain (in 34% CP-CML of patients, with 7% of grade 3-4).

Non-haematologic SAEs were pancreatitis (5% of patients, with 69% of cases occurred in the first month), abdominal pain (2% of patients) and increased lipase level (2% of patients).

Ponatinib has recently been withdrawn from the market because of

an excessive risk of severe vascular events. A recent warning by the FDA shows that 245 of patients in the Phase 2 clinical trial[29] (median treatment duration 1.3 years) and approximately 48% of patients in the phase 1 clinical study[27] (median treatment duration 2.7 years) experienced SAEs such as myocardial infarction, stroke and venous and arterial thrombosis. These SAEs occurred in those patients with and without established cardiovascular risk factors and in all age ranges.[29] New safety measures were adopted, including narrower indications and more attention about the optimal dosage, even though 45mg is still the initial recommended dose.[29] In the PACE Trial, the initial planned dose in the study was 45mg per day, but this seems questionable given that the doses of 15mg and 30mg per day is sufficient in many cases to reach the blood concentration required to suppress the emergence of new mutations.[30-31]

Conclusions

Several drugs are now available for the treatment of patients affected by CML. Although imatinib is a very effective treatment, it is associated with AEs both in the short- and long-term period. As described in many papers, imatinib AEs can lead the patient to treatment discontinuation or low-adherence behaviour, offering the possibility of achieving a deep and sustained molecular response.[14,15] Persistent low grade AEs may adversely affect patients' QoL, and consequently their adherence.[14] If AEs lay the patient on the line of low adherence, we strongly believe that the switch to a 2nd generation TKIs should be an advisable option. Nilotinib and dasatinib showed an improved tolerability profile over imatinib, supporting the use of second generation TKIs not only as a choice in case of intolerance but also as first-line therapy in frontline.[17,19] Their AEs, such as pleural effusion with dasatinib and increased fasting glucose with nilotinib, seems to have no impact on the efficacy, being easily manageable.[17] "Off-target" complications, such as PAH with dasatinib and PAOD with nilotinib, even though very rare, should always be kept in mind; it seems reasonable to look for pre-disposing conditions before starting patients on them. Bosunitib has a unique toxicity and tolerability profile, with diarrhoea occurring in up to 82% patients. It is transient, self-limited and easily manageable. Ponatinib seems irreplaceable in certain subgroups of patients, but recent cardiovascular issues narrowed its indication and have raised the need for additional data in order to truly assess its role outside of salvage therapy. With the increase of available drugs the difficulty in choosing the most suitable one for the first and second or subsequent lines of therapy is emerging. Rarely mutation analysis and more often comorbidites are still, along with the discussion with the patient about different AEs, our best guide to the choice of the correct TKI.

Disclosure

There are no conflicts of interest.

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Monitoring Viral Immunity in HSCT Patients

Liselotte Brix
COO and Co-Founder, Immudex

Introduction

Viral infection and disease pose a major risk in immunosuppressed patients. Reactivation of latent viruses, such as herpes viruses are frequently observed post transplantation but community acquired viruses may also cause disease.

Cytomegalovirus (CMV) is the major infectious agent threatening HSCT patients and most knowledge regarding viral immunity post-transplant has been achieved looking at this virus. CMV infects and establishes persistent lifelong infections in 50-85% of adults.[1,2] In healthy individuals the virus is controlled by CMV-specific T cells but following transplantation reactivation of the virus is a frequently occurring complication due to immunosuppression and can significantly contribute to morbidity and mortality. CMV replication and disease is also associated with a higher risk for bacterial and fungal infections and has been linked with increased risk of graft versus host disease (GVHD).[3,4]

CMV infection is managed by preemptive antiviral drug therapy. However, medications currently available are expensive and hampered by side-effects as renal toxicity and immune suppression. Development of drug-resistant strains has also been observed. Side effects can be cumulative, limiting usage.

Preemptive anti-viral treatment is guided by monitoring CMV viral load using real-time PCR or monitoring CMV pp65 expression in leukocytes. However, this approach disregards the patients' immune status. Many patients with low-level CMV viremia will have spontaneous clearance of the infection if CMV-immunity is adequately recovered. Antiviral drug therapy of the patient capable of immune-mediated viral control unnecessarily exposes the patient to potential side effects and high costs of the currently available antiviral medication. Thus, patients with reestablished CMV-immunity may tolerate higher viral loads, even in the absence of anti-viral treatment and without increased risk of CMV disease or may be managed with shorter courses of antiviral drugs.

New types of anti-viral therapy are in development. Several of these are based on delivery or induction of CMV-specific cytotoxic T cell (CTL) immunity in the treated patient, increasing immune-mediated viral control. Adoptive transfer of virus-specific T cells is actively investigated and also CMV-specific vaccines have reached the clinical stage of development.[5-8] These strategies are very promising and could overcome some of the limitations and toxicities of current long-term antiviral treatments.

Why Monitor Viral Immunity?

Viral-specific immunity plays a critical role in development and control of viral disease. In healthy individuals equilibrium is achieved where viral-specific T cells control persisting virus. When T-cell function is impaired and equilibrium is not established, viral reactivation and clinical disease may develop.

Reconstitution of antiviral immune responses and control of viral reactivation is mainly achieved by anti-viral CD8+ T cells. In transplant patients a low number of CMV-specific CD8+ T cells are predictive for development of CMV-related complications.[9] Additional components of the immune response, such as CD4+ T cells, contribute to viral control.

The adaptive immune response to a viral infection is a complex process involving multiple cell subsets and a multitude of secreted effector molecules. Most immune monitoring strategies seek to simplify this complexity by focusing on regular measurements on one or a selected group of parameters that reflects the state of

Liselotte Brix is COO at and co-founder of Immudex, a company specialized in monitoring antigen-specific T-cell responses. She holds a Ph.D in immunology and biotechnology and has 15+ years scientific experience with TCR-MHC interactions in relation to cancer, infectious and autoimmune disease. She joined the Danish diagnostic company Dako, Copenhagen in 2005 and was before that assistant professor at the Danish Pharmaceutical University, Copenhagen and at Aarhus University. In her current position she is heading discovery and development activities at Immudex.

immunity in a given subject. What simple marker would be more obvious than monitoring the cells responsible for eradication of the virus, the virus-specific CD8+ T cells?

Knowledge of the level of viral-specific T-cell immunity in a given patient allows evaluation of the patient's preparedness for fighting the viral infection. Patients with slow versus fast recovery of viral-specific immunity may be identified as illustrated in Figure 1, permitting patient-by-patient stratification with respect to withdrawal, initiation or duration of anti-viral therapy.

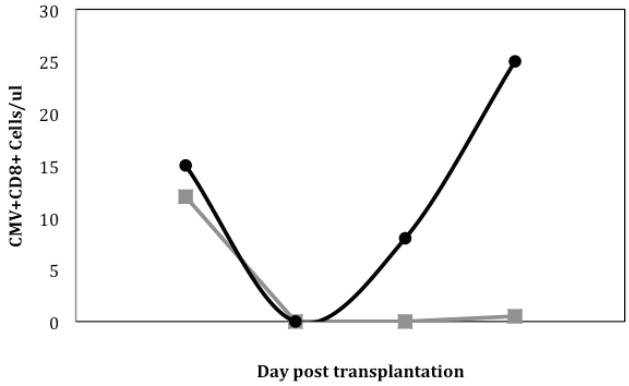

Figure 1. Immune monitoring of T-cell responses in HSCT patients. Schematic drawing of CMV-specific immunity in two hematopoietic stem cell transplanted patients using MHC multimers. Patients were monitored pre-transplantation (PreBMT), and at day 30, 60 and 90 post transplantation. One patient shows fast reconstitution of CMV immunity (black) and the other patient recovers slowly (grey).

Experience with viral immune monitoring from clinical studies has consolidated the importance of measuring T cell-mediated immunity. Several groups have shown that failure to recover CMV-specific CD8+ T cells post-HSCT is associated with higher incidence of recurrent or severe CMV reactivation and increased risk for the development of CMV disease.[9-14] The threshold level of measured CMV-specific T cells per ul peripheral blood that confer protection against CMV disease has evolved only slightly over the course of these studies, ranging from 2 – 10 CMV-specific T cells per ul blood.[9-11] Borcher *et al*. further suggested that combining measurement of the level of CMV-specific T cells in blood with measurement of CMV-specific T cell expansion may be an even better predictive risk marker.[12] The two larger multicenter studies performed to date agree that sequential monitoring of CMV-specific T cells at regular intervals post transplantation is necessary.[9, 10] The CMV-specific CD8+ T-cell response at any single time point post-transplant provides insufficient information in it selves to base clinical decisions.

Methods for Monitoring Viral Immunity

Today several assays exist for monitoring CMV-specific cellular immunity. The ideal assay should be easy to perform in a routine diagnostic laboratory, be highly standardized, has broad population coverage and a short assay turnaround time.

Current strategies rely on the direct detection and enumeration of viral-specific T cells in blood or on measurement of cytokines secreted by specific T cells upon viral-specific stimulation.

Direct detection methods use viral-specific MHC multimers for labelling and visualization of viral-specific T cells in blood. MHC multimers are reagents that carry multiple MHC-peptide complexes, the natural ligand for the T cell receptor (TCR). Cooperative binding of the many MHC-peptide complexes to TCRs on the T cell surface enables efficient, highly specific detection of T cells with the specificity of interest. Each multimer also carries fluorochromes facilitating single cell visualization by flow cytometry. The original MHC multimer reagent was termed the 'tetramer'[15] and has been used in several clinical studies evaluating reconstitution of cellular immunity in hematopoetic transplant patients. The tetramer has been followed by pentamers and streptamers, differing in the number of MHC-peptide pairs and method of multimerization. More recently the highly sensitive Dextramer was developed, which allow easier enumeration and identification of even low-affinity T cells.[16]

MHC multimer assays are specific and robust assays, easy to perform, require little sample material and are highly reproducible. A short assay time (1-2 hours) enables timely results. Assay protocols harmonized between laboratories have been established through proficiency panel testing and validated assay procedures.[17, 18] Today MHC multimer assays yield very consistent results between labs worldwide as illustrated by data obtained in the latest MHC multimer proficiency panel (to be published, see http://www.immudex.com/proficiency-panels.aspx).

MHC multimer assays are limited by the need to know the patients HLA-type, since the MHC-type expressed on the MHC multimer must be matched to the HLA-type of the patient being tested. Large panels of multimers broadly covering HLA-types of the target population must be available to apply this technology in a routine setting. Recently, a commercial MHC multimer assay for monitoring of CMV-specific cellular immunity was CE-marked for in vitro diagnostic use in Europe (Dextramer® CMV Kit, Immudex, Denmark), which includes Dextramers covering more than 95% of the European population. Such validated kits make it easier to introduce routine CMV cellular immune monitoring in the hematopoietic transplant setting.

Several assay platforms measuring secreted cytokines, upon viral-specific in vitro stimulation, have been developed for monitoring

of CMV-specific cellular immunity. Most of these assays rely on detection of secreted interferone-γ (IFN-γ). IFN-γ is measured in supernatant by ELISA, from single cells via Elispot or by intracellular cytokine staining (ICS) using flow cytometry. The advantage of these assays is that they detect only those cells capable of secreting cytokine. Disadvantages are long assay turnaround time (running over 2 days), no differentiation between CD8+ and CD4+ T cell subsets and strict requirements to sample freshness and cell viability. Both Quantiferone and Elispot assays have been standardized to some degree but assay variation between labs still exist. Two of these IFN- γ release assays are commercial available, the Quantiferone®-CMV assay and the T-Track® CMV assay, both CE-marked for commercial use in Europe. No ICS based assay is commercially available.

Table 1 summarises principles, advantages and disadvantages of CMV cellular immune monitoring assays.

Both measurement of upon virus-specific *in vitro* stimulation and direct detection of virus-specific T cell subsets have shown to be valuable tools for monitoring reconstitution of cellular immunity to CMV.[9, 12, 13] The MHC multimer technology can be combined with measurement of cytokine secretion and/or expression of activation surface markers allowing measurement of functionality of individual viral-specific T cell subsets detected by the multimer.

	MHC multimer	Quantiferon	Elispot	ICS
Sample	Whole blood/ PBMCs	Whole blood	PBMC	Whole blood/ PBMCs
Sample Volumen	0,5-1 ml /1x10^6 cells	3 ml	2x10^6 cells	0,5-1 ml /1x10^6 cells
Antigen	Individual peptides (pp65, IE-1, pp50)	Pool of 22 different peptides (from pp65, pp50, IE-1, IE-2, gB)	Individual or pools of peptides/ whole antigens/virus lysates	Peptides, whole antigens
Assay Turnaround Time	2 hours	24 hours	24-48 hours	10-24 hours
Measurant	Presence of CMV-specific T cells	Secreted cytokine from pool of cells	Secreted cytokine from individual cells	Secreted cytokine from individual cells
Principle	Direct detection	*In vitro* stimulation	*In vitro* stimulation	*In vitro* stimulation
Advantages	Easy to perform, highly standardized, specific and sensitive, can be combined with functional analysis, differentiates phenotypes	Functional analysis, simple to perform, standardized, sensitive	Functional analysis, sensitive	Functional analysis on single cell level, can be combined with MHC multimer analysis, differentiates phenotypes
Limitations	Knowledge on individual HLA-types required	Sensitive to lymphopenia, restricted to most widespread HLA alleles, high sample viability required, no phenotypic differentiation	Need purified PBMC, limited technical standardization, high sample viability required, labor intensive, no phenotypic differentiation	Labor intensive, lack technical standardization, high sample viability required
Commercial Test	Dextramer® CMV kit (CE-marked)	Quantiferone®-CMV (CE-marked)	T-Track® CMV	No

Table 1. Characteristics of methods for monitoring CMV-specific T cell responses

Clinical Applications

Detectable CMV-specific T cell responses are correlated with appropriate control of CMV in transplant patients. Thus, in HSCT recipients CMV-specific T cell monitoring can be used to identify patients at risk of CMV complications at different time points pre- and post-transplant:

- Regular monitoring of reconstitution of CMV-specific immunity in the first 100 days post transplantation identifies patients at higher risk of CMV reactivation in the early phase.

- Assessment of immune competence post-primary CMV infection in patients after transplantation identifies patients at risk of additional CMV reactivation after cessation of antiviral prophylaxis.

- Assessment of CMV-specific immune competence in patients beyond day 100 after transplantation identifies who are at risk of developing late CMV disease.

- Assessment of viral-specific immunity in recipients prior to transplantation identifies recipients with no preexisting immunity. Such patients will have the highest risk of CMV disease if they acquire an infection.

The immune response in HSCT patients is also strongly dependent on the immune response present in the donor. Patients receiving a graft from a CMV positive donor has significant lower risk of CMV reactivation and disease compared to patients receiving a graft from a CMV negative donor.[19] The donor CMV-specific T cells will confer protection to the recipient and decrease risk of later CMV reactivation. The number and functionality of CMV-specific T cells transferred with the graft may therefore be an important parameter to measure prior to transplantation as well as following their establishment in the recipient. Monitoring the level of CMV-specific T cell immunity in a given donor may also help optimize recruitment of donors having high numbers of CMV-specific CD8+ T cells and identify donors optimal for CMV CTL adoptive therapy.

Finally, measurement of CMV immunity is relevant for the new therapeutic approaches aiming at inducing CMV CTL responses patients by adoptive transfer of CMV CTLs or through virus-specific vaccination. Monitoring the level of induced viral-specific immunity help evaluate the effectiveness of such therapies and may be easily accomplished by the methods described.

Summary and Future Perspectives:

CMV-specific T cells are readily quantifiable and such data may be used to answer important clinical questions regarding risk of CMV-reactivation in individual patients and their possibility of progressive CMV replication and disease. Current CMV handling in HSCT patients are based on preemptive anti-viral treatment and prophylaxis. The ability to monitor CMV immunity is an important progress that can improve CMV prevention and treatment through patient and donor stratification.

Other viruses like EBV, adenovirus and BK virus may also contribute to infectious complications post transplantation. Monitoring reconstitution of immunity to these viruses could improve viral prevention and handling, by predicting patients at risk.

The level of CMV immunity may be a surrogate marker for general immune status, and therefore an indication of the patient's preparedness to combat infections in general. Thus, reconstitution of CMV immunity may correlate with reconstitution of general immunity. This would allow prediction of the immune system's preparedness to fight other viral, bacterial and fungal infections.

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Graham Collins
Consultant Haematologist, Oxford University Hospitals NHS Trust, Honorary Senior Clinical Lecturer, Oxford University, Oxford, UK

Classical Hodgkin Lymphoma is unusual in that it largely affects young people with a median age of presentation in the 15-35 age group. The treatment of Hodgkin Lymphoma is generally a success story, with the majority of those affected being cured of their disease. In children a risk-stratified, combination chemoradiotherapy approach has resulted in 5 year event free survival (EFS) rates of over 85% and 5 year overall survival (OS) rates of over 95%.[1] Results in adults are also impressive. Even in advanced stage disease, overall survival rates generally exceed 90%.[2-4] However a minority do still relapse. In the UK, figures from Cancer Research UK indicate that there are approximately 1500 new cases of classical Hodgkin lymphoma diagnosed per year. Of these, approximately 1300 occur in adults and approximately 35-40% will be advanced stage (Ann-Arbor stage III or IV). This equates to roughly 500 adult cases of advanced Hodgkin per year. Most data suggests that the cure rate of first line ABVD (the most commonly used first line regimen used in adults in the UK) is approximately 75% [2,3] meaning that 125 relapses would occur each year. Of course this is an underestimate since some early stage patients will relapse after first line treatment and the cure rate from first line chemotherapy in elderly patients is much less good.[5] For a typical academic centre with a catchment area for stem cell transplantation of 2 million, it would therefore expect to see 4-5 patients per year. The majority of these patients would be young and fit and eligible for potentially curative second line treatment.

Second Line Treatment

There are two goals of second line treatment in relapsed Hodgkin patients who are fit for potentially curative regimens: induction of 2nd remission and mobilisation of autologous haematopoietic stem cells. A number of different regimens have been used in relapsed Hodgkin lymphoma (Table 1). No randomised trials have compared different regimens and phase II data suggests that there is no one standard regimen. Most importantly then, centres should use regimens with which they are familiar. There are also no comparative trials assessing the efficacy of salvage regimens to mobilise autologous stem cells. Some studies suggest there maybe differences. For example, a retrospective study comparing the efficacy of mini-BEAM with GDP in collecting > $5x10^6$ CD34+ cells/kg showed a superior result with GDP (97% compared with 57%).[6] Similarly, a retrospective analysis suggested stem cell mobilisation following IVE was more successful than after ICE.[7] Again, no phase III randomised studies have been performed to formally test these results. Concern has also been raised over a possible negative effect of alkylating agents on the capacity to mobilise stem cells. Two studies implicated the use of mini-BEAM prior to stem cell collection as having a negative impact on subsequent collections[8,9] so it is generally recommended to avoid such regimens until after successful stem / progenitor cell collection.[10]

Previous studies have identified Hodgkin lymphoma as being associated with poor stem / progenitor cell collection[11] although this may have been due to extensive use of alkylating agents and radiotherapy which would not equate with more modern first line management. Even so, failure to collect sufficient progenitors at the first attempt has been reported to occur, depending on the definition, at a rate of approximately 10-20%.[12,13] Recently plerixafor, an antagonist to the chemokine receptor CXCR4 has been licensed for use in combination with GCSF in patients with lymphoma or myeloma who have poor stem cell mobilisation. Although most data is with non-Hodgkin lymphoma patients, a number of studies also support its use in Hodgkin lymphoma. For example, in a compassionate use study of 115 patient who had failed one previous mobilisation attempt, GCSF and plerixafor resulted in successful collection in 76% of Hodgkin patients.[14] Therefore use in mobilisation failure does appear to increase the likelihood of subsequent successful progenitor cell collection. More recently, protocols have been using plerixafor in a pre-emptive fashion in an attempt to optimise the success of the first mobilisation attempt.[15]

The Importance of Second Remission

An important aim of 2nd line treatment is to achieve a complete

Graham Collins is consultant haematologist and honorary senior clinical lecturer at the Oxford Cancer and Haematology Centre which is part of Oxford University Hospitals NHS trust. After training in Cambridge and London, he underwent haematology training in Oxford which included a DPhil investigating the molecular biology of the Reed-Sternberg cell. Since taking up his consultant post he has focused on clinical research and has an active interest in lymphoma trials (both early and late phase) and in developing and validating predictive biomarkers. He sits on the NCRI lymphoma Clinical Studies Group and has recently co-authored the national guidelines for relapsed or refractory Hodgkin Lymphoma.

remission by FDG-PET scan. This has been highlighted by a number of studies, especially by the group from Memorial Sloan-Kettering. A seminal paper by this group reported on consecutive patients from 1994 to 2003 treated with ICE based second line treatment.[27] The exact second line treatment did vary according to era of treatment with earlier patients treated with standard ICE whereas later patients received a risk-based approach with higher risk patients receiving at least 1 cycle of augmented ICE. The pathway was also slightly unusual in that patients received IFRT to all sites of residual or relapsed nodal based disease prior to stem cell transplantation. In addition, the conditioning used was not that normally used in the UK, consisting of high dose cyclophosphamide and etoposide for radiation-naïve and CBV (cyclophosphamide, BCNU and etoposide) for those previously irradiated. Patients received functional imaging (initially gallium scanning but more latterly FDG-PET scanning) prior to second line treatment, and pre-ASCT. The overall 5 year EFS and OS for the group were 63% and 71% respectively. Importantly, on multivariate analysis the only factor predictive of outcome was disease status pre-ASCT as determined by functional imagine. The 5y EFS for functional imagine positive and negative patients was 31% and 75% respectively with no difference seen between type of functional imaging. The significance of pre-ASCT FDG-PET has also been confirmed by other studies.[28-31]

A natural question is what should be done if the PET scan remains positive after first salvage treatment? This was addressed in a prospective, risk-adapted study whereby patients with relapsed Hodgkin lymphoma received 2 cycle of either ICE or augmented ICE (depending on clinical risk factors).[32] After 2 cycles, a PET scan was performed. If deemed negative, patients went on to receive an ASCT. If positive, patients received 4 cycles of biweekly gemcitabine, vinorelbine and liposomal doxorubicin. A further PET scan was performed and all patients with responsive disease (irrespective of PET status) proceeded to ASCT. The key message from this trial is that EFS curves for those patients who obtained a negative PET scan after either only ICE /aICE or GVD were superimposable. Those who were PET positive after GVD had much worse outcomes. The message seems to be that achieving PET negativity with salvage chemotherapy prior to ASCT, should be a high priority.

Autologous Stem Cell Transplantation

High dose chemotherapy with autologous stem cell transplantation is currently the gold standard treatment for relapsed cHL after response to 2nd line treatment. There are 2 randomised clinical trials which support this approach. The first was reported by the British National Lymphoma Investigation (BNLI) group.[33] They randomised patients between BEAM chemotherapy with autologous bone marrow transplantation, or up to 3 cycles of mini-BEAM. Only 40 patients were randomised and there was no statistically significant difference in overall survival. However, 3y event free survival was 53% for the BEAM group and 10% for the mini-BEAM cohort. The second study was larger and with a slightly different design. All patients received 2 courses of Dexa-BEAM chemotherapy. Those randomised to high dose therapy underwent this procedure if they were responding after the 2 cycles of salvage. For those randomised to standard chemotherapy, they received 2 further cycles of dexa-BEAM. Again, no overall survival difference was demonstrated but the 3 year freedom from treatment failure was 55% for the transplant group versus 35% for the chemotherapy group.[34] In both trials, a lack of overall survival benefit maybe due to patient failing on the chemotherapy arm and then progressing to high dose therapy.

Much work has aimed at optimising the autologous stem cell transplant approached. One very significant study is the H96 trial which reported on the use of tandem ASCTs in high risk patients.[35] For the purposes of this study, high risk was defined as time to relapse of < 12 months, stage III or IV relapse and relapse within a previously irradiated site. Conditioning

Table I. Chemotherapy Regimens Trialled in Relapsed cHL

Regimen	ORR (%)	CRR (%)
ICE (ifosfamide, carboplatin, etoposide)[16]	88	26
IVE (ifosfamide, epirubicin, etoposide)[17]	85	37
DHAP (dexamethasone, cytarabine, cisplatin)[18]	89	21
GDP (gemcitabine, dexamethasone, cisplatin)[19]	70	52
GemCis (gemcitabine, cisplatin, dexamethasone)[20]	94	*65*
IGEV (ifosfamide, gemcitabine, vinorelbine)[21]	81	54
GVD (gemcitabine, vinorelbine, liposomal doxorubicin)[22]	70	19
Mini-BEAM (carmustine, etoposide, cytarabine, melphalan)[23]	84	32
DexaBEAM (dexamethasone, carmustine, etoposide, cytarabine, melphalan)[24]	69	62
MINE (mitoxantrone, ifosfamide, vinorelbine, etoposide)[25]	75	34
ASHAP (doxorubicin, methylprednisolone, cytarabine, cisplatin)[26]	70	34

for the 2 transplants was different, with the 2nd occurring between 45-90 days after the first in those patients who were not showing signs of progression. The 5 year freedom from 2nd treatment failure and overall survival were 46% and 57% respectively, which for such a high risk group are encouraging results. However a randomised trial is required to confirm superiority of this approach.

Allogeneic Stem Cell Transplantation

The role of allogeneic stem cell transplantation in relapsed Hodgkin lymphoma is controversial. There is however, reasonable data supporting its use in those relapsing after autologous stem cell transplantation. Data from registries and larger series suggest a long term progression free survival of between 20-40%[36-38] although the non-relapse mortality of up to 20% does off-set this benefit to a degree. A few reports suggest that those who progress to allogeneic stem cell transplantation do in fact have superior outcome than those who do not, providing indirect evidence in support of this approach.[39,40] Recently, a risk-adapted strategy has been trialed in which allogeneic stem cell transplants are performed in high risk patients at first relapse, defined as those who fail to achieve a metabolic complete remission after first line salvage therapy. The original report showed a 3 year PFS of 68% with the interesting observation that some relapsing patients could be brought back into a PET negative remission by the use of donor lymphocyte infusions.[41] This strategy has recently been tested in a multi-centre trial although results are awaited.

Novel Agents

Brentuximab vedotin is a novel anti-CD30 immunoconjugate in which a monoclonal antibody is attached to a potent tubulin toxin (monomethy auristatin E) via a protease cleavable linker. CD30 is widely expressed by Hodgkin / Reed-Sternberg cells and in an initial phase I study for relapsed/refractory CD30-positive malignancies, 54% showed an objective response with 39% achieving a complete remission.[42] In a subsequent phase II study focusing on relapsed/refractory Hodgkin lymphoma in patients who had received an autologous stem cell transplant, the overall response rate rose to 75% with a 34% complete remission rate. The median duration of response was 5.6 months but for those in CR, this rose to an impressive 20.5 months.[43] The most common grade 3 or more adverse events were neutropenia and peripheral sensory neuropathy. Brentuximab has now been licensed by the EMEA for classical Hodgkin lymphoma relapsing after ASCT or for those patients relapsing after 2 or more lines of treatment not suitable for high dose therapy. In practice, this means that brentuximab is frequently being used as a bridge to allogeneic stem cell transplantation in patients fit enough and with a suitable donor. Retrospective data suggests it is a useful therapy to achieve this goal.[44-46] Although the license for brentuximab is for 8-16 courses, most centres are using fewer than this when subsequent transplantation is planned as progression can be seen after an initial response to 3 or 4 doses. Brentuximab has also been used following an allogeneic transplant and it appears safe and effective in this context, often allowing administration of donor lymphocyte infusions when patients are in a better remission.[47]

A variety of other novel agents have been tried in relapsed / refractory Hodgkin although none have shown quite the promise of brentuximab vedotin. The bifunctional alkylator and purine nucleoside analogue bendamustine, for example, was associated with an overall response rate of 53% and 58% in 2 reports although the duration of remission was disappointingly short at 5 months.[48,49] The histone deacetylase inhibitor vorinostat produced only a 4% response rate[50] although the response rate with a different HDAC inhibitor, mocetinostat, was better at 35%.[51] Finally, the inhibitor of Mammalian Target of Rapamycin (mTOR) everolimus was associated with an encouraging response rate of 47% although the time to progression was just over 7 months.[52] In summary then, brentuximab vedotin clearly represents the most encouraging novel agent for Hodgkin lymphoma in recent years although as a single agent in high risk disease it is unlikely to produce a cure.

Summary

Classical Hodgkin Lymphoma is sometimes considered a 'good cancer' to have. While clearly there are no 'good cancers' the sentiment represents the curability of first line treatment. However at relapse the cure rate falls significantly although modern functional imaging is becoming increasing useful at identifying those likely to do well from standard approaches involving autologous stem cell transplantation. For those that fail to reach a PET-defined complete remission, novel approaches are warranted, such as use of second line chemotherapy, brentuximab vedotin, or possibly an allogeneic stem cell transplantation. For second or subsequent relapses, the outcome is much less certain. Although the patient numbers are low, they are frequently young and fit and novel agents used either alone, or used in intelligent, preferably biomarker-driven combinations could offer hope for future therapy.

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The Effect of JAK Inhibitors in Myelofibrosis

Francesco Passamonti
Division of Hematology, Department of Medicine, University Hospital Ospedale di Circolo e Fondazione Macchi, Varese, Italy

The ELN guidelines recommended to use hydroxyurea (HU) as drug of choice when an anti-myeloproliferative effect is needed in MPNs.[1] However, data available on HU are scant. The most complete study on HU in MF evaluated retrospectively 40 patients.[2] Reasons for treatment were constitutional symptoms (55%), symptomatic splenomegaly (45%), thrombocytosis (40%), leukocytosis (28%), pruritus (10%), and bone pain (8%). Responses on different symptoms/clinical findings were as follows: bone pain in 100%, constitutional symptoms in 82%, pruritus in 50%, splenomegaly in 40%, and anaemia in 12.5%. According to the IWG-MRT criteria, clinical improvement was achieved in 16 patients (40%). Despite the high rate, the median duration of response was 13.2 months. Worsening of anaemia or appearance of pancytopenia wase observed in half of the patients.

In the last few years several medicines with anti JAK properties, named JAK inhibitors (JAKi) have been studied.[3] Among these, ruxolitinib is the only approved in many States and available for clinical practice. Other compounds entered phase-3 investigation (fedratinib, momelotinib, pacritinib), while others are being tested in phase 1-2 studies (www.clinicaltrials.gov). For the practical purpose of this review only ruxolitinib, fedratinib and momelotinib will be discussed in detail as only data reported as a full paper will be taken into account.

Ruxolitinib

A phase I/II trial with ruxolitinib (oral drug) was conducted in 152 patients with PMF or post-PV/post-ET MF.[4] Eligible subjects were therapy-requiring patients, refractory, relapsed, intolerant to previous therapy, or patients with intermediate or high-risk Lille score, if at diagnosis. Main exclusion criteria were thrombocytopenia (platelets <100 x10^9/L) and neutropenia. Applying IWG-MRT criteria, 44% of patients obtained a clinical improvement of spleen size (≥50% reduction from baseline, measured by palpation) at 3 months and responses were maintained at 12 months in more than 70% of patients. The majority of patients had ≥50% improvement in constitutional symptoms mostly due to the activity against pro-inflammatory cytokines. The reduction of the JAK2 (V617F) allele burden was modest. This study was mainly conducted at MD Anderson Cancer Center (MDACC), Houston, and at Mayo Clinic, Rochester. Two comparisons of outcomes from this phase I/II trial with historical controls have been performed separately in the two centers to test the effect of ruxolitinib on survival.[5,6] Mayo Clinic investigators compared 51 patients who received ruxolitinib at Mayo Clinic with 410 patients from the Institutional database not showing any difference in term of survival.[6] The second study compared 107 patients treated with ruxolitinib at MDACC with 310 patients (from three different centers) matched for the phase 1-2 study entry criteria, as controls.[5] A survival benefit for patients treated with ruxolitinib was demonstrated. In addition, the study demonstrated that patients treated with ruxolitinib who obtained a reduction of spleen size greater than 50% have a significantly better survival than those who did not.

Two prospective randomised trials with ruxolitinib have been published: COMFORT-1 (155 ruxolitinib vs. 151 placebo)[7] and COMFORT-2 (146 ruxolitinib vs. 73 best available therapy, BAT).[8] In COMFORT-1, the primary endpoint (reduction of spleen volume by MRI equal to or greater than 35%) at week 24 was reached in 42% of patients in the ruxolitinib arm and in 1% of those in the placebo arm. At week 24, 46% of patients receiving ruxolitinib and 5% of those receiving placebo experienced symptom alleviation by at least 50%, as measured by the modified Myelofibrosis Symptom Assessment Form (MF-SAF).[9] Patients treated with ruxolitinib experienced relief of abdominal discomfort, early satiety, night sweats, itching,

Francesco Passamonti is Head of the Division of Hematology of the University Hospital of Varese, Italy. He obtained his medical degree from the University of Pavia, subsequently becoming a specialist in Hematology. He worked as Assistant Professor of Hematology at the University of Pavia and as hematologist at the Division of Hematology of Policlinico San Matteo, Pavia, Italy. He is principal investigators in many clinical trials in hematological malignancies, however, his main area of interest is in Philadelphia-negative myeloproliferative neoplasms (MPN), polycythemia vera, essential thrombocythemia, and myelofibrosis.

and musculoskeletal pain. In the COMFORT-2 trial the primary endpoint (the same as the COMFORT-1 study but evaluated at week 48) was reached in 28% of patients treated with ruxolitinib and in 0% of those receiving BAT; at week 24 the figures were 32% and 0%, respectively. Mean improvements from baseline in FACT-LymS (Functional Assessment of Cancer Therapy–Lymphoma System) were greater in the ruxolitinib arm.

Recently, the long-term (median time, 2 years) data from the COMFORT-1[10] trial has been published: 100 of 155 patients randomised to ruxolitinib were still receiving treatment. Mean spleen volume reductions in the ruxolitinib group were 32% at week 24 and 35% at week 96; improvements in quality of life measures were also maintained. Improved survival was observed for ruxolitinib (n=27 deaths) versus placebo (n=41 deaths) with a hazard ratio of 0.58. Dose-dependent anaemia and thrombocytopenia were the most common adverse events in the ruxolitinib group, but these events rarely led to discontinuation. The incidence of new-onset grade 3 or 4 anaemia (29% and 11%, respectively) and thrombocytopenia (9% and 3%, respectively) reported in the first 6 months of therapy decreased over time to less than 5% for anaemia and less than 2% for thrombocytopenia. Mean hemoglobin values reached a nadir of 10-12% below baseline between weeks 8 and 12 and stabilised over time to a new steady state slightly below baseline by week 24, and then remained stable throughout the remaining follow-up. In the first 6 months of treatment, the most common non-hematologic adverse events that occurred more frequently in the ruxolitinib group compared with the placebo group were ecchymosis, headache and dizziness. Under ruxolitinib the rate of non-hematologic adverse events reduced over time. Two patients originally randomised to receive ruxolitinib developed BP at the time of the primary analysis, and no further cases were reported in this group.

COMFORT-2 trialists updated the 3 year-follow with 45% (66 of 146) of those originally randomised to ruxolitinib remaining on treatment.[11] The 3-year probability to maintain spleen response (greater than 35%, by MRI) was 50% among patients achieving such degree of response. Ruxolitinib continues to be well tolerated. Anaemia and thrombocytopenia were the main toxicities, but they were generally manageable, improved over time, and rarely led to treatment discontinuation (1% and 3.6% of patients, respectively). Other adverse events of special interest included leukopenia, bleeding, infections, thromboembolic events, elevated transaminase levels, increased systolic blood pressure, and weight gain. The rate of these events generally decreased with longer exposure to ruxolitinib treatment, with the highest rates occurring within the first 6 months of treatment. Among these events, infections occurred in 50% of patients between weeks 0-24 and included bronchitis, gastroenteritis, nasopharyngitis, urinary tract infections. The rate of infections becomes 25% in weeks 144-168. Over the entire course of the study, 2 patients (1.4%) in the ruxolitinib arm had tuberculosis. No single non-hematologic adverse event led to definitive ruxolitinib discontinuation in more than one patient. Finally, patients randomised to ruxolitinib showed longer overall survival than those randomised to BAT (hazard ratio, 0.48).

As in both studies all patients crossed over to ruxolitinib, it is difficult to compare the effect on survival in the long term. In a recent study,[12] survival from diagnosis of PMF patients (n=100) who received ruxolitinib in the COMFORT-2 at intermediate-2 and high risk IPSS[13] was compared to that of a comparable group of conventionally treated PMF patients (n=250), namely the DIPSS cohort,[14] when at the same risk. With an appropriate and ad hoc statistical analysis, investigators found that patients treated with ruxolitinib at some point during their disease history had a better survival when compared to those who continued standard treatment for the whole duration of follow-up, ultimately suggesting that ruxolitinib affects PMF natural history.

Taken together the COMFORT trials showed that ruxolitinib, a drug with a good safety profile, improves two clinical needs of patients: splenomegaly and MF-related symptoms. However, reactivation of infections such as tubercolosis or viral hepatitis has been reported in very few case reports and this underlines the need for a careful observation of patients during follow-up. *In vitro* data[15] demonstrated that ruxolitinib significantly affects dendritic cell differentiation and function leading to impaired T-cell activation,[16] potentially resulting in increased infection rates in ruxolitinib-treated patients. Though requiring adequate monitoring for these potential side effects, data on survival advantage are really interesting and place this drug as a new potential first line therapy in MF patients at higher risk.

Fedratinib, SAR302503

In a phase I-II trial, fedratinib was administered orally once a day to 59 patients with intermediate and high-risk MF.[17] By six and 12 cycles of treatment, 39% and 47% of patients, respectively, had achieved a spleen response per IWG-MRT criteria. The majority of patients with leukocytosis or thrombocytosis at baseline achieved normalization of blood counts after six (57% and 90%, respectively) and 12 (56% and 88%, respectively) cycles. Beside the effect on splenomegaly, the majority of patients with constitutional symptoms, fatigue, pruritus had a durable resolution. Grade 3 to 4 hematologic adverse events included anaemia (occurring in 35% of 37 patients who were not RBC transfusion dependent at baseline), thrombocytopenia (24%) and neutropenia (10%). At doses ranging between 240 mg and 520 mg, two of five RBC transfusion-independent patients

became RBC transfusion-dependent and two of nine had grade 3/4 thrombocytopenia. The main non-hematologic adverse events included all grades nausea (69%), diarrhea (64%) vomiting (58%), all self-limited and controlled by symptomatic treatments. Asymptomatic increase of lipase, AST, ALT, and creatinine have been reported in roughly one quarter of patients. A randomised, blinded, placebo-controlled study of fedratinib (dose 400 mg or 500 mg daily), named JAKARTA, in patients with intermediate-2 or high risk MF has been started with very promising results meeting the endpoint (reduction of spleen volume by MRI equal to or greater than 35%) and demonstrating an advantage of fedratinib against BAT. However, few cases consistent with Wernicke's encephalopathy have been reported in patients participating in fedratinib trials and following a thorough risk-benefit analysis, the risk to patient safety was considered to outweigh the benefit that fedratinib would bring to patients. So, all clinical trials involving fedratinib have been halted.

Momelotinib, CYT387

Momelotinib was studied in a phase 1/2 trial in patients with high or intermediate risk MF.[18] Pre-planned safety and efficacy analysis has been completed for the initial 60 patients. In the dose-escalation phase, the maximum-tolerated dose was 300 mg/day based on reversible grade 3 headache and asymptomatic hyperlipasemia. Twenty-one and 18 additional patients were accrued at two biologically effective doses, 300 mg/day and 150 mg/day, respectively. Anaemia and spleen responses, per IWG-MRT criteria, were 59% and 48%, respectively. Among 33 patients who were RBC-transfused in the month prior to study entry, 70% achieved a minimum 12-week period without transfusions. Most patients experienced constitutional symptoms improvement. Grade 3/4 adverse reactions included thrombocytopenia (32%), hyperlipasemia (5%), elevated liver transaminases (3%) and headache (3%). New-onset treatment-related peripheral neuropathy was observed in 22% of patients (sensory symptoms, grade 1). A phase 3 study to determine the efficacy of momelotinib versus ruxolitinib in MF patients naive of JAKi is ongoing.

Conclusions

Taking together all available clinical data on MF, one may conclude that JAKi give a benefit to patients with MF, by reducing spleen size of ~50% in approximately 40-50% of patients and by abolishing symptoms in the vast majority of patients. However, effect on these disease manifestations should be balanced with the safety profile. Anaemia and thrombocytopenia are on-target toxicities expected with all JAKi. Infections should be monitored with ruxolitinib, drug with the longest time of observation, but might be expected with all JAKi.

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Current and Future Treatments for Paroxysmal Nocturnal Hemoglobinuria

Serena Marotta, Simona Pagliuca and **Antonio M Risitano**
Hematology, Department of Clinical Medicine and Surgery, Federico II University, Naples, Italy

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH)[1-3] is a rare hematological disorder due to a somatic mutation in the phosphatidyl-inositol glycan class A (PIG-A) gene,[4,5] which impairs the biosynthesis of the glycosyl-phosphatidyl-inositol (GPI) anchor and the subsequent expression of a number of surface proteins. The PIG-A mutation occurs in one (or a few) hematopoietic stem cells, which eventually expand over normal hematopoiesis leading to the clinical phenotype. The reasons for such expansion lie on an immune pressure which may damage normal hematopoietic stem cells (as in aplastic anaemia), possibly sparing the PIG-A mutated hematopoiesis, as first postulated by Rotoli and Luzzatto in the "dual pathophysiology theory".[6,7] Recently, a further proof of this hypothesis came from the observation that NK-T cells specific for the GPI anchor itself are increased in PNH patients,[8,9] and they may represent the auto-reactive immune cells accounting for the selective expansion of GPI-deficient hematopoiesis. However, irrespective of the underlying bone marrow disorder, it is the aberrant phenotype of the mutated mature blood cells which accounts for the typical manifestation of the disease. Indeed, the lack of GPI-linked proteins from the cell surface include some physiological complement regulators (a regulator of early activation – CD55,[10] and a regulator of the terminal effector complement – CD59)[11] which are essential for the survival of erythrocytes, and possibly are involved in the thrombophilia typical of PNH (which may occur both in patients with classic PNH and in those with concomitant bone marrow failure – the so-called aplastic anaemia/PNH syndrome)[12]. Bone marrow failure, complement-mediated hemolytic anaemia and thrombophilia:[1-3] this clinical triad may require a complex therapeutic management, which may include different therapies.[13] The natural history of PNH has been described in three large studies;[14-16] the most recent from France reported a median survival of 22 years, with thrombosis and bone marrow failure accounting for the majority of deaths.[16] Here we briefly review treatment strategies for PNH patients, focusing on current established options as well as on the novel strategies under development. Before starting, it is important to remind that one of the most challenging issue in PNH remains the prompt diagnosis:[17] nowadays, flow cytometry is the standard diagnostic tool for PNH. However, it has to be remarked that the clinical suspicion comes before flow cytometry itself, and on the other hand the final diagnosis remains a clinical tool which combines flow cytometry with other hematological findings.

Antonio Risitano graduated in Naples in 1997, where he started his residency in Hematology under the mentorship of Prof. Bruno Rotoli. In 2000 he moved to NIH, where he joined Dr. Neal Young lab doing research in aplastic anaemia (AA) and paroxysmal nocturnal hemoglobinuria (PNH). In 2004 he moved back to Naples, to complete his PhD in Medical Biotechnologies. Since 2010 he is Assistant Professor at the Federico II University of Naples, where he is the Head of Bone Marrow Transplantation Clinical Unit. Prof. Risitano research activities mostly focused on basic science and clinical research in the field of bone marrow failure and bone marrow transplantation. His main contribution to the field where in the dissection of pathogenic mechanisms of AA and PNH, and in the pre-clinical and clinical development of novel treatment strategies for these diseases.

Supportive Care

Supportive care has been the only available treatment for decades in PNH. However, even in the era of novel treatment options, supportive measures remain pivotal for all PNH patients, and sometimes the only needed treatment.[13,17] As for other interventions, even supportive care should be driven by specific disease manifestations. Mild anaemia is the most common reason for supportive care in PNH; because of the compensatory erythropoiesis, patients should receive folate and possibly iron supplementation (because of the chronic iron loss through the urine – "perpetual hemosiderinuria" as defined by Marchiafava – PNH patients are prone to develop iron deficiency rather iron overload, even if they receive blood transfusions).[17] In case of severa anaemia, blood transfusions are needed; they represented the main treatment for PNH until complement inhibitors became available. No symptomatic treatment for chronic hemolysis or paroxysms exists: steroids have been used, but actually their use must be avoided for the remarkable side effects which are not counterbalanced by any demonstrated efficacy.[13,17] The most relevant supportive care in PNH should aim to prevent fatal

thrombotic complications; however, whereas it is well established that secondary prophylaxis is mandatory in all PNH patients who have experienced a thrombotic event (by either low molecular weight heparin or warfarin), there is still no consensus on the use of primary prophylaxis. Some groups recommend oral anti-coagulants to PNH patients with high risk of thrombosis (high proportion of PNH cells, high level of hemolysis),[18] while others feel not reasonable exposing a remarkable proportion of patients to unacceptable risk of hemorrhagic complications (about two out of three patients will never experience a thrombotic event during their disease course).[19]

Treatment of Hemolysis: Anti-Complement Therapy

The treatment of PNH has dramatically changed since the availability of the first complement inhibitor eculizumab (Soliris®, Alexion Pharmaceuticals). Eculizumab is a humanized monoclonal antibody (mAb)[20] which binds the terminal complement component 5 (C5), thereby inhibiting its cleavage to C5a and C5b and preventing the assembly of the Membrane Attack Complex (MAC). Two large multi-national trials have documented that eculizumab is highly effective in controlling intravascular hemolysis of PNH patients, leading to reduced transfusional need, hemoglobin stabilization and resolution of all hemolysis-related symptoms.[21,22] The sustained control of the terminal effector complement and subsequent intravascular hemolysis may result in transfusion-independence in about half of the treated patients, with some patients also achieving substantial increase of their Hb levels. Furthermore, eculizumab seems to reduce the risk of thromboembolic events in this patient cohort,[23] possibly due to the pathogenic linkage between intravascular hemolysis and thrombosis (e.g., nitric oxide consumptions,[24] pro-thrombotic micro-vesicles) or to any direct effect on complement-medieted thrombophilia (i.e., on PNH platelets).[25] Given that thrombosis (together with complications of marrow failure) is the leading cause of death in PNH, it is not surprising that eculizumab seems to result in a remarkable improvement of survival, as recently shown in a study from United Kingdom.[26] Remarkably, eculizumab exhibited an excellent safety profile, with negligible side effects; concerns about possible infectious risk were almost ruled out, even if all patients need to be vaccinated against Neisseria Meningiditis. Thus, nowadays eculizumab is the standard of care for the treatment of hemolysis of PNH; patients who qualifies for anti-complement treatment should have transfusion-dependent (or in any case severe) anaemia, and/or anaemia-related symptoms (e.g., frequent painful paroxysms, smooth muscle dystonia, etc). For the close link between thrombosis and complement-mediated hemolysis, PNH patients with life-threatening thrombosis may also have indication to anti-complement therapy, regardless the severity of anaemia. Conversely, patients who exhibit evident bone marrow failure (eventually contributing to anaemia) should be classified as aplastic anaemia patients with a PNH clone, and should be managed aiming to treat the concomitant aplastic anaemia (see below).

Development of Novel Anti-Complement Strategies

As usually in medicine, new therapies may carry new insights in the pathophysiology of disease they treat; eculizumab does not make an exception. Indeed, we and others have demonstrated that, leaving the early phases of the complement cascade intact, eculizumab prevents the lysis of PNH erythrocytes, but finally result in their progressive opsonization with C3 fragments.[27,28] This is due to the fact that complement regulation remains impaired on PNH erythrocytes due to the lack of CD55; more relevant, these C3 fragments may work as opsonins with subsequent entrapment of C3-bound PNH erythrocytes in the hepatosplenic macrophages. This biological observation matches with the current unmet clinical needs as approximately one third of PNH patients insufficiently respond to the drug and remain dependent on transfusions.[19,29] C3-mediated extravascular hemolysis is considered the most important cause of insufficient response to eculizumab, even if concomitant bone marrow failiure and/or "breakthrough" intravascular hemolysis may also contribute. Nevertheless, upstream complement inhibition at the level of C3 or of the C3 convertase is now considered a promising approach for alternative complement therapeutics.[30] So far, successful in vitro studies have been conducted with: i. an antibody against C3b (mAb 3E7);[31] ii. the fusion protein TT30 that combines regulatory domains of FH with the iC3b/C3d-binding domains of CD21;[32] iii. a novel engineered inhibitor that links the regulatory and surface recognition areas of FH (mini-FH);[33] iv. small peptide inhibitors members of the compstatin family.[34] TT30 is already in a phase I clinical trial enrolling PNH patients,[35] whereas some of the others (especially the compstatin analogs, that may be particularly cost-effective) are close to start their translational plans. The hope is that some of these candidate agents may lead to a second generation of anti-complement drugs which may improve the current outcome of anti-complement treatment in PNH.

Treatment of Bone Marrow Failure: Immunosuppressive Therapy

PNH patients with clinically meaningful bone marrow failure (namely moderate or severe aplastic anaemia - AA) should be treated as AA patients irrespective of the presence of the PNH clone (and at some extent even of clinically meaningful intravascular hemolysis). The treatment of moderate AA is mostly supportive, even if immunosuppressive therapy (IST) with cyclosporine may be considered (intensive IST is usually recommended only for transfusion-dependent moderate AA); androgens may be also considered. The treatment of severe AA is out of the purpose of this paper; however, in brief, it is based on either bone marrow transplantation (BMT) or intensive IST.[35-38]

The choice between the two options depends on patient age and on the availability of a suitable HLA-matched related donor. For patients older than 50 years or lacking a matched donor BMT is considered as a second-line treatment after possible failure of IST (one or two courses). Intensive IST for severe AA is based on the association of anti-thymocyte globuline (ATG) and cyclosporine; based on recent data,[39] horse-ATG is strongly recommended over rabbit ATG, resulting in an overall response rate of 60-70% (followed by relapse in about one third of patients). At the moment, no alternative IST regimen has proven effective to improve these results, either alone (cyclophosphamide, alemtuzumab) or in combination with the platform ATG + cyclosporine. Preliminary data from refractory patients may suggest some efficacy of the thrombopoietin mimetic agent eltrombopag;[40] clinical trials are currently ongoing to investigate its effect in combination with IST.

Bone Marrow Transplantation

BMT has proven effective in eradicating the abnormal PNH clone, possibly leading to the cure of PNH;[41-44] however, morbidity and mortality remain a major limitation. Giving the rarity of the disease, prospective studies are lacking and only 3 large retrospective studies are available. In the 1999, the International Bone Marrow Transplant Registry (IBMTR) reported on 57 consecutive allogeneic BMT for PNH registered between 1978 and 1995.[45] The 2-year overall survival was 56% in HLA-identical sibling transplants (n=48; 16 patients grafted because of AA/PNH). The most common causes of treatment failure were inadequate engraftment (n=7) and infections (n=3). The incidence of graft versus host disease was 34% for its acute form (grade II or higher) and 33% for its chronic form. These data were confirmed by an Italian retrospective study on 26 PNH patients (4 AA/PNH) transplanted between 1998 and 2006, that showed a 10-year survival of 57%, with acute and chronic GvHD rates of 42% (grade III-IV 12%) and 50% (extensive 16%), respectively. This series also included reduced intensity conditioning (RIC) BMT (n=11), which showed a higher transplant related mortality in comparison to sibling BMT (63% versus 26%), possibly due to comorbidity and higher number of unrelated donor in the RIC group.[46] More recently, the European Bone Marrow Transplation (EBMT) Group, together with the French PNH Registry, has reported a retrospective studies comparing the outcome of all BMT performed in Europe with the natural history of PNH (in the pre-eculizumab era). This is the largest cohort of BMT for PNH,[47] that included 211 transplants (136 from HLA-identical siblings; 135 using bone marrow as stem cell source) performed in 83 EBMT centers from 1978 to 2007; patients were subgrouped according to the presence of recurrent haemolytic anaemia, AA and thrombosis. The rejection rate was 7%; the cumulative incidence of acute GvHD (grade II-IV) was 40%, that of chronic GvHD 29%. The 5-year overall survival rate was 68%, without difference between recipients grafted from a sibling or an unrelated donor. The indication to BMT significantly affected the outcome, since the 5-year survival was worse in patients grafted for thrombosis (54%) in comparison to those grafted for AA (69%) or haemolytic anaemia only (86%). This study also aimed to identify which PNH patients may benefit from a transplant procedure, by comparing these transplants with a cohort of 402 non-transplanted PNH patients diagnosed between 1950 and 2005 in 92 French centers. PNH patients with previous thrombosis showed a better survival when not receiving a BMT (even if non-transplant treatment did not include eculizumab therapy), whereas immunosuppression and BMT did not result in any significant different outcome for patients with AA/PNH. These data support the notion that BMT is a worth option for PNH patients, with the exception of those with previous life-threatening thrombosis. Thus, concomitant AA remains the main indication to BMT for PNH patients, according to the AA treatment algorithm described above. Nowdays BMT seems not appropriate for clinically significant hemolytic anaemia and life-threatening thrombosis (this latter may actually represent a contra-indication to BMT), which rather represent indication to anti-complement treatment. However, given that eculizumab is not a curative treatment and clinical benefit may be heterogeneous, BMT remains a worthy second-line therapy for the few patients not achieving a good response to eculizumab (and possibly in Countries where eculizumab is not available yet). As for the specific BMT procedure, whereas the role of unrelated donor seems similar to what established for AA (i.e., unrelated BMT only as second-line therapy), the debate on the conditioning regimen remains open. Based on available data, AA/PNH patients should parallel conditioning regimens used for AA (i.e., cyclophosphamide/ATG for sibling transplants, fludarabine-based RIC for unrelated transplants);[48] non-hypoplastic PNH patients receiving BMT should rather benefit from a myeloablative conditioning (e.g., busulphan-based),[49] with RIC regimens (fludarabine-based)[50] to be considered in case of comorbidities or of older age.

Conclusions

PNH is a disorder of hematopoiesis clinically appearing as a complement-mediated disease, with hemolysis and possibly thrombophilia. Treatment options remain elusive for decades, but nowadays different strategies are available for most patients. The only curative option for PNH patients remains BMT, which in any case is restricted to a limited number of patients. Even if BMT is becoming less risky, its broader use seems hampered by the continuous progress in supportive and etiologic therapies targeting the complement. Indeed, anti-complement treatment has changed the natural history of PNH, resulting in substantial clinical benefit in the almost totality of patients. PNH continues to be at the forehead of medicine, with the ongoing development of novel anti-complement agents which may further improve the outcome of current treatment of PNH.

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THALASSAEMIA INTERNATIONAL FEDERATION

In official relations with the World Health Organization

Thalassaemia International Federation (TIF) is a non-governmental, patient-oriented association, established in 1986 to safeguard the rights of patients with haemoglobin disorders, thalassaemia, and sickle cell disease globally and to ensure their access to quality medical care. Today, TIF represents the voice of patients in 117 National Patients/Parents Associations, registered in 56 countries across the world and works in official relations with the World Health Organisation since 1996.

In addition, TIF collaborates with a wide range of official health, and policy oriented bodies and agencies across the world and a wide range of professional medical bodies and other disease-oriented organisations (see our website http://www.thalassaemia.org.cy/collaboration-partnerships-networks/collaboration-partnerships-networks.shtml). TIF's vision defines its mission which is the establishment of national strategies for the prevention and management of thalassaemia.

TIF's work is based on four pillars of activities:

1. Educational Programme
2. Collaboration, Partnerships, Networks
3. Advocacy & Policy Development
4. Communication & Awareness

TIF's Educational Programme is one that stands out and one for which TIF can claim having a truly measurable impact on a global scale. The Educational Programme encompasses three important elements:

1. The preparation and organisation of Educational Events at a national, regional, and international level (so far TIF has organised over 67 local, national, regional, international conferences and other events (workshops, seminars, delegation visits in 60 countries worldwide).

International Conferences on Thalassaemia and other Haemoglobinopathies are organised exclusively by TIF in the context of two parallel sessions, one for medical specialists and health professionals and another for patients and parents.

The latest International Conference has been carried out with great success in Abu Dhabi, United Arab Emirates, 20-23 October 2013. The next one is scheduled for 2017.

In this context, we are delighted to announce that TIF is organising the 4th Regional Pan-European Conference, which will take place in Athens, 7-8 November 2014.

Other regional conferences in Asia and the Middle East are scheduled to take place in 2015, and 2016 respectively.

2. The preparation, translation, publication and distribution of material to the medical community, the patients' community, and the community at large as a free of charge service (19 publications translated in 20 languages, distributed in over 60 countries).

3. The development of academic post-graduate courses including: a) an e-Msc course in Haemoglobinopathies for medical professionals and b) the Experts-Patients Programme for the patient community

TIF was the first body to publish:

1. Guidelines on the management of Thalassaemia and other haemoglobin disorders Guidelines for the Clinical Management of Thalassaemia (2005) Cappellini M-D, Cohen A, Eleftheriou A, Piga A, Porter J, Taher A, ISBN: 978-9963-623-70-9

2. Guidelines on Prevention of Haemoglobin Disorders: Prevention of Thalassaemias and other Haemoglobin Disorders, Vol 1, 2nd Edition (2013) - Old J, Galanello R, Eleftheriou A, Traeger-Synodinou J, Petrou M, Angastiniotis M, ISBN 9963 623 39 5 Prevention of Thalassaemias and Other Haemoglobin Disorders, Vol. 2: Laboratory Protocols (2012)- Old J, Harteveld C L, Traeger-Synodinos J, Petrou M, Angastiniotis M, Galanello R, ISBN 978-9963-717-01-9

3. Guidelines for Haemoglobinopathy Nurses A Guide for the Haemoglobinopathy Nurse (2013) - Aimiuwu E, Thomas A, Roheemun N, Khairallah T, Nacouzi A N, Georgiou A, Papadopoulou C, ISBN 978-9963-717-02-6

4. Guidelines for the Management of the Non-Dependent Transfusion Thalassaemias Guidelines for the Clinical Management of Non-Transfusion Dependent Thalassaemias (2013)- Cappellini M D, Taher A, Musallam K, ISBN 978-9963-717-03-3

5. The first book for the patient and the parent about Thalassaemia About Thalassaemia (2007) Eleftheriou A, ISBN: 9963-623-40-9

6. The first cartoon book for children, also released as a cartoon animation. All About Thalassaemia (2010) Dr Eleftheriou A, ISBN 978-9963-623- 95-2

Upcoming Congresses and Meetings

Swiss Stem Cells Network (SSCN) Annual Meeting

4 June 2014

Geneva, Switzerland

The SSCN is a non-profit group of scientists, which was designed to foster interactions among scientists and between scientists and society, provide information on current stem cell research and advance the discovery of basic molecular, cellular and organismic mechanisms of embryonic and adult stem cell functions in human and all model systems.

19th Congress of the European Hematology Association (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 International Society for Stem Cell Research (ISSCR) 12th Annual Meeting

18 – 21 June 2014

Vancouver BC, Canada

The International Society for Stem Cell Research (ISSCR) is an independent non-profit organisation founded to foster the exchange of information on stem cell research worldwide. The ISSCR Annual Meeting provides an excellent opportunity for scientists to present and discuss their latest research with participants from academic, industry and government settings from around the world.

International Congress on Stem Cells and Tissue Formation

8 – 11 July 2014

Dresden, Germany

Stem Cell researchers from all over the world will convene for the International Conference on Stem Cell and Tissue Formation. The congress will combine symposia on general principles and mechanisms of stem biology with more topically focused subjects in the area of hematopoietic stem cells, neural stem cells and diabetes.

43rd Annual International Society of Hematology Meeting

21-24 August 2014

Montreal, Canada

The Society for Hematology and Stem Cells (ISEH) is dedicated to the promotion of the scientific knowledge and clinical application of basic hematologic and immunologic disorders through research, publications, and scientific programs. The Annual meeting provides a forum for the exchange and discussion of original, unpublished and significant research advances in the areas of hematology and stem cell biology.

German Society for Transfusion Medicine and Immunohematology (DGTI) Annual Meeting

9 – 12 September 2014

Dresden, Germany

The DGTI is a non-profit organisation, which aims to promote transfusion medicine and the development of cooperation within the specialized areas in the field of science and research, and public health. This year the Scientific Committee has made efforts to include a variety of symposia in conjunction with other scientific organisations with a focus on cell therapy.

German Stem Cell Network (GSCN) Annual Conference

3 – 5 September 2014

Heidelberg, Germany

The GSCN aims at creating synergies between all areas of basic and applied stem cell research and to provide an interface between science, education, politics and society as a whole. This year the conference will focus on the application of stem cells in regenerative therapies, disease modelling and drug development are topics that will be discussed. In addition the latest findings in stem cell biology, including genetic and epigenetic mechanisms of reprogramming, maintenance of pluripotency, differentiation and of differentiated organs will be covered.

The Society for Immunotherapy of Cancer (SITC) 29th Annual Meeting

6 – 9 November 2014

Maryland, USA

The SITC is a non-profit society of medical professionals that has created a place for interaction and innovation in the cancer biologics community. Attendees will be a part of more than 1100 basic, clinical and translational scientists from academia, government and

industry from around the world, all dedicated to improving cancer patient outcomes through cancer immunotherapy. The meeting will include cutting-edge research and educational sessions to help advance the field and bridge the gap between bench to bedside translation research, development and clinical practice.

The European Molecular Biology Laboratory (EMBL) - Stem Cells in Cancer and Regenerative Medicine

9 -12 October 2014

Heidelberg, Germany

The EMBL is an intergovernmental organisation specialising in basic research in the life sciences. The conference integrates basic, translational and clinical aspects of stem cell research into a single framework. Participants get an overview of the model systems and scientific and logistic framework involved in gaining and exploiting basic knowledge of stem cell biology in cancer research and regenerative medicine settings.

7th Trends in Medical Mycology (TIMM) Meeting

9-12 October 2015

Lisbon, Portugal

This meeting 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 provides a forum where researchers from all over the world present the most important advances and research findings in mycology. The program will include plenary sessions on fungal infections in all aspects led by an internationally renowned faculty, round table sessions, and meet-the-experts meetings.

40th Annual American Society for Histocompatibility and Immunogenetics (ASHI) Meeting

20 – 24 October 2014

Denver, Colorado

Sessions will include roundtable discussions, symposia and interactive workshops on topics such as Current Trends in Haematopoietic Stem Cell Transplantation and New Horizons in Complement.

World Stem Cells & Regenerative Medicine Congress Asia 2014

21 – 23 October 2014

Seoul, South Korea

World Stem Cells & Regenerative Medicine Congress Asia 2014 brings together leading pharmas, biotechs, regulators, research institutes and investors from Asia and beyond to unveil key strategies for commercialising a stem cell based therapy, streamlining clinical development, navigating the regulatory landscape and gaining funds to expedite innovative R&D efforts.

ESH - 6th International Conference on Myeloproliferative Neoplasms

23 - 25 October 2014

Estoril, Portugal

The 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. This event will bring together scientists, and clinicians with new data as well as information on diagnosis and treatment.

ESH - 2nd International Conference on Multiple Myeloma

7-9 November 2014

Athens, Greece

The aim of this educational meeting is to address the latest developments in the field of multiple myeloma, as part of the quest for more effective and better-tolerated therapies, and better understanding of the disease pathophysiology. With a number of prestigious speakers this meeting is expected to be a great success, reflecting the exciting and most recent achievements.

The World Stem Cell Summit (WSCS) 2014

3-5 December 2014

Texas, USA

The World Stem Cell Summit is the largest interdisciplinary, networking meeting of stem cell science and regenerative medicine. Produced by the Genetics Policy Institute (GPI) the purpose of the meeting is to foster biomedical research, funding and investments targeting cures. This year's agenda features more than 200 speakers and 65 hours of in-depth presentations.
The Summit builds a foundation to advance cell therapies by establishing a supportive environment of regulation, legislation, financing, reimbursement and patient advocacy.

56th American Society of Hematology (ASH) Annual Meeting and Exposition

6 - 9 December 2014

San Francisco, USA

ASH aims to further the understanding, diagnosis, treatment, and prevention of disorders affecting the blood, bone marrow, and the immunologic, hemostatic and vascular systems, by promoting research, clinical care, education, training, and advocacy in hematology. 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.

41st Annual Meeting of the European Group for Blood and Marrow Transplantation (EBMT)

22 - 25 March 2015

Istanbul, Turkey

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, standardisation 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.

40th ANNUAL MEETING

DENVER, COLORADO

MONDAY, OCTOBER 20 – FRIDAY, OCTOBER 24, 2014

Sheraton Denver
Downtown Hotel
Denver, Colorado
ashi-hla.org

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