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HEMATOLOGY

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Reviews of the
18th **EHA** Annual Congress
Stockholm, Sweden,

and the 39th **EBMT** Annual
Meeting, London, UK

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Welcome

Kelly-Ann Lazarus, Editor

A bright summer's welcome to the latest edition of *European Medical Journal - Hematology,* which aims to provide the latest information on healthcare developments, clinical advancements and breaking technological news across the haematological spectrum.

The 18th Congress of the European Hematology Association (EHA) took place in the picturesque city of Stockholm on the 13th- 16th June 2013, uniting delegates from around the world to discuss issues pertaining to 'age and ageing in blood disorders'. In addition to the biological aspect, the impact on society, healthcare costs, and the overall ethics in cases where patients are either too old or too young to be treated, were also discussed. In the coming year, more attention will be drawn to the developments that involve ageing and the need for further research. For extensive coverage of the EHA Congress, please refer to our Congress Review section of this publication.

This edition also contains a stimulating range of topics to peak anyone's haematological interest. Some thought-provoking topics includes hypertension in children, bioengineering veins, and further issues related to haemophilia, leukaemia, myeloma and thalassaemia. New studies into a hormone that minimises iron overload, drug resistance issues, and new concerns with educational approaches for hematology graduates are also featured in this publication.

Our sincere gratitude is extended to the esteemed members of our editorial board and peer review panel. Their loyal support to the *European Medical Journal - Hematology* ensures the maintenance of the high quality which we always strive for. I hope this issue will be a point of reference guide to any healthcare professional in providing exciting news within the haematological field.

Kelly-Ann Lazarus, **Editor**

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Foreword

Professor Emili Montserrat

Professor of Medicine, University of Barcelona

Dear colleagues,

It is my great pleasure to introduce to you the *European Medical Journal - Hematology*, a member of the medical specialties series launched by the *European Medical Journal*.

The *European Medical Journal - Hematology* is enthusiastically joining the community of those interested in the education and research of haematologic disorders. Hematology is a fascinating specialty that covers benign and malignant diseases of blood and its forming elements.

The progress operated in the understanding of blood conditions and their treatment in the last few years is unprecedented, it is one of the clearest examples that modern medicine requires the collaboration of professionals with different backgrounds and expertise but also, and very importantly, of individuals with a broad understanding of hematology, and capable of putting together in a clear and meaningful manner, advances operated from different sub-specialities.

The *European Medical Journal - Hematology* hopes to be useful to all biologists, chemists, pathologists, epidemiologists, pharmacologists, and clinicians, by providing a tool where to disseminate original research along with review articles, position papers, guidelines, case reports, and forum discussions, among many others. All articles are peer-reviewed by a large panel of experts to ensure that published contributions offer new insights into hematology or useful updates on different areas. The *European Medical Journal - Hematology* also reports on the most important congresses and meetings within the hematology field to ensure that the latest information is accessible.

I look forward to the development of the *European Medical Journal - Hematology* as an instrument to facilitate, in both a friendly and rigorous manner, education and research in hematology.

Most sincerely,

Prof Emili Montserrat

Professor of Medicine, University of Barcelona, CLL and Lymphoma Program, Hospital Clinic, President, European Research Initiative on CLL (ERIC). Barcelona, Spain.

Click here to see an exclusive interview with Prof Emili Montserrat held at the EHA Congress 2013.
'What is happening in the world of CLL?'

EHA ANNUAL CONGRESS 2013

STOCKHOLMSMÄSSAN, STOCKHOLM, SWEDEN

13TH-16TH JUNE 2013

Welcome to the *European Medical Journal* review of the 18th Annual **EHA** Congress of Hematology

EMJ EUROPEAN MEDICAL JOURNAL

Welcome to the *European Medical Journal* Review of the EHA Annual Congress 2013

The European Hematology Association (EHA) Annual Congress is the most important meeting for haematologists worldwide, aiming to promote excellence in clinical practice, research and education. The EHA has 3,000 members from over 100 different countries, all of whom are provided with the latest results in both basic and clinical research, as well as state-of-the-art diagnostic and therapeutic education sessions.

EHA have used these principles as a basis for their 18th Annual Congress, which was dedicated to age and ageing in blood disorders, while endeavouring to answer the question: "Who is old?". The Congress brought together not only haematologists with an interest in ageing patients suffering from blood disorders, but also those with an interest in ageing cells. This collaboration ensures both extensive research concerning ageing, and also enables insight into the process of intervention.

The Congress was held between June 13th-16th in Stockholm, a city filled with beautiful architecture and one which prides itself on its creative spirit. It can also boast to be the location of the most innovations per capita in the world.

The EHA organised a Hematology-in-Focus Session; three programs concerned with delivering high-quality medical education. The EHA also collaborated with 16 Scientific Working Groups, presenting sessions which focused on the progress of lymphoma and myeloma research. Multiple myeloma was a hot topic discussed at the congress this year; two presentations, one focusing on the drug Daratumumab, and the other on Pomalidomide, delved into the treatment for multiple myeloma patients.

New research findings, concerned with treating chronic lymphocytic leukaemia (CLL), were also presented. One session displayed how using next-generation sequencing

will help doctors discover mutations in cancerous cells, while another presented how using combination therapy will help patients with aggressive CLL. In order to achieve optimal didactic results throughout the congress, interactivity and multimedia approaches were used.

In keeping with their aim to promote excellence in education, the EHA showcased an exciting medical education program. This was helpful for both young haematologists and more advanced clinicians. The Advocacy Track program, for example, discussed ways to deliver healthcare in times of economic crises, how to explain the unavailability of treatment to a patient, how to personalise medicine, and how to involve patients further as partners in clinical research. Their innovative learning program, combined with online learning tools, ensured a harmonised basic curriculum for training young haematologists, while also integrating all existing online learning tools to enable doctors to find any relevant materials within seconds.

For haematologists-in-training, an Early Career Track program was available. This session focused specifically on career development, which aimed to support young scientists involved in both basic and clinical research. To do this, the EHA offered a number of fellowships and training opportunities, many of which are host institutes throughout Western Europe.

The EHA provides a platform for all professionals with an active interest in hematology, enabling them to present their findings, research and innovations. In an era when people are developing more age-related blood disorders, the importance of research and developments come to the fore. It is for this reason that the EHA is ever-growing in size and in importance.

NGS technology provides valuable insight

Next-generation sequencing (NGS) has been developed and refined to discover mutations that evolve in cancer cells, according to research presented in Stockholm during the European Hematology Association's Annual Congress.

Mutations can be inherited or acquired during the pre-clinical stages of cancer and throughout its life. Over time, the mutational landscape changes, and various mutations arise in different cells leading to intra-tumour heterogeneity.

As cancer cells are not homogeneous, NGS helps to dissect their subclonal architecture. A study has shown how this can be effective in patients with chronic lymphocytic leukaemia (CLL). CLL is a disease which constantly undergoes tumour evolution. It has a silent background, and many carriers are unaware that they have it because they have almost no symptoms. However, the disease can be very aggressive and rapidly fatal.

In this way, NGS estimates the fraction of DNA molecules harbouring each mutation, and also estimates the number of cells in the tumour carrying it. This Darwinist concept of the evolution of cancer helps to provide insight into tumour biology and will help to facilitate personalised medicine strategies.

Understanding variables within cancer will refine the treatment for it. For example, many samples displayed that post-treatment subclonal mutations were found. Thus suggesting that treatment has differential cytotoxic effects across different subclones of the tumour, decreasing competition and allowing the growth of smaller, fitter and chemo-resistant clones above the detection threshold.

Mutations of the clonal group known as the driver, frequently contribute directly towards CLL emergence. Whereas, the subclonal group aids CLL progression as it shifts to form a clonally over time. This highlights that timing and treatments do have a positive effect on the CLL subclones which harbour driver mutations. For example, the presence of subclonal drivers suggested that treatment was needed earlier compared to samples without subclonal drivers. Whereas, clonal evolution was characterised by shorter times to the next treatment.

This knowledge, a direct result of using NGS, has the potential to improve the quality of care that is delivered to patients. The technology has provided a strong rational to 'wait and watch' in many asymptomatic diseases. It also provides further information concerning the development of combinational chemotherapies.

A solution to autoimmune disease?

New research has found that T-cells, a white blood cell and a key player in the body's immune system, could be converted into a treatment for many autoimmune diseases.

Treating autoimmune diseases often compromises a patient's immune system. Chang Kim, a member of Prude's Center for Cancer Research and Weldon School of Biomedical Engineering, Professor of Comparative Pathobiology and a university faculty scholar, aims to "catch the thief without taking down the house."

Suppressive T-cells will block the development of painful inflammation, while maintaining the number of T-cells needed for correct immune function.

Unlike other immune-suppressive drugs, there are no toxic side-effects. They are developed from the patient's blood, thus the treatment is not rejected and remains in the body for longer. Furthermore, it is much more precise, though much more research is needed to determine the correct amount of dosage needed for human patients.

Combination therapy improves outcomes for elderly CLL patients

A combination of the chemotherapeutic drug chlorambucil with a monoclonal CD20 antibody, might have a greater effect on outcome compared a purely chlorambucil-based treatment in chronic lymphocytic leukaemia (CLL) cases. Another possibility exists that these patients might benefit from novel CD20 antibody obinutuzumab (GA101) instead of the approved CD20 antibody rituximab.

A CLL11 trial conducted by German CLL study Group (GCLLSG), in collaboration with Roche, compared the aforementioned treatments in the following combination: GA101 plus chlorambucil, rituximab plus chlorambucil and chlorambucil alone.

CLL is a blood disorder which affects the B cell lymphocytes. Statistics have shown that a significant proportion of people affected are elderly patients. These patients also have a range of comorbidities which makes it difficult for aggressive standard treatments.

The results of this investigation revealed that combination therapies (GA101 or rituximab with chlorambucil) showed good prognosis in comparison to just the use of chlorambucil alone. An acceptable safety profile was also demonstrated with the combination therapies. These significant findings are a step forward in improving the treatment of CLL in elderly patients with comorbidities.

Breaking news on lymphoma and myeloma reported

The meeting of Scientific Working Groups (SWK) has yielded substantial news on lymphoma (lymph node cancer) and myeloma (plasma cell cancer) during the European Hematology Association's Congress.

To maintain the high quality of research, EHA collaborated with 16 Scientific Working Groups (SWG) to focus on fostering activities directed towards basic and translation research. The progress of lymphoma and myeloma research was discussed in simultaneous sessions on Thursday June 13th in Sweden.

The European Mantle Cell Lymphoma Network extensively discussed developments in new biological prognostic factors, which might prevent over- and under-treatment of patients.

The results of treatments involving molecular approaches/smart molecules on younger patients were also presented, a regime which potentially could lead to an increase in survival rates. Special attention were paid to new treatments options for the elderly with the aim of achieving a better prognosis.

'Models for drug resistance' was a priority of the SWG, which is one of the main problems in maintaining today's effective treatments. New insights into the resistance mechanisms which also included identification and prediction were also covered.

A focus was also on genetic signature of defined resistant subgroups of patients. A new disease model mimicking the clinical condition provided the opportunity to accelerate the development of new drugs, along with the mechanism of evolution to aggressive disease.

New EHA Medical Education

Programme looks to assist students

To correspond with EHA's mission statement, "to promote excellence in clinical practice, research and education in European Hematology", a revamped Medical Education Programme has been launched.

Ulrich Jäger, former President of EHA, suggested the previous medical programme was too divided, and as a result they have now "tried to put an umbrella over this kind of education programme. It's now a comprehensive programme which serves all topics of hematology." The programme develops "from oral education to web-based education."

This beneficial, varied, comprehensive programme covers all topics of hematology. It serves various targeted groups, from highly trained and qualified clinicians, to new starters. It is evidenced-based, developed, reviewed and delivered by a faculty who are experts in their fields. It also incorporates a career development programme, for all levels, promoting research in hematology.

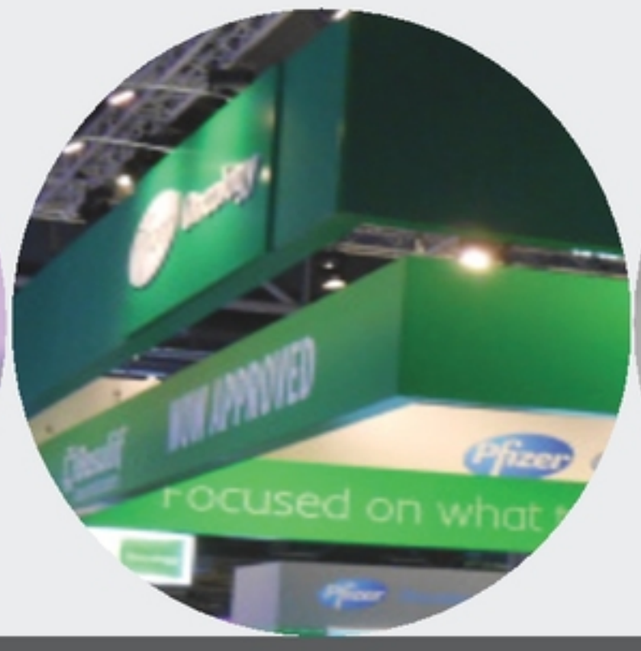
EHA's curriculum is a target-specific programme. EHA identified certain knowledge gaps in many hematology graduates. The use of this information has helped to tailor their education programme, adjust their learning activities, and have developed new tools to cover any knowledge gaps.

Evidence for this is clearly seen in the EHA's European Hematology Curriculum which harmonises hematology in Europe by endorsing 27 national hematology societies. As Jäger highlighted: "We believe that learning and a mixture of personal education and eagerness is important, and all of our programmes, of course, are attached to our curriculum."

EHA have also developed a blended learning program, an online learning course for small groups of students facilitated by mentors. For more senior hematologists, EHA performs needs assessments and constantly monitors trends in medical education and explores these possibilities to further improve and extend the education programme.

A number of online learning tools have also been designed to facilitate easier access to knowledge for doctors across the continents. As a direct result, EHA's learning resources have expanded significantly with an extensive library of online sharing tools accompanying the website. To fully benefit everyone, all of the online learning resources are free of charge and are easily accessible in EHA's learning centre.

Owing to this success, EHA established an outreach programme which enables them to share educational opportunities with less developed countries. Each event is specifically tailored to each specific host region, and is based on the input of local specialists. It is aimed at doctors who wish to further their knowledge but do not have the opportunity to attend events.



First guidelines for treating non-transfusion-dependent thalassaemia

The management on non-transfusion-dependent thalassaemias (NTDT) was addressed, in new guidelines presented by the Thalassaemia International Federation (TIF) at EHA.

The guidelines provide a consensus of expert opinion, are based on the most recent data, and were developed to give clinicians a comprehensive set of protocols. It is hoped they will help to identify and manage patients who are often overlooked and underdiagnosed.

NTDT is used to describe patients who do not require blood transfusions for their entire lifetime, but rather need occasional or frequent transfusions in certain clinical situations and for definite periods of time.

Non-transfusion-dependent thalassaemias include: thalassaemia intermedia, HbE/thalassaemia and α-thalassaemia (HbH), and predominately affects people of South and South-East Asian, Mediterranean, and Middle Eastern origin.

> "NTDT affects an equal or even larger population than β-thalassaemia major on a global scale."
>
> *- Dr Androulla Eleftheriou, Executive Director, TIF*

"NTDT affects an equal or even larger population than β-thalassaemia major on a global scale, because NTDT is more prevalent in some of the most populous regions of the world," said Dr Androulla Eleftheriou, Executive Director of TIF.

Overall, the new guidelines will contribute to a better understanding of NTDT, and will shed light on the fact that patients are still at risk of developing chronic iron overload due to increased iron absorption in the stomach and intestines, triggered by the body's need for more red blood cells.

"Without proper disease management, people with NTDT can still develop dangerous iron build-up in vital organs, that can cause serious and potentially life-threatening complications," Dr Michael Angastiniotis, TIF Medical Advisor, said.

Some key highlights in the new guidelines include the warning that iron chelation therapy should be initiated when liver iron concentration (LIC) is >5 mg Fe/g dry weight.

Patients who are below the recommended LIC should be taken off the chelator. Patients who require iron chelation therapy, meanwhile, should be given oral formulations, since this has shown to improve the patient's quality of life, when the burden of regular prolonged infusions can negatively impact on psychological wellbeing.

"Over the last decade, we have made great strides to better understand the management of the disease, most notably the role of transfusion therapy and ion chelation therapy," added Dr Elftheriou.

"The TIF NTDT guidelines offer the most current medical evidence to help clinicians make more informed therapeutic decisions to better influence patient outcomes and avoid disease complications."

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Pomalidomide testing is positive

A phase III study testing a new drug, Pomalidomide (POM), for the treatment of multiple myeloma (MM), revealed positive results. The study compared POM in combination with low-dose dexamethasone (POM+LoDex) and high-dose dexamethasone in MM patients with the later stages of MM who previously failed other treatments. The result was, the patient's quality of life improved, and both progression free survival and overall survival significantly improved with POM+LoDex, compared to using Dex in higher doses.

Pomalidomide will improve the management of advance myeloma for patients who no longer respond to other treatments. Pomalidomide was launched in the USA, and is currently undergoing review in Europe, with a decision expected soon.

New ways to treat B-cell lymphoma

Dr Lou Staudt has shown significant developments in treating B-cell lymphoma. Diffuse large B-cell lymphoma (DLBCL) comprises of three different diseases, all with critical abnormalities which allows them to survive as a malignancy. It has been discovered that by attacking B-cell receptor (BCR) signalling the tumours will die.

The investigators trialled a potent inhibitor of Bruton Tyrosine Kinase (BTK) called ibrutinib. BTK blocks the abnormal BCR signalling in DLBCL. The study also highlights that tumours in the BCR signalling are the most sensitive to ibrutinib. This proof of principle trials will help identify those patients most likely to benefit from this drug.

Mini-hormone proved to fight iron overloading

A potential new therapy for iron overload disorders involves hepcidin replacement. Hepcidin is difficult to synthesise naturally and has unfavourable pharmacological properties. By maintaining the active structure of the hormone, minihepcidins were produced. This is a peptide mimic of the hormone which is engineered to improve their bioavailability and reduce production and economical costs.

Current iron overload treatments are troublesome or can cause unpleasant side-effects. The cause of iron overload is the deficiency of hormone hepcidin in diseases such as hereditary haemochromatosis and thalassaemia.

Hepcidin functions to regulate dietary iron absorption and utilisation of iron from stores. The lack of function of this hormone results in the excessive iron absorption from the diet and iron loading of vital organs.

Initial studies have shown that minihepcidins completely prevented iron loading in a hepcidin knockout mice that has a severe form of haemochromatosis. Minihepcidin prevents both anaemia and iron overload in a mouse model that has been afflicted with thalassaemia.

The study, as a whole, was deemed a good indication that minihepcidin may be useful for human iron overload problems.

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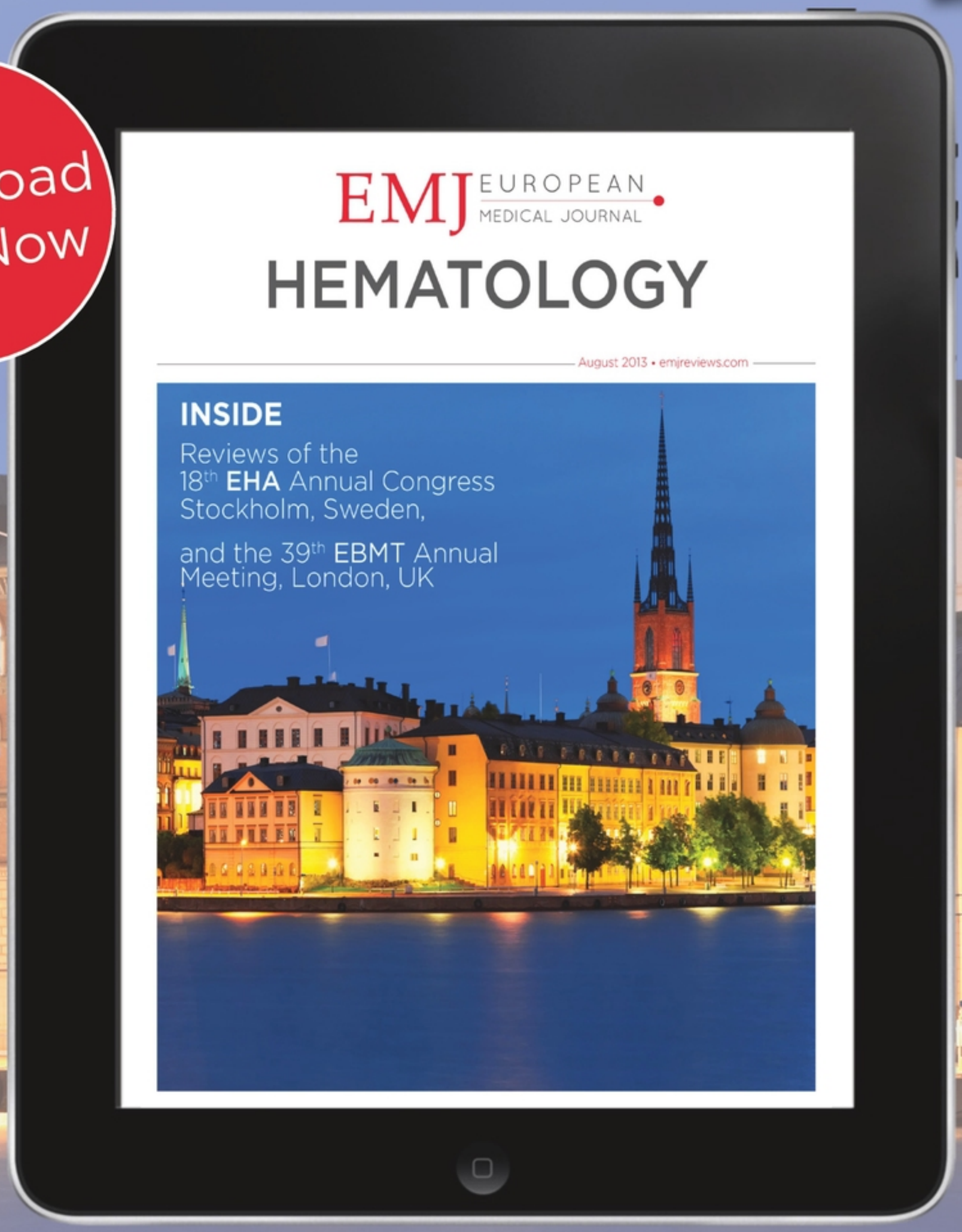

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Bone Marrow Transplant Day:
The uplifting experiences of patients

The EHA Annual Congress was not the only European meeting for haematologists this year. Between the 7th-10th April, the 39th annual meeting of the European Group for Blood and Marrow Transplantation (EBMT) was hosted in London's ExCeL Exhibition Centre.

With members from over 50 countries worldwide, the EBMT enjoyed a very successful and informative meeting at this year's event. The Congress delivered state-of-the art symposiums to over 4,300 delegates over the course of three days in England's capital city, and a comprehensive scientific programme which did not disappoint.

One of the many symposia held at the meeting, was dedicated to biologists and technicians who were interested in cell processing. There was also an education day which welcomed 594 nurses. This event dealt with 'Management of complications of HSCT (haematopoietic stem cell transplantation) patients'. The meeting also included a 'Paediatric Day', an integral part of the scientific programme, which aimed at attracting specialists from other fields in getting involved with haemato-oncology for children and adolescents.

Finally, around 200 attendees gathered for the EBMT 'Patient and Family Day.' This involved a series of patient-related educational sessions, and also individual workshops for patients with specific diseases. The 'Patient and Family Day' welcomed a number of patients, all at different stages in their recovery.

The event, organised by Imperial College London and the blood cancer charity Anthony Nolan, brought together patients with blood cancer and disorders and gave them a chance to meet both patients and medical experts. The day was organised after the announcement that the one millionth transplant worldwide had taken place was made by the World Marrow Donor Association (WMDA).

It is estimated that one person is diagnosed with blood cancer every 20 minutes, with around 1,700 people in the UK in need of a bone marrow transplant, but 70% of them unable to find matching donors in their families.

Jayne Snell, an attendee and AML patient, was very positive about the event, hoping that talking about her experiences gave "people hope for the future."

"It was great to see so many patients and families relating to each others' experiences in a very positive and supportive way," she added.

There were four topics presented throughout the day: 'life after transplant', 'what's new in transplantation', 'state-of-the-art treatment for late effects', and 'how do we find the right donor at the right time?'

Professor Jane Apperley, the EBMT 2013 Congress President and Chair of the Department of Haematology at Imperial College, educated patients on how to cope with the challenge ahead. Making her aim clear by stating: "Patients are our focus, as they are at the heart of what we do."

To allow for the growing success of blood and marrow transplantation, a Management and Resource System for Stem Cell Therapy (MARCELL) has been developed, and was introduced during the event. The software product allows for data to be centrally held, so that it may be used in all stem cell transplantation phases. It can produce a sample treatment plan which can be adapted to suit individual needs.

"Patients are our focus, as they are at the heart of everything we do."

- Professor Jane Apperley, Congress President

"It was great to hear other people ask the questions that we wanted to ask, but never had the courage to."

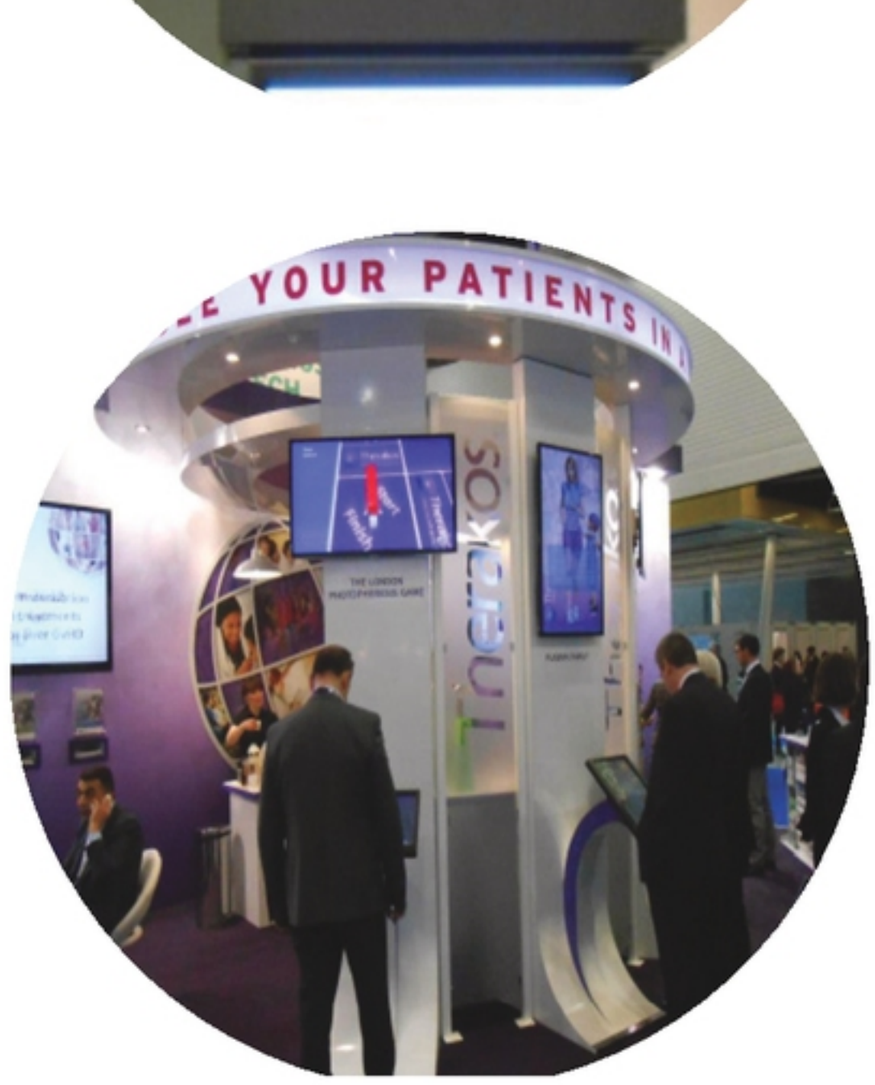
- Jodie McGauley, attendee and transplant patient

Furthermore, the Anthony Nolan charity, which helps to save the lives of people with blood cancer, gave 1,000 people the chance of life through using their register to match bone marrow donors. One attendee expressed: "30 years ago, I didn't think that I would see my children grow up. Now I have seven grandchildren, which is just amazing."

The efforts of the EBMT have resulted in the compilation of the largest transplant registry in the world. Events such as these have helped diagnosed patients, even after their transplant, learn that they have a support group around them. Jodie McGauley, who attended the event, received her transplant in December 2011 after being diagnosed in the October of the same year, expressed that she had learnt a lot more about the whole process.

She said: "It was great to hear other people ask the questions that we wanted to ask but never had the courage to. It was also reassuring to hear that all of my experiences and feelings about the bone marrow transplant were normal and that other people had had similar experiences to me."

Owing to the success of this year's EBMT congress, the 40th annual meeting of the EBMT will be held from the 30th March–2nd April 2014, in Milan, Italy. During this event, many historic events, such as the 8th Patient and Family Day and also the 1st Donor Day, will be celebrated. Coupled with the fact that topics covered include cell therapy, gene therapy, stem cell mobilisation, and graft engineering, this congress promises to be a high-profile, unforgettable event.



DELAYED ERYTHROID AND PLATELET RESPONSE TO ECULIZUMAB IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA - A CASE REPORT AND LITERATURE REVIEW

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Disclosure: No potential conflict of interest.

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ABSTRACT

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired clonal disorder of haemopoiesis characterised by haemolytic anaemia, thrombophilia and variable cytopaenias. Complement-mediated blood cell damage leads to the main clinical features of PNH, including anaemia, haemoglobinuria, other haemolysis-related symptoms, thrombosis, and thrombocytopaenia. The treatment of PNH has remained supportive until the development of the first complement inhibitor, eculizumab. This antibody efficiently blocks terminal complement activity, quickly halting intravascular haemolysis. However, both the time course and the magnitude of erythroid and platelet responses to this drug are highly variable. Here, we report a case illustrating both delayed erythroid and platelet responses to eculizumab, and review mechanisms and therapeutic options for partial responses.

Keywords: Paroxysmal nocturnal haemoglobinuria, intravascular haemolysis, anaemia, thrombocytopaenia, bone marrow failure, eculizumab.

INTRODUCTION

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare clonal haematological disorder caused by an acquired deficiency of the glycosylphosphatidylinositol (GPI) anchor in a haematopoietic stem cell. The defect is transmitted to all its progeny with consequent absence or reduction in all plasma membrane GPI-anchored proteins in a subpopulation of every blood cell type.[1,2] The absence of the GPI-anchored complement inhibitory proteins, CD55 and CD59, renders affected blood cells susceptible to complement attack, which causes intravascular haemolysis and damage to other blood cells. The consequences are haemolytic anaemia, haemoglobinuria, thrombosis, dysphagia, abdominal pain, and other PNH clinical symptoms.[1,2]

A clinically evident or subclinical bone marrow failure is frequently the background where a PNH clone appears and expands, sometimes to become responsible for a meaningful proportion of haemopoiesis.[2] Thus, besides haemolytic anaemia, PNH is also characterised for cytopaenia, thrombocytopaenia and/or neutropaenia of variable severity. These cytopaenias may involve multiple physiopathological pathways, such as bone marrow failure and/or complement-mediated cell damage.

Classically supportive measures are employed to treat cytopaenias, including red blood cell (RBC) transfusions and iron and folic acid supplementation.[3] 6 years ago, both the US Food and Drug Administration and European Medicines Agency (FDA and EMA), American and the European medical regulatory agencies, approved eculizumab to treat PNH. It is a humanised monoclonal antibody that binds to complement fraction 5, blocking its cleavage to C5a and C5b, mediated by the C5 convertases. The assembly of the terminal complement complex is thus inhibited.[1] Eculizumab treatment results in haemolysis blockade, stabilisation of haemoglobin

Table 1. Haematological responses to 4 months corticosteroids (03/2011) and to long-term eculizumab (06/2011).

Date	Hb (g/dL)	Reticulocytes /μL	WBC /μL	Platelets /μL	I. Bilirubin (mg/dL)	LDH (⊥: ≤480 U/L)	RBC (units transfused)*
10/2008	9.4	-	4,260	138,000	0.82	1,725	0
03/2009	9.2	-	4,130	161,000	1.79	1,777	0
09/2009	9.3	84,500	3,750	144,000	1.7	3,247	0
07/2010	9	53,460	3,500	100,000	1.01	4,380	0
12/2010	6.6	121,800	2,700	45,000	2	2,593	0
02/2011	4.7	84,500	2,650	30,000	3.1	2,944	4
03/2011	6.1	475,000	3,240	16,000	3.5	4,380	4
04/2011	7.3	179,250	4,100	41,000	1.3	1,281	7
06/2011	7.5	258,700	5,030	49,000	1.4	2,234	0
10/2011	7.6	62,140	2,440	31,000	1.5	544	6
02/2012	7.2	157,560	3,470	68,000	2.2	520	6
06/2012	7.1	184,240	3,050	89,000	2.2	504	2
10/2012	8.2	389,480	3,720	98,000	2.7	479	0
02/2013	8.7	201,840	3,910	141,000	2	380	0
04/2013	10.6	183,040	5,960	157,000	2.5	373	0

* RBC units transfused in the interval from the previous control date until the following one.

levels, resolution or decrease in transfusion requirements, improvement in quality of life,[4] and sometimes an increase in platelet counts.[5] However, the time and magnitude of maximal erythroid and platelet response to the drug is variable in every patient.

We report here a case with a delayed bilineal response to eculizumab, showing a long-term effect of complement blockade.

CASE REPORT

A 20-year-old woman was diagnosed in July 2002 with infectious mononucleosis. She later experienced more episodes of jaundice. In March 2005, a haemolytic crisis with fever, back pain, and dark urine took place. Her initial blood analysis revealed bicytopaenia, haemolysis and haemoglobinuria.

An abdominal ultrasound showed normal suprahepatic and portal veins, as well as a normal spleen. A flow cytometry of peripheral blood was positive for PNH (October 2005), with clone sizes of 68.5% in granulocytes, measured as CD66b negative neutrophils, and 27.02% in RBC, as shown by CD59 negative erythrocytes.

The patient was treated with folic acid and iron. Her clinical course was characterised by chronic haemolysis, with acute exacerbations every 1 to 3 months, with or without an identified trigger. She also experienced episodic dysphagia and abdominal pain, and subsequently her cytopaenias worsened (Table 1 and Figure 1). In January 2011, a disabling fatigue compelled her to leave work, with RBC transfusions required. A new peripheral blood flow cytometry showed a PNH clone size of 92% in granulocytes and 22% in erythrocytes.

A bone marrow biopsy showed a 20% cellularity with relative erythroid hyperplasia and without fibrosis (Figure 2). The cytogenetic study was normal. On March 2011, severe thrombocytopaenia with gingival bleeding and menorrhagia appeared. Eculizumab was prescribed and corticosteroids were administered. A more immunosuppressive therapy was postponed until the response to eculizumab could be evaluated.

Corticosteroid treatment (meprednisone 40 mg daily) was associated with both a decrease in transfusion requirements and increases in leukocyte and platelet counts, but at the expense of a pharmacological Cushing's syndrome.

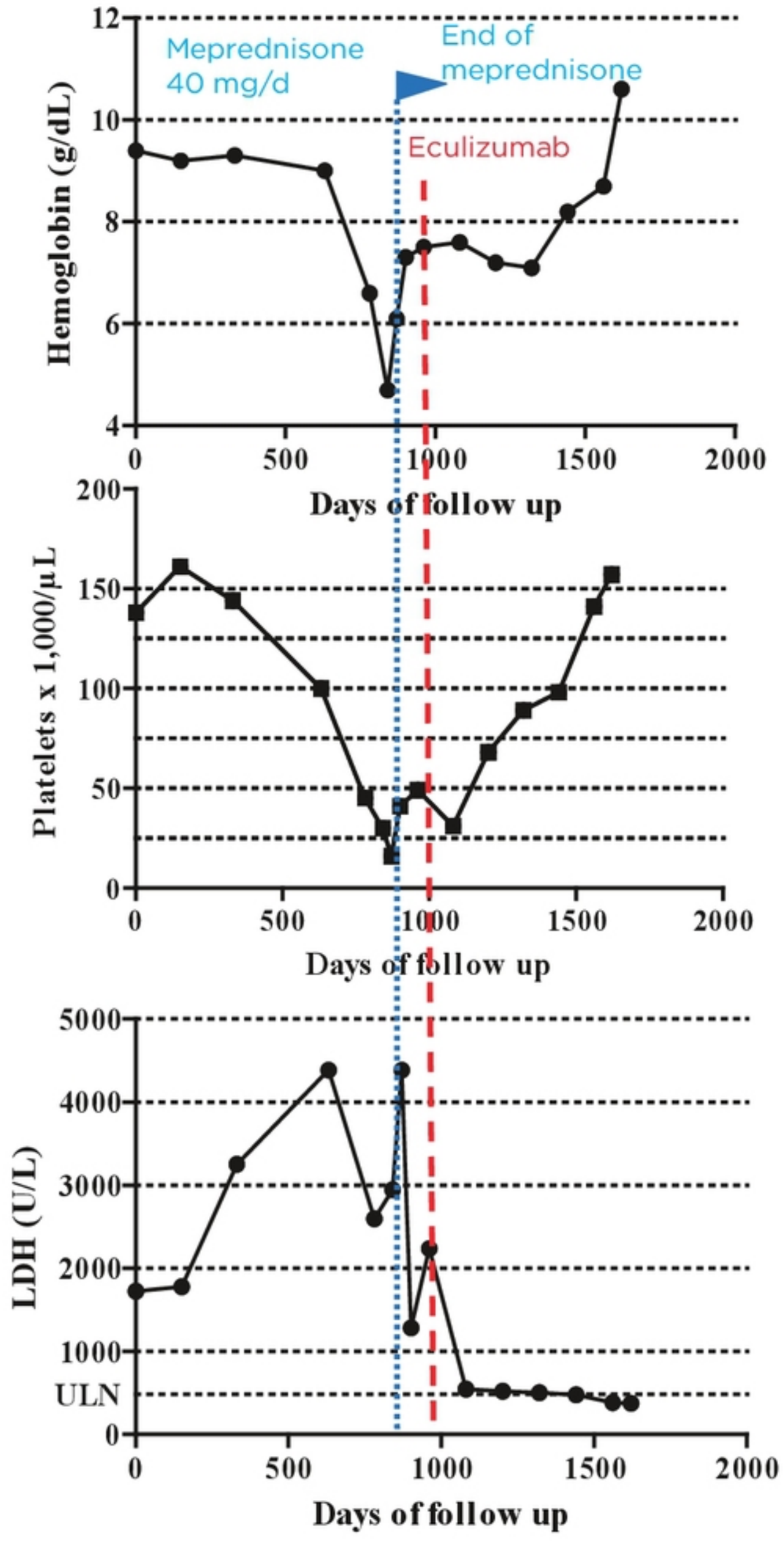

Figure 1. Graphs showing spontaneous and treatment-related changes in haemoglobin, platelet counts and LDH levels, start of meprednisone and eculizumab treatments and end of corticosteroids. ULN: upper limit of normal of LDH levels.

In June 2011, eculizumab treatment was started at the prescribed doses: 600 mg weekly for 4 doses, 900 mg at the fifth week, and then 900 mg every 14 days. Doses of meprednisone were progressively lowered and halted, lactate dehydrogenase (LDH) values sharply decreased, but RBC transfusion requirements persisted.

An erythrocyte survival study showed two RBC populations differing in their half-lives, seen at 12.5 and 23 days respectively, with a normal RBC lifespan range of 25-31 days for this study, with erythrocyte destruction taking place mainly in the spleen. Erythropoietin levels were very high at >750 mU/mL (normal range 9-19 mU/mL), as were ferritin levels (between 761 and 1,275 ng/mL), attesting to iron overload secondary to RBC transfusions. A liver Magnetic Resonance Imaging (MRI) scan confirmed an increase in hepatic iron.

Three therapeutic options were considered to address the ongoing transfusion requirements:

a) Low doses of corticosteroids to reduce extravascular haemolysis.

b) Splenectomy to remove the main site of PNH RBC destruction.

c) Erythropoietin or danazol to stimulate erythropoiesis. However, as reticulocytes counts and erythropoietin levels were high, their chance to achieve transfusion independence was judged very low.

An alternative option was to continue with RBC transfusions and to begin iron chelation.

The patient refused low doses of corticosteroids and chose to continue with eculizumab and supportive treatment. In the following months, her transfusion requirements diminished progressively and her platelet counts increased (Table 1). After 1 year on eculizumab, she required no more transfusions and her platelet counts increased above 80,000/µL in the last year and above 100,000/µL in the last 4 months (Table 1 and Figure 1). A new flow cytometry performed in March 2013 showed a PNH clone size of 95.72% in granulocytes and 56.84% in erythrocytes.

DISCUSSION

Eculizumab treatment blocks intravascular haemolysis in PNH patients. The results are an increase of PNH RBC counts and a consequent improvement of anaemia.[6] In the present case, PNH red cell counts increased in a 24 month period (March 2011 to March 2013 respectively) from 651,000/µL to 1,415,316/µL, accounting for both transfusion independence and improved haemoglobin levels.

The persistence of anaemia in PNH patients treated with eculizumab is mainly due to two mechanisms:

a) Activation of the proximal alternative pathway of complement, not blocked by eculizumab, leading to the deposition of C3b and its catabolites on PNH RBC surface. These C3 peptides are opsonines,

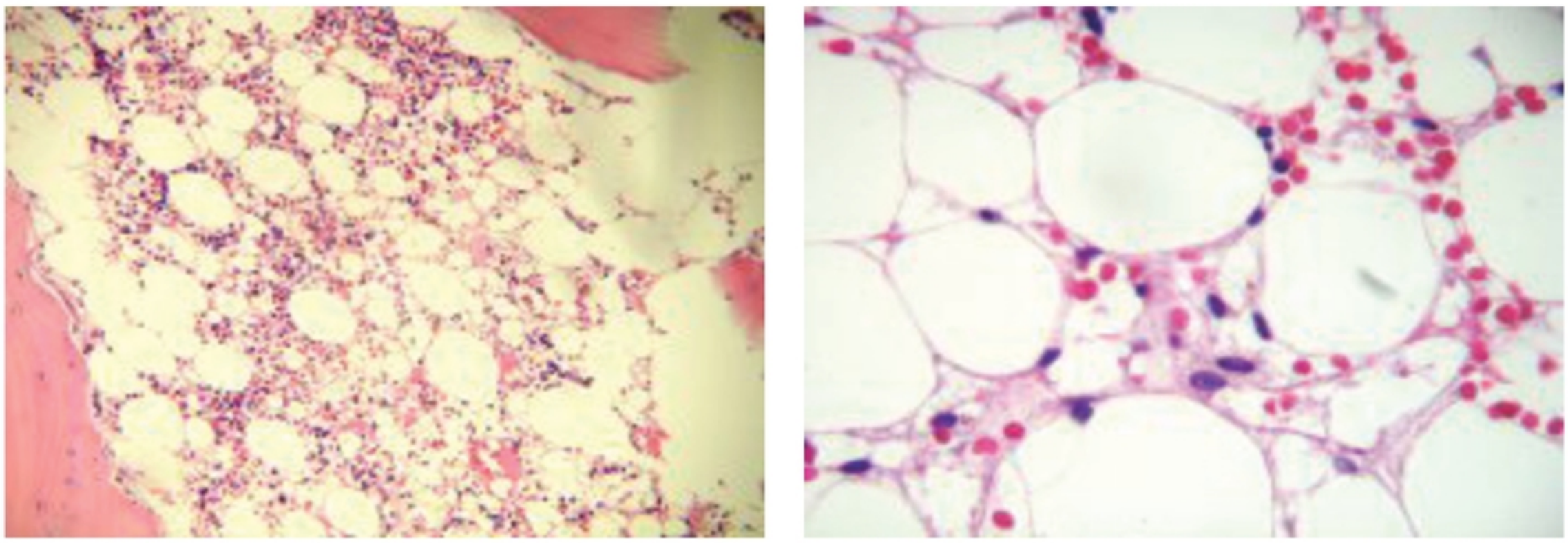

Figure 2. Bone marrow biopsy shows a 20% cellularity with relative erythroid hyperplasia (HE A: 100x B: 400x).

recognised by complement receptor 2 present in the surface of reticuloendothelial system macrophages. PNH erythrocyte phagocytosis then takes place, accounting for the extravascular haemolysis associated to eculizumab treatment.[7,8]

b) Bone marrow failure, present in nearly every patient with PNH, with deficient erythropoiesis, inadequate to fulfill patient requirements.[2]

In every case, the therapeutic options will be different. In the setting of bone marrow failure, erythropoietin or danazol may be tried to stimulate erythropoiesis. With predominant extravascular haemolysis, options include splenectomy, corticosteroid use, or blood transfusions associated with iron chelation. Transfusions with packed RBCs do not stop haemolysis, only transiently improve anaemia-related symptoms, cause iron overload, and may transmit multiple infections. Corticosteroids and androgen therapy, on the other hand, may lead to well-known adverse effects. Splenectomy has halted transfusion requirements in a case with significant extravascular haemolysis, taking place mainly in the spleen.[9] However, the risks (thrombosis, life-long immunosuppression) and benefits should be carefully balanced before this surgery is prescribed.

As shown by the present case, the deadline to deciding whether to remove a spleen as a rescue therapy to avoid transfusions in an eculizumab-treated patient should extend to more than a year (between 12 and 16 months). Due to corticosteroidal side effects (obesity, acne, hirsutism) the patient refused to receive them, which in turn allowed a late erythroid and platelet response to eculizumab to appear.

Thrombocytopaenia in PNH also has several physiopathological mechanisms: bone marrow failure, ineffective thrombopoiesis, platelet activation mediated by complement, with or without haemostatic consumption (microthrombosis), or platelet margination in an enlarged spleen or in the vascular bed.[10] In some, but not all, PNH patients with thrombocytopaenia, platelet survival is reduced.[10,11] Furthermore, in eculizumab-treated patients a study showed an inverse relationship between blood levels of both thrombosis and inflammation markers, on one hand, and platelet counts on the other.[12] So, in some PNH patients, thrombocytopaenia may be partially due to thrombin-mediated consumption of platelets, as attested by the quick rise in platelets seen in a previous case.[13]

Eculizumab treatment does not globally produce an increase in platelet levels. However, PNH patients with severe thrombocytopaenia experienced a highly significant rise in platelet counts, at odds with what happened to the less affected ones. This platelet response in a subgroup of patients was not paralleled with an increase in marrow blood cell production.[5] Although more research is required, all these findings suggest that terminal complement activity results in PNH-platelet activation, as has been shown *in vitro*,[12] frequently causing platelet consumption with thrombocytopaenia and thrombosis.

This case also shows that, instead of a shorter platelet half-life as compared to RBC, a platelet response to

eculizumab may also be a delayed phenomenon, requiring months, or even more than a year, to show its full magnitude. The mechanisms underlying such a late improvement are not clear. A spontaneous improvement in marrow hypoplasia may have played a role, but the initial response of both RBC and platelets to corticosteroids and subsequently to eculizumab suggests a PNH-related reduced survival as the main cause of bicytopaenia.

CONCLUSION

In conclusion, this case illustrates that both erythroid and platelet responses to eculizumab can be delayed phenomena. Therefore, many months may be required before undertaking alternative, more toxic, or invasive treatments.

REFERENCES

1. Brodsky RA. Narrative review: paroxysmal nocturnal hemoglobinuria: the physiology of complement-related hemolytic anemia. Ann Intern Med. 2008;148:587-95.

2. Risitano AM, Rotoli B. Paroxysmal nocturnal hemoglobinuria: pathophysiology, natural history and treatment options in the era of biological agents. Biologics Targets and Therapy. 2008;2(2):205-22.

3. Parker C, Omine M, Richards S, et al. International PNH Interest Group. Diagnosis and management of paroxysmal nocturnal hemoglobinuria. Blood. 2005;106:3699–709.

4. Hillmen P, Roth A, et al. Long-term safety and efficacy of sustained eculizumab treatment in patients with paroxysmal nocturnal haemoglobinuria. Br J Haematol. 2013;e-pub doi:10.1111/bjh.12347.

5. Socie G, Muus P, Schrezenmeier H, et al. Terminal complement inhibitor eculizumab improves complement-mediated platelet consumption and thrombocytopenia in patients with paroxysmal nocturnal hemoglobinuria (PNH). Blood. 2009;114[Abst 4030].

6. Schubert J, Hillmen P, Röth A, et al. Eculizumab, a terminal complement inhibitor, improves anaemia in patients with paroxysmal nocturnal haemoglobinuria. Br J Haematol. 2008;142:263–72.

7. Risitano AM, Notaro R, Marando L, et al. Complement fraction 3 binding on erythrocytes as additional mechanism of disease in paroxysmal nocturnal hemoglobinuria patients treated by eculizumab. Blood. 2009;113:4094-100.

8. Hill A, Rother RP, Arnold L, et al. Eculizumab prevents intravascular hemolysis in patients with paroxysmal nocturnal hemoglobinuria and unmasks low-level extravascular hemolysis occurring through C3 opsonization. Haematologica. 2010;95(4):567-73.

9. Risitano AM, Marando L, Seneca E, et al. Hemoglobin normalization after splenectomy in a paroxysmal nocturnal hemoglobinuria patient treated by eculizumab. Blood. 2008;112:449–51.

10. Louwes H, Vellenga E, de Wolf J.T.M. Abnormal platelet adhesion on abdominal vessels in asymptomatic patients with paroxysmal nocturnal hemoglobinuria. Ann Hematol. 2001;80:573–6.

11. Devine DV, Siegel RS, Rosse WF. Interactions of the platelets in paroxysmal nocturnal hemoglobinuria with complement. Relationship to defects in the regulation of complement and to platelet survival *in vivo*. J. Clin. Invest. 1987;79:131-7.

12. Weitz IC, Razavi P, Rochanda L, et al. Eculizumab therapy results in rapid and sustained decreases in markers of thrombin generation and inflammation in patients with PNH independent of its effects on hemolysis and microparticle formation. Thromb Res. 2012;130(3):361-8.

13. Brodsky AL, Mazzocchi O, Sanchez F, et al. Eculizumab in paroxysmal nocturnal hemoglobinuria with Budd-Chiari syndrome progressing despite anticoagulation. Experimental Hematology & Oncology. 2012;1:26.

14. Wiedmer T, Hall SE, Ortel TL, et al. Complement-induced vesiculation and exposure of membrane prothrombinase sites in platelets of paroxysmal nocturnal hemoglobinuria. Blood. 1993;82(4):1192-6.

THE CHEMOKINE CCL17/TARC AS A BIOMARKER IN HODGKIN LYMPHOMA

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ABSTRACT

Classical Hodgkin lymphoma (cHL) is a lymphoproliferative disorder hallmarked by a distinctive type of neoplastic cells, the Hodgkin and Reed/Sternberg (H/RS) cells. H/RS cells represent only a minor cell population of the total tumour mass and are surrounded by an infiltrate composed of mostly inflammatory cells. This composition results from the reciprocal release of soluble factors, such as cytokines and chemokines and other growth factors. In this context, the chemokine CCL17, also known as thymus and activation-related chemokine (TARC), emerges to have important biological functions, as it is expressed in high amounts by H/RS cells and highly elevated in the serum of cHL patients. CCL17 recruits Th2 cells and regulatory T cells that account for a beneficial microenvironment for H/RS cells. In this review, we summarise the current knowledge on CCL17 in cHL and other leukaemias and lymphomas and provide an outlook into clinical applications of CCL17 as a disease biomarker and as a therapeutic target in cHL.

Keywords: Hodgkin lymphoma, TARC, CCL17, serum biomarker.

Hodgkin Lymphoma: Dependent on Microenvironment

Hodgkin Lymphoma (HL), with an incidence of 3/100,000 per year in the Western world, is a disease which affects mostly young adults. Although HL is regarded as a curable disease as therapy is successful in more than 90% of cases,[1] many patients relapse at later points or suffer from treatment-related secondary malignancies. Two forms of HL exist, nodular lymphocyte predominant HL (which is not linked to elevated CCL17 levels),[2] and classical HL (cHL) which manifests different clinical signs and biology. This review focuses on CCL17 in cHL. The tumour cells in cHL are the mononucleated Hodgkin cells and the multinucleated Reed/Sternberg cells that evolve from Hodgkin cells undergoing endomitosis.[3,4] Interestingly, these Hodgkin and Reed/Sternberg (H/RS) cells constitute only about 1% of the tumour mass and are hugely outnumbered by infiltrating inflammatory cells. These surrounding bystander cells support the tumour and provide survival signals to the H/RS cells, making them highly dependent on their surroundings. The cHL microenvironment includes macrophages, mast cells, plasma cells, B cells, dendritic cells, fibroblasts, neutrophils, eosinophils, and T cells. Of the latter, CD4 T cells often show a tumour-promoting Th2 or regulatory T cell type. For review of HL and its microenvironment see Steidl et al.[5]

CCL17 Recruits Tumour-Promoting Th2 and Treg Cells

Chemokines are small secretory molecules acting as chemoattractants for leukocytes. CCL17, a CC type chemokine, is also commonly addressed as thymus and activation-related chemokine (TARC).[6] CCL17 is encoded in a gene cluster with CX3CL1 and macrophage-derived chemokine (MDC, CCL22) on 16q13 (for review see Colobran et al.[7]). CCL17 is constitutively expressed in the thymus[6] and physiologically secreted by dendritic cells,[8] some endothelial and epithelial cells,[9,10] as well as in some instances by fibroblasts and keratinocytes.[11] CCL17 is expressed by M2a macrophages, a subtype

of macrophages, which can act in a tumour-promoting manner,[12] and inhibits classical (M1) macrophage activation.[13]

CCL17 and CCL22 bind to their receptor CCR4,[14,15] which is characteristically expressed on Th2 cells and regulatory T cells.[16,17] Th2 type immune cells are commonly approved to provide tumour-promoting signals to cancer cells, and regulatory T cells keep reactive immune cells in check, preventing tumour immunosurveillance inter alia by secretion of immunosuppressive cytokines as IL-10 and TGF-β. CCL17 (together with CCL22[18]) recruits these cells into the proximity of H/RS cells in cHL patients. As patients display highly elevated serum levels of these two chemokines, both can be regarded as suitable biomarkers for cHL, with CCL17 potentially being the more potent one as its mean serum values for healthy individuals and patients are set wider apart as compared to CCL22.[19]

Although many studies reveal a purely tumour-beneficial role of CCL17-dependent recruitment of Th2 and regulatory T cells, two groups also report contradicting results. One is that cHL patients with high numbers of Th2 cells in the tumour tissue have a favourable prognosis and many regulatory T cells accompanied by low numbers of Th2 cells account for a poorer prognosis.[20] The second study even showed that many regulatory T cells, together with a low reactive cytotoxic T lymphocyte count, correlate with better prognosis for the patients.[21] Consequently, there is still a need for further experimental/clinical evidence to better understand the tumour-promoting or possibly tumour-opposing roles of Th2 cells and regulatory T cells in cHL.

While this review focuses on the significance of CCL17 in cHL and its role in other haematologic malignancies, it is worth mentioning that CCL17 has also been linked to several other diseases. Among these are skin diseases such as atopic dermatitis (for review see Saeki and Tamaki[22]), allergic diseases as asthma,[23] pulmonary fibrosis,[24] and some solid tumours in which CCL17 might promote metastasis.[25-27]

CCL17 is Highly Elevated in Hodgkin Lymphoma

First hints on CCL17 secretion by H/RS cells were found about 15 years ago, published by the Poppema group.[2,18,28] CCL17-positive H/RS cells have been found in patient tissue and CCL17 serum levels are significantly elevated in cHL patients compared to healthy individuals.[2,18,19,28-32] Moreover, cHL cell lines express and secrete high CCL17 levels.[18,28,32] It was shown that this elevated CCL17 secretion correlates with recruitment of CCR4-positive T cells into the tumour.[33,34]

CCL17 serum levels of cHL patients are dependent on the Ann Arbor stage of disease[35-37] and correlate with tumour burden,[37,38] providing CCL17 as a suitable biomarker for evaluation of response to treatment. Indeed, several studies, one of them published in the *New England Journal of Medicine*, already used CCL17 as a marker for response to therapy.[39-41]

With the largest cHL cohort so far evaluated for CCL17 levels, we established a multivariate model of response to treatment including CCL17 and established risk factors. Following this model, patients with baseline serum CCL17 above a certain threshold have a threefold aggravated risk of therapy failure compared to patients with CCL17 values below that threshold.[36]

In line with these data is Weihrauch's study,[35] which revealed elevated CCL17 levels in 90% of patients. Complete responders exhibit lower CCL17, while cHL patients with progressive disease exhibit higher CCL17 before and after treatment, and high CCL17 levels after therapy are a risk factor for poorer survival.

Plattel et al.,[37] and our own unpublished results, have shown that CCL17 levels drop after treatment in most patients and as early as after one cycle of chemotherapy. In the study performed by Plattel and colleagues,[37] non-responders are the only patients not showing this intense reduction after treatment. Furthermore, this study reveals elevated CCL17 levels in all included recurrent patients at the time of relapse. This implies that monitoring CCL17 serum levels after therapy might be a handy method for early identification of relapse patients. Nevertheless, the impact of CCL17 monitoring to evaluate the freedom of the disease needs further confirmation as Plattel's study investigated a relatively small cohort only.

The Role of CCL17 in Other Leukaemias and Lymphomas

Besides the indisputable significance of CCL17 in cHL, this cytokine also seems to play a role in multiple other leukaemias and lymphomas. While CCL17 attracts a Th2 type microenvironment in cHL, hence acting as an endocrine factor, in several other diseases, the tumour cells secrete CCL17 and express

its receptor CCR4 at the same time, suggesting an autocrine, tumour-promoting mechanism.

Tumour cells with expression of CCL17 and CCR4, can be found in adult T cell leukaemia/lymphoma (ATLL)[31,42-46] as well as in cutaneous T cell lymphoma (CTCL).[47-52] Here, CCR4 expression on the tumour cells often results in skin homing, Treg-like functions of the tumour cells themselves and can be correlated to poor prognosis. At least in CTCL, CCL17 serum levels have prognostic relevance as they correlate with tumour stage and lead to further recruitment of CCR4-positive T cells.[47,48]

It might be noteworthy that at least one report claims CCR4 expression in cHL cell lines as well,[28] while others do not find CCR4 on H/RS cells in tumour tissue.[29] It would seem that the textbook lines have yet to be written, but if there are indeed CCR4-positive H/RS cells, this is likely of functional and therapeutic relevance.

In anaplastic large cell lymphoma (ALCL), which is CD30-positive just like cHL, CCR4 is expressed on tumour cells in some cases, while CCL17 was not found to be elevated, providing CCL17 as a marker for differential diagnosis in morphologically similar tumour types.[2,29,50]

Lastly, only few reports exist on CCL17 secretion by leukaemic cells. In acute and chronic lymphocytic leukaemia (ALL and CLL) some CCL17 production can be measured, which might depend on CD40 ligation.[53-56] In acute myeloid leukaemia (AML), CCL17 levels might even correlate with stage of disease.[57]

CCL17 as a Therapeutic Target in Haematological Malignancies

These data hint to the feasibility of CCL17 and its receptor as a potential therapeutic target in cHL as well as in CCL17 or CCR4-positive lymphomas. There are a few studies demonstrating beneficial effects of such treatment strategies. Ishida et al.[34] were able to inhibit migration of CCR4-positive T cells *in vitro*, potentially impeding the favourable Th2 type microenvironment.

Another study used T cells carrying a chimeric antigen receptor (CAR) specific for the HL tumour antigen CD30. These CAR T cells additionally expressed CCR4 to direct them to the tumour. When they subcutaneously engrafted tumours composed of cHL cell lines in immunocompromised mice, the so engineered T cells exhibited enhanced tumour control.[58]

For immunotherapy of CCR4-expressing ATLL and CTCL cells, CCR4 antibodies are being developed and the first studies have shown promising results.[43,59] In another approach, CCL17 was fused to a toxin and has been tested in mice.[60]

CONCLUSION AND FUTURE REMARKS

Here, we summarise the current knowledge about the biomarker CCL17 in cHL and other leukaemias and lymphomas. In cHL, CCL17 is secreted by H/RS cells, and has important biological functions as it recruits Th2 cells and regulatory T cells that account for a beneficial microenvironment for the tumour cells. CCL17 serum levels are significantly increased in Hodgkin patients, and advanced disease stages exhibit higher CCL17 levels. Adding to that, a multivariate model, taking into consideration pre-treatment CCL17 levels together with established risk factors, showed a three-fold enhanced risk for therapy failure if CCL17 was above a certain threshold. Other studies show rapid normalisation of serum CCL17 immediately after the first cycle of chemotherapy in responding patients; while in non-responders and relapse patients, CCL17 fails to drop. This underlines the impact of CCL17 as a biomarker for therapy outcome in cHL.

Alongside its role as a serum marker, several promising studies have been performed indicating a role for CCL17 (and its receptor CCR4) as an (immuno) therapeutic target. Efforts have been made to inhibit T cell recruitment or to use the CCL17 gradient in cHL patients to direct genetically modified effector T cells into the tumour.

Summarising the overall information on CCL17 in cHL, this chemokine can be regarded as a key player in cHL. Being elevated in about 90% of patients, its levels correlating with stage of disease and predicting if therapy will be successful, makes CCL17 a suitable serum marker that can be analysed quickly and inexpensively by enzyme-linked immunosorbent assay (ELISA). Determination of CCL17 levels should be performed in all cHL patients and be included in clinical studies. Monitoring CCL17 levels throughout and beyond therapy will help to identify non-responders. After treatment completion, measuring CCL17 every couple of months will likely help with the early identification of patients suffering from relapse. All in all, it is beyond question that CCL17 should be kept in mind when thinking about cHL.

REFERENCES

1. Eichenauer DA et al. Hodgkin's lymphoma: current treatment strategies and novel approaches. Expert Rev Hematol. 2008;1(1):63-73.

2. Peh SC, Kim LH, Poppema S. TARC, a CC chemokine, is frequently expressed in classic Hodgkin's lymphoma but not in NLP Hodgkin's lymphoma, T-cell-rich B-cell lymphoma, and most cases of anaplastic large cell lymphoma. Am J Surg Pathol. 2001;25(7):925-9.

3. Drexler HG et al. Formation of multinucleated cells in a Hodgkin's-disease-derived cell line. Int J Cancer. 1989;43(6):1083-90.

4. Re D et al. Cell fusion is not involved in the generation of giant T-cells in the Hodgkin-Reed Sternberg cell line L1236. Am J Hematol. 2001;67(1):6-9.

5. Steidl C, Connors JM, Gascoyne RD. Molecular pathogenesis of Hodgkin's lymphoma: increasing evidence of the importance of the microenvironment. J Clin Oncol. 2011;29(14):1812-26.

6. Imai T et al. Molecular cloning of a novel T-cell-directed CC chemokine expressed in thymus by signal sequence trap using Epstein-Barr virus vector. J Biol Chem. 1996;271(35):21514-21.

7. Colobran R et al. The chemokine network. I. How the genomic organization of chemokines contains clues for deciphering their functional complexity. Clin Exp Immunol. 2007;148(2):208-17.

8. Sallusto F et al. Distinct patterns and kinetics of chemokine production regulate dendritic cell function. Eur J Immunol. 1999;29(5):1617-25.

9. Campbell JJ et al. The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T-cells. Nature. 1999;400(6746):776-80.

10. Sekiya T et al. Inducible expression of a Th2-type CC chemokine thymus- and activation-regulated chemokine by human bronchial epithelial cells. J Immunol. 2000;165(4):2205-13.

11. Yu B et al. Differential regulation of thymus- and activation-regulated chemokine induced by IL-4, IL-13, TNF-alpha and IFN-gamma in human keratinocyte and fibroblast. J Dermatol Sci. 2002;30(1):29-36.

12. Mantovani A et al. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004;25(12):677-86.

13. Katakura T et al. CCL17 and IL-10 as effectors that enable alternatively activated macrophages to inhibit the generation of classically activated macrophages. J Immunol. 2004;172(3):1407-13.

14. Imai T et al. The T-cell-directed CC chemokine TARC is a highly specific biological ligand for CC chemokine receptor 4. J Biol Chem. 1997;272(23):15036-42.

15. Imai T et al. Macrophage-derived chemokine is a functional ligand for the CC chemokine receptor 4. J Biol Chem. 1998;273(3):1764-8.

16. D'Ambrosio D et al. Selective up-regulation of chemokine receptors CCR4 and CCR8 upon activation of polarized human type 2 Th cells. J Immunol. 1998;161(10):5111-5.

17. Iellem A et al. Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by CD4(+)CD25(+) regulatory T-cells. J Exp Med. 2001;194(6):847-53.

18. Maggio EM et al. Common and differential chemokine expression patterns in rs cells of NLP, EBV positive and negative classical Hodgkin lymphomas. Int J Cancer. 2002;99(5):665-72.

19. Niens M et al. Serum chemokine levels in Hodgkin lymphoma patients: highly increased levels of CCL17 and CCL22. Br J Haematol. 2008;140(5):527-36.

20. Schreck S et al. Prognostic impact of tumour-infiltrating Th2 and regulatory T-cells in classical Hodgkin lymphoma. Hematol Oncol. 2009;27(1):31-9.

21. Alvaro T et al. Outcome in Hodgkin's lymphoma can be predicted from the presence of accompanying cytotoxic and regulatory T-cells. Clin Cancer Res. 2005;11(4):1467-73.

22. Saeki H, Tamaki K. Thymus and activation regulated chemokine (TARC)/ CCL17 and skin diseases. J Dermatol Sci. 2006;43(2):75-84.

23. Sugawara N et al. TARC in allergic disease. Allergy. 2002;57(2):180-1.

24. Belperio JA et al. The role of the Th2 CC chemokine ligand CCL17 in pulmonary fibrosis. J Immunol. 2004;173(7):4692-8.

25. Al-Haidari AA et al. CCR4 mediates CCL17 (TARC)-induced migration of human colon cancer cells via RhoA/ Rho-kinase signaling. Int J Colorectal Dis. 2013[Epub ahead of print].

26. Li JY et al. The chemokine receptor CCR4 promotes tumor growth and lung metastasis in breast cancer. Breast Cancer Res Treat. 2012;131(3):837-48.

27. Olkhanud PB et al. Breast cancer lung metastasis requires expression of chemokine receptor CCR4 and regulatory T-cells. Cancer Res. 2009;69(14):5996-6004.

28. van den Berg AL, Visser, and S. Poppema. High expression of the CC chemokine TARC in Reed-Sternberg cells. A possible explanation for the characteristic T-cell infiltratein Hodgkin's lymphoma. Am J Pathol. 1999;154(6):1685-91.

29. Vermeer MH et al. Differential expression of thymus and activation regulated chemokine and its receptor CCR4 in nodal and cutaneous anaplastic large-cell lymphomas and Hodgkin's disease. Mod Pathol. 2002;15(8):838-44.

30. Beck A et al. Expression of cytokine and chemokine genes in Epstein-Barr virus-associated nasopharyngeal carcinoma: comparison with Hodgkin's disease. J Pathol. 2001;194(2):145-51.

31. Ohshima K et al. Imbalances of chemokines, chemokine receptors and cytokines in Hodgkin lymphoma: classical Hodgkin lymphoma vs. Hodgkin-like ATLL. Int J Cancer. 2003;106(5):706-12.

32. Ma Y et al. Proteomics analysis of Hodgkin lymphoma: identification of new players involved in the cross-talk between HRS cells and infiltrating lymphocytes. Blood. 2008;111(4):2339-46.

33. Ohshima K et al. Infiltration of Th1 and Th2 lymphocytes around Hodgkin and Reed-Sternberg (H&RS) cells in Hodgkin disease: Relation with expression of CXC and CC chemokines on H&RS cells. Int J Cancer. 2002;98(4):567-72.

34. Ishida T et al. Specific recruitment of CC chemokine receptor 4-positive regulatory T-cells in Hodgkin lymphoma fosters immune privilege. Cancer Res. 2006;66(11):5716-22.

35. Weihrauch MR et al. Elevated serum levels of CC thymus and activation-related chemokine (TARC) in primary Hodgkin's disease: potential for a prognostic factor. Cancer Res. 2005;65(13):5516-9.

36. Sauer M et al. Baseline serum TARC levels predict therapy outcome in patients with Hodgkin lymphoma. Am J Hematol. 2013;88(2):113-5.

37. Plattel WJ et al. Plasma thymus and activation-regulated chemokine as an early response marker in classical Hodgkin's lymphoma. Haematologica. 2012;97(3):410-5.

38. Jones K et al. Serum CD163 and TARC as disease response biomarkers in classical Hodgkin lymphoma. Clin Cancer Res. 2013;19(3):731-42.

39. Reiners KS et al. Effects of the anti-VEGF monoclonal antibody bevacizumab in a preclinical model and in patients with refractory and multiple relapsed Hodgkin lymphoma. J Immunother. 2009;32(5):508-12.

40. Younes A et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med. 2010;363(19):1812-21.

41. Fehniger TA et al. A phase 2 multicenter study of lenalidomide in relapsed or refractory classical Hodgkin lymphoma. Blood. 2011;118(19):5119-25.

42. Harasawa H et al. Survey of chemokine receptor expression reveals frequent co-expression of skin-homing CCR4 and CCR10 in adult T-cell leukemia/lymphoma. Leuk Lymphoma. 2006;47(10):2163-73.

43. Ishida T,Ueda R. CCR4 as a novel molecular target for immunotherapy of cancer. Cancer Sci. 2006;97(11):1139-46.

44. Ishida T et al. Clinical significance of CCR4 expression in adult T-cell leukemia/lymphoma: its close association with skin involvement and unfavorable outcome. Clin Cancer Res. 2003;9(10 Pt 1):3625-34.

45. Shimauchi T et al. Production of thymus and activation-regulated chemokine and macrophage-derived chemokine by CCR4+ adult T-cell leukemia cells. Clin Cancer Res. 2005;11(6):2427-35.

46. Yoshie O et al. Frequent expression of CCR4 in adult T-cell leukemia and human T-cell leukemia virus type 1-transformed T-cells. Blood. 2002;99(5):1505-11.

47. Abou El-Ela M et al. Thymus and activation-regulated chemokine in different stages of mycosis fungoides: tissue and serum levels. Australas J Dermatol. 2011;52(3):167-71.

48. Ferenczi K et al. Increased CCR4 expression in cutaneous T-cell lymphoma. J Invest Dermatol. 2002;119(6):1405-10.

49. Fierro MT et al. Expression pattern of chemokine receptors and chemokine release in inflammatory erythroderma and Sezary syndrome. Dermatology. 2006;213(4):284-92.

50. Jones D et al. Expression pattern of T-cell-associated chemokine receptors and their chemokines correlates with specific subtypes of T-cell non-Hodgkin lymphoma. Blood. 2000;96(2):685-90.

51. Kakinuma T et al. Thymus and activation-regulated chemokine (TARC/CCL17) in mycosis fungoides: serum TARC levels reflect the disease activity of mycosis fungoides. J Am Acad Dermatol. 2003;48(1):23-30.

52. Miyagaki T et al. Serum soluble CD26 levels: diagnostic efficiency for atopic dermatitis, cutaneous T-cell lymphoma and psoriasis in combination with serum thymus and activation-regulated chemokine levels. J Eur Acad Dermatol Venereol. 2013;27(1):19-24.

53. Ghia P et al. Chronic lymphocytic leukemia B cells are endowed with the capacity to attract CD4+, CD40L+ T-cells by producing CCL22. Eur J Immunol. 2002;32(5):1403-13.

54. Ghia P et al. Chemoattractants MDC and TARC are secreted by malignant B-cell precursors following CD40 ligation and support the migration of leukemia-specific T-cells. Blood. 2001;98(3):533-40.

55. Scielzo C et al. The functional in vitro response to CD40 ligation reflects a different clinical outcome in patients with chronic lymphocytic leukemia. Leukemia. 2011;25(11):1760-7.

56. Yan XJ et al. Identification of outcome-correlated cytokine clusters in chronic lymphocytic leukemia. Blood. 2011;118(19):5201-10.

57. Olsnes AM et al. T lymphocyte chemotactic chemokines in acute myelogenous leukemia (AML): local release by native human AML blasts and systemic levels of CXCL10 (IP-10), CCL5 (RANTES) and CCL17 (TARC). Cancer Immunol Immunother. 2006;55(7):830-40.

58. Di Stasi A et al. T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. Blood. 2009;113(25):6392-402.

59. Chang DK et al. Humanization of an anti-CCR4 antibody that kills cutaneous T-cell lymphoma cells and abrogates suppression by T-regulatory cells. Mol Cancer Ther. 2012;11(11):2451-61.

60. Baatar D et al. CCR4-expressing T-cell tumors can be specifically controlled via delivery of toxins to chemokine receptors. J Immunol. 2007;179(3):1996-2004.

PAIN MANAGEMENT IN PATIENTS WITH SICKLE CELL DISEASE - A REVIEW

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ABSTRACT

Pain is defined, by the International Association for the Study of Pain (IASP), as an 'unpleasant sensitive and emotional experience, associated with or described in terms of tissue lesion'. It may be classified as acute or chronic when it becomes a symptom that worsens the quality of life, and thus loses its protective function. Pain may also be considered as chronic when it lasts over 3 to 6 months. Acute painful episodes, are the most common cause for patients with sickle cell disease (SCD) to seek medical attention. The causes of chronic pain are diverse in this population. In a sense, chronic sickle cell pain is a spin off the recurrent acute painful episodes. Pain management in patients with SCD presents unique challenges and opportunities.

Keywords: Pain, sickle cell disease, opioids, NSAID.

INTRODUCTION

Sickle cell anaemia (SCA), an autosomal recessive disease, results from a valine for glutamic acid substitution at position six of the β-globin gene of haemoglobin (Hb). When the sickle haemoglobin (HbS) molecule is deoxygenated, there is a hydrophobic interaction between this and other haemoglobin molecules that trigger an aggregation into large polymers, resulting in sickle-shaped deformities of the red blood cell (RBC).[1] When RBCs sickle, the common critical manifestations are vaso-occlusive, sequestration, haemolytic, and aplastic crises. Acute, painful episodes are the most common cause for patients with sickle cell disease (SCD) to seek medical attention. While the annual incidence rate of pain episodes increases with age, rates in adults with SCD are underestimated because the majority of such episodes are managed at home. Sickle cell pain syndromes include an unusual triumvirate of acute, chronic and neuropathic pain that occur sequentially or simultaneously with age.

Unlike other diseases associated with chronic pain, sickle cell acute pain manifests itself in infancy and continues to recur throughout the life span. SCD is the most common globin gene disorder: across the world, about 300,000 children are born with it each year.[2,3] The pain of sickle cell crisis is excruciating and, in global terms, a major health problem. No evidence-based guidelines exist for the treatment of SCD-associated acute pain episodes, either in the hospital or at home.

TYPES OF PAIN IN SICKLE CELL DISEASE

Pain caused by sickle cell disease can be acute, chronic or a mixture of the two.[4] The acute pain of tissue infarction, in skeletal or soft tissue, tends to be sudden, unpredictable in onset and intense. Most adults with SCD are aware of their triggers for vaso-occlusive pain episodes and are sensitive to avoiding them. Established precipitants of pain include extremely cold temperatures, change in weather, over-exertion, dehydration, onset of menses, direct or indirect exposure to tobacco smoke, and concomitant exacerbation of co-morbid conditions.[5]

Clinically, acute sickle pain is typically sharp and/or throbbing in nature, of sudden or gradual onset. It may last from hours to weeks in duration. The average duration of an acute painful crisis in adults, based on the length of hospital stay, is about 7 days.[6] Acute sickle cell pain is believed to be secondary to vaso-occlusion by sickled erythrocytes that adhere

Figure 1a. The Wong-Baker faces scale for assessment of intensity of pain in children.
"These faces show how much something can hurt. This face [point to left-most face] shows no pain. The faces show more and more pain [point to each from left to right] up to this one [point to right-most face] - it shows very much pain. Point to the face that shows how much you hurt [right now]." "Score the chosen face 0, 1, 2, 3, 4, or 5, counting left to right, so '0' = 'no pain' and '5' = 'very much pain.' Do not use words like 'happy' and 'sad.' This scale is intended to measure how children feel inside, not how their face looks."

to vascular endothelium. Vascular occlusion leads to ischaemia and consequent damage of the tissues supplied by the occluded vessel. Tissue damage creates a state of inflammation with the release of several inflammatory mediators. Each inflammatory mediator, released peripherally after tissue damage, binds to a specific receptor on the neurons of peripheral nerves. These neurons are crowded with multiple receptors for inflammatory mediators and for descending inhibitory pathways from the central nervous system (CNS). This state of affairs of one neuron with multiple receptors implies that inhibition of the transmission of the painful stimulus at the level of the peripheral nerve entails the blocking of all these receptors.[7]

Chronic pain[8] is simply pain that does not go away for 3 or more months. It is usually described as pain that is deep, persistent and there all the time. Chronic sickle cell pain is of two types. The first is due to obvious causes such as leg ulcers and avascular necrosis. The second type is due to persistent or frequently recurrent acute painful episodes that lead to chronic pain syndrome, especially without the proper treatment.

Assessment of Pain

Assessment is the gold standard of effective pain management.[9] It should be conducted before and periodically after the administration of analgaesics. The patient's self-report is the most important factor in the hierarchy of pain management. Other factors in the process of assessment should include the presence or absence of other complications of the disease. The patient's self-report[10] should include multidimensional scales describing intensity, quality, location, distribution, onset, duration, mood, sedation, pain relief, and factors that aggravate or relieve pain. Periodic assessment with rating and categorising of pain will delineate mixed pain syndromes, which may occur as the pain progresses over time.

Four stages of a pain event have been described in adults with SCD: prodromal, initial, established, and resolving. No combination of clinical and laboratory findings exists to determine if an individual is currently in pain.

The intensity of pain can be assessed using any of several available scales,[11,12] such as the visual analogue scale, verbal scale, numerical scale, or Wong-Baker faces scale for children (Figure 1a). Explain to the child that each face is for a person who feels happy because he has no pain or sad because he has some or a lot of pain. Face 0 is very happy because he does not hurt at all. Face 5 on the other hand, hurts as much as you can imagine, although you do not have to be crying to feel this bad. Ask the child to choose the face that best describes how he/she is feeling. The rating scale is recommended for persons aged 3 years and older.

The most common measurement for adults is the Visual Analog Scale (VAS), a continuous line, 100 mm in length, ranging from no pain to severe pain (Figure 1b). When treated in the emergency department (ED), adults with SCD report that a change in the VAS of 13.5 mm is the minimum objective change that relates to a clinically significant subjective change in a vaso-occlusive pain episode.

Other guidelines have used a scale to assess response to therapy (0=none, 1=little, 2=moderate, 3=good, 4=complete).

It is important, however, to stick to one scale and use it routinely, so that both the patient and provider become familiar with it and with its significance to a particular patient.

Management of the Acute Pain Crisis

Pain is a common experience in children beginning as early as 4 to 6 months of age, and dactylitis in infants is an early prognostic indicator for increased risk of complications in children. The failure to address the pain early can have lifelong implications on their health, generating a vicious cycle of fear, avoidance of pain, and poor coping strategies. Unrelieved pain can have more than negative consequences such as missed days of school and other life activities, or fear or mistrust of health care providers, but also can lead to amplified responses to subsequent pain experiences and sensitivity to pain later in life. Frequent typical vaso-occlusive pain may involve limbs, abdominal viscera, ribs, sternum, vertebrae, and sometimes skull bones.[13] Pain episodes can start suddenly, or they may follow an illness along with decreased activity, loss of appetite, and increased jaundice. Painful episodes are associated with early mortality in adults with SCD.

General principles of management[14]

Fluid replacement therapy: Fluid balance should be monitored in all patients. The patient should be encouraged to take oral fluids (60 ml/kg/24 h in adults). If the patient is unable to drink sufficient amounts or is vomiting, intravenous or nasogastric fluids are necessary at a similar rate. Central lines should be avoided unless needed for life-saving.

Oxygen: Oxygen should be given if pulse oximetry shows the oxygen saturation is below the patient's known steady-state level. Some patients have low steady-state oxygen saturations, which appear to be well tolerated without oxygen.

Thromboprophylaxis:[15] Patients with SCD appear to have a hypercoagulable state at baseline and they often have other factors that further increase the risk of venous thromboembolism (VTE) (e.g. indwelling catheter, immobility, infection). For all adults (those >18 years) with SCD who are admitted to the hospital for an acute medical condition, thromboprophylaxis is recommended with low dose of unfractionated heparin (e.g. 5000 units SQ three times a day).

There are no objective measurements of pain severity, and analgaesia should be titrated against the patient's reported pain, as recorded on a pain chart.

Initial management should be aimed at providing rapid pain control.[16] Analgaesia should then be maintained with long-acting oral or parenteral analgaesia, with provision for bolus analgaesia if breakthrough pain occurs. The choice of analgaesia will depend on how far along the 'analgaesic ladder' the patient has already progressed, and treatment

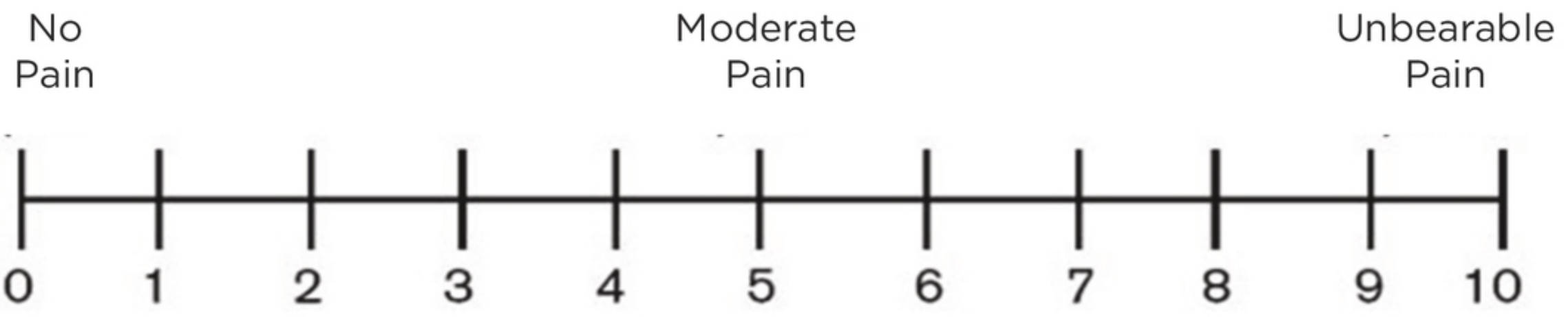

Figure 1b. The Visual Analog Scale (VAS) for pain measurement in adults.

The 0 to 10 pain scale is commonly and successfully used with hospitalised and nursing home patients, even those with mild to moderate dementia. The scale is often displayed as a line numbered from 0 to 10. This scale asks the person in pain to assign a number, from 0 to 10, to the severity of their pain. It is important to properly instruct the person in how to rate their pain. 0 means you have no pain at all. 10 means the worst possible pain you can image. The values on the pain scale correspond to pain levels as follows:

1 – 3 = mild pain
4 – 6 = moderate pain
7 – 10 = severe pain

should generally start with the next step. Once pain is controlled, the underlying cause should be assessed more comprehensively.

Paracetamol and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Paracetamol (acetaminophen)[17] is an analgaesic and antipyretic with little anti-inflammatory effect, whose exact mode of action is currently unknown. It is the most widely used drug for pain relief. In order of increasing effectiveness, paracetamol can be administered rectally, orally and intravenously. Paracetamol, at therapeutic doses, rarely results in adverse effects and, unlike NSAIDs, does not cause gastrointestinal ulceration or bleeding.

NSAIDs[17,18] are analgaesic, anti-inflammatory, antiplatelet, and antipyretic. They exert their analgaesic effect by reducing the production of prostaglandins responsible for pain and inflammation. NSAIDs achieve this by inhibiting the enzyme COX-2, which is essential in the synthesis of prostaglandins. NSAIDs vary in whether they selectively inhibit COX-2. Non-selective NSAIDs, such as ibuprofen and diclofenac, inhibit not only COX-2 but also cyclo-oxygenase 1 (COX-1). COX-1 is involved in the synthesis of prostaglandins that have a role in the maintenance and protection of the gastrointestinal (GI) tract, platelet adhesion, and renal function. Non-selective NSAIDs are therefore associated with adverse GI effects, renal toxicity, prolonged bleeding time, bronchospasm and oedaema.

One complication of SCD is nephropathy—characterised by proteinurea, ranging from microalbuminuria to massive excretion (with nephrotic syndrome). The nephropathy can be worsened by NSAIDs, so treatment with these agents should be stopped after a week at the most. NSAIDs can be used to control mild to moderate pain and may have an additive role in combination with opioids for severe pain. The doses and frequency of treatment should be monitored every 3 to 6 months in chronic users.

Opioid treatment

Opioids are used for severe pain in SCD.[19] The choice of an opioid, its dose, and route of administration should be individualised, based on past history and experience. No one opioid constitutes a panacea for all patients. The general trend today is to avoid the use of meperidine and to administer opioids orally for mild pain and intravenously or subcutaneously for severe pain, and avoid the intramuscular route if possible.

Opioid agonists produce their effect by binding into μ receptors. It is the L-isomers of opioids that exert their analgaesic activity. The binding affinity or strength with which a drug binds to its receptors varies considerably among opioids, with fentanyl, for example, having a higher binding affinity than morphine. The binding affinity of opioids seems to correlate well with their analgaesic potency.

Morphine

Morphine[19,21] is a strong μ-opioid agonist and the gold standard for the treatment of acute sickle cell painful crisis. It may be administered by any route and is available in both immediate and extended release formulation. Morphine is highly histaminergic and is often associated with pruritus that may be severe. Other recently reported side-effects of morphine include increased risk of acute chest syndrome in patients with SCD and acceleration of renal injury, especially in combination with NSAIDs.

The standard dosing[22] interval for morphine injections and rapid release preparations is 4-6 hours, but some individuals become so tolerant to opioids that doses are needed 2-hourly.

Pethidine

Pethidine[23] is short-acting with poor bioavailability and is metabolised to norpethidine, which is a renally-excreted cerebral irritant, causing dysphoria, clonus and seizures. It is usually given by repeated, high-dose intramuscular injections and has resulted in severe muscle damage. Pethidine should only be used in exceptional circumstances when there is a severe allergy to morphine and diamorphine (heroin). Continuous infusions[24] of pethidine should be avoided and it should not be used for more than 48 hours, or at doses greater than 600 mg/24 hours (American Pain Society, 1999). After 48 hours, the pethidine should be stopped and an alternative opiate used if necessary.

There should be no weaning of opioids[25] in the first 24 hours of a hospital admission unless there are signs of respiratory depression, increased lethargy, or other side effects associated with excessive amounts of opioids. Opioids should be weaned by decreasing the dose about 10% to 20% at a time, rather than by increasing the interval between doses. Conversion to oral pain medication should occur

when the intravenous dose is roughly equivalent to home doses of oral medications.

Management of Chronic Pain in Sickle Cell Disease

Management of the chronic underlying pain, requires a multifaceted approach to ensure patient adherence to treatment and adequate management of symptoms. The principles of treating chronic pain are different than those of acute pain. The goal of managing acute pain is to heel the acute injury or precipitating factors. The goal of treating chronic pain is to restore function. Once chronic pain sets in it is joined by other maladies that enhance its chronicity. These include depression, anxiety, suffering, despair, insomnia, loneliness, helplessness, and dependence on pain medications.

Oral opiates such as methadone, morphine, codeine, oxycodone, and hydroxycodone provide alternatives for outpatient treatment and pain management for patients discharged from the hospital.[26] Although these drugs are administered routinely to patients with SCD, their efficacy and safety have not been evaluated for treatment of acute pain crisis. These drugs are given as an oral analgaesic for treatment of mild-to-moderate pain at home, as transitional therapy between hospital treatment and home management, or for management of chronic pain.

Medications that can alter the perception of pain in the spinothalamic tract include opioids, serotonin norepinephrine reuptake inhibitors (SNRIs),[27] and tricyclic antidepressants.[28] The SNRIs duloxetine and milnacipran have indications for chronic pain although not for sickle cell pain. No tricyclics are FDA approved for chronic pain, though they are routinely used for this purpose as an adjunct to non-pharmacologic therapy for chronic neuropathic pain.

Paradoxically, the chronic administration of opioid analgaesics to treat pain may lead to similar confusion by also contributing to or causing pain.[29] Increased sensitivity to pain may be observed in any clinical setting where recurrent acute or chronic pain occurs. This is often erroneously attributed to either more disease-related pain, or to aberrant drug-seeking or addictive behaviors. There are basic steps to be taken when opioid neurotoxicity exists. First, recognise the syndrome; delirium, agitation, or restlessness may make the patient seem to be irrational or to be exaggerating the pain. This usually offends opioids which are frequently used to immediately-release (morphine, hydromorphone oxycodone) opioids, or very high doses of sustained release formulations of morphine, hydromorphone, and oxycodone.

An early sign may be clonus, which can be seen while the patient is asleep, before it becomes clinically overt. Allodynia and hyperalgaesia cause the pain to occur all over and do not follow a reasonable distribution. Rapidly increasing the opioid makes the pain worse. Second, discontinue the offending opioid and rotate to another drug. Third, add additional non-opioid adjunctive medications. Fourth, begin hydration to clear opioid metabolites. Fifth, consider benzodiazepines to decrease neuromuscular irritability but avoid sedation.

Transdermal fentanyl[30,31] patches are effective in chronic pain. These patches are easy to administer and contain multi-day dosage, but stable plasma levels may not be reached for 12 hours after application. The main disadvantages of the patches are that analgaesia is slow in onset and difficult to titrate against response, and that a residual depot is left after removal of the patch. We have used fentanyl patches successfully in a few patients in the later stages of admission for acute sickle cell crisis and have discharged the patients with them to take home. Fentanyl is released at a nearly constant rate from the transdermal matrix system into the skin, where it accumulates; this results in a depot of fentanyl in the outer layer of skin. Fentanyl is absorbed into systemic circulation from the depot. This results in a gradual increase in serum concentration over the first 12-24 hours, followed by fairly constant concentrations for the remainder of the dosing interval.

Methadone[32]

Methadone is a synthetic potent-opioid agonist. It has a long half-life (at least 36 hours) but a short duration of analgaesic effect (4 to 6 hours). It is associated with cardiotoxicity due to prolongation of the QTc interval with arrhythmia that could be fatal. It is associated with mortality more than any other opioid. Other medications such as antibiotics and antidepressants contribute to its cardiotoxic effect, and their use with methadone should be avoided or monitored carefully, although, methadone is an excellent analgaesic that is useful in treating chronic pain.

Cannabis[33,34]

Cannabis contains a mixture of phytocannabinoids whose synthetic congeners have been extensively investigated in the laboratory for their effects on pain sensation.

The hypothesis is that vaporised cannabis can induce dose-dependent antinociceptive changes in spontaneous and evoked pain in subjects with neuropathic pain. The second hypothesis is that the higher dose employed induces a greater degree of antinociception that is not independent of differences in mood, cognition, and psychomotor performance. Finally, it is hypothesised that an interaction with time will occur such that antinociception will outlast changes in cognitive impairment and psychomotor performance.

Psychological interventions

According to the cognitive-behavioural model,[35] an individual's interpretation of external events and bodily sensations directly affects their emotional reaction to these events and subsequent behaviour. Every cognitive behavioural approach starts by identifying and modifying unhelpful thinking patterns that are believed to increase distress. Dichotomous thinking, catastrophisation, and overgeneralisation are considered dysfunctional cognitive patterns because they typically arise from limited information and do not entirely reflect reality.[36]

Cognitive-behavioural interventions are an important part of a multimodal approach to pain management. They help the patient obtain a sense of control and develop coping skills to deal with the disease and its symptoms.[37] Guidelines by a National Institutes of Health assessment panel suggest integration of pharmacologic and behavioural approaches for treatment of pain and insomnia. Other studies suggest that behavioural interventions targeted to specific symptoms, such as pain and fatigue, can significantly reduce symptom burden and improve the quality of life for patients with chronic pain.

Non-pharmacological therapies

Non-pharmacological methods used in pain management can be classified in different ways. Meditation, progressive relaxation, dreaming, rhythmic respiration, biofeedback, therapeutic touching, transcutaneous electrical nerve stimulation (TENS), hypnosis, musical therapy, acupressure and treatments are just some of them. Acupuncture[38] is accepted as a scientific treatment method that provides the body to restore its balance by means of stimulating some special points on the body with needles. Mechanism of action for the acupuncture could not be completely understood until now. Chiropractics[39] is the neck-pulling movement used in treatment of the disorders in connective tissues and musculoskeletal system which consists of muscles, joints, bones, tendon, cartilage and ligaments. It is focused on the connection between body structure and the functions of the neural system, and manipulation of bones and joints to regain the health.

CONCLUSION

The act of taking care demands an overload of attention and intense dedication, especially as the healthcare team has to ensure that patients and their families have the conditions to reorganise their lives physically and emotionally. Ineffective pain control prior to discharge may also contribute to high early revisit rates. It is concluded that opioid dose requirements vary widely in patients with uncomplicated vaso-occlusive crisis and often exceed guideline recommendations.

REFERENCES

1. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. Lancet. 2010;376(9757):2018–31.

2. Dampier C, LeBeau P, Rhee S et al. Health-related quality of life in adults with sickle cell disease (SCD): a report from the comprehensive sickle cell centers clinical trial consortium. Am J Hematol. 2011;86(2):203-5.

3. Quinn CT, Rogers ZR, Buchanan GR. Survival of children with sickle cell disease. Blood. 2004; 103(11):4023-7.

4. Jacob E, Beyer JE, Miaskowski C et al. Are there phases to the vaso-occlusive painful episode in sickle cell disease? J Pain Symptom Manage. 2005;29(4):392-400.

5. Jones S, Duncan ER, Thomas N, et al. Windy weather and low humidity are associated with an increased number of hospital admissions for acute pain and sickle cell disease in an urban environment with a maritime temperate climate. Br J Haematol. 2005;131(4):530-3.

6. Brandow AM, Brousseau DC, Panepinto JA. Postdischarge pain, functional limitations and impact on caregivers of children with sickle cell disease treated for painful events. Br J Haematol. 2009;144(5):782-8.

7. Okpala I, Tawil A. Management of pain in sickle-cell disease. J R Soc Med. 2002;95(5):456-8.

8. American Pain Society. Guideline for the management of acute and chronic pain in sickle cell disease. 1999.

9. Ho K, Spence J, Murphy MF. Review of pain-measurement tools. Ann Emerg Med. 1996;27(4):427-32.

10. Benjamin LJ, Swinson GI, Nagel RL. Sickle cell anemia day hospital: an approach for the management of uncomplicated painful crises. Blood. 2000;95(4):1130-6.

11. Luffy R, Grove SK. Examining the

validity, reliability, and preference of three pediatric pain measurement tools in African-American children. Pediatr Nurs. 2003;29(1):54-9.

12. Smith WR, Penberthy LT, Bovbjerg VE, et al. Daily assessment of pain in adults with sickle cell disease. Ann Intern Med. 2008;148(2):94-101.

13. Westerman MP, Bailey K, Freels S, et al. Assessment of painful episode frequency in sickle-cell disease. Am J Hematol. 1997;54(3):183-8.

14. Co JP, Johnson KB, Duggan AK, et al. Does a clinical pathway improve the quality of care for sickle cell anemia? Jt Comm J Qual Saf. 2003;29(4):181-90.

15. Westerman MP, Green D, Gilman-Sachs A, et al. Coagulation changes in individuals with sickle cell trait .Am J Hematol. 2002;69(2):89-94.

16. Benjamin LJ, Swinson GI, Nagel RL. Sickle cell anemia day hospital: an approach for the management of uncomplicated painful crises. Blood. 2000;95(4):1130-6.

17. McGrath PJ, Finley GA, editors. Chronic and recurrent pain in children and adolescents. Seattle, WA: International Association for the Study of Pain. (1999); IASP Press.

18. Schaller S, Kaplan BS. Acute nonoliguric renal failure in children associated with nonsteroidal antiinflammatory agents. Pediatr Emerg Care. 1998;14(6):416-8.

19. Gupta M, Gupta K. "Opioids as promoters and regulators of angiogenesis". Maragoudakis ME, Papadimitriou E, eds. Angiogenesis: Basic Science and Clinical Applications (2007), Kerala, India: Transworld Research Network, pp. 303–18.

20. Bijur PE, Kenny MK, Gallagher EJ. Intravenous morphine at 0.1 mg/kg is not effective for controlling severe acute pain in the majority of patients. Ann Emerg Med. 2005;46(4):362-7.

21. van Beers EJ, van Tuijn CF, Nieuwkerk PT, et al. Patient-controlled analgesia versus continuous infusion of morphine during vaso-occlusive crisis in sickle cell disease, a randomized controlled trial. Am J Hematol. 2007;82(11):955-60.

22. Sear JW, Hand CW, Moore RA, McQuay HJ. Studies on morphine disposition: influence of renal failure on the kinetics of morphine and its metabolites. Br J Anaesth. 1989;62(1):28-32.

23. Mercadante S, Bruera E. Opioid switching: a systematic and critical review. Cancer Treatment Reviews. 2006;32(4):304–15.

24. Gonzalez ER, Bahal N, Hansen LA, et al. Intermittent injection vs patient-controlled analgesia for sickle cell crisis pain. Comparison in patients in the emergency department. Arch Intern Med. 1991;151(7):1373-8.

25. Maxwell LG, Kaufmann SC, Bitzer S, et al. The effects of a small-dose naloxone infusion on opioid-induced side effects and analgesia in children and adolescents treated with intravenous patient-controlled analgesia: a double-blind, prospective, randomized, controlled study. Anesth Analg. 2005;100(4):953-8.

26. Ballas SK. Current issues in sickle cell pain and its management. Hematology Am Soc Hematol Educ Program. 2007:97-105.

27. Perronne V, Roberts-Harewood M, Bachir D, Roudot-Thoraval F, Delord JM, Thuret I, Schaeffer A, Davies SC, Galacteros F, Godeau B. Patterns of mortality in sickle cell disease in adults in France and England. Hematol J. 2002;3(1):56–60.

28. Hetrick SE, et al. Selective serotonin reuptake inhibitors (SSRIs) for depressive disorders in children and adolescents. Cochrane Database of Systematic Reviews. 2007;3:CD004851.

29. Saarto T, Wiffen PJ. Antidepressants for neuropathic pain. Cochrane Database of Systematic Reviews. 2007;4:CD005454

30. The American Pain Society in Conjunction with The American Academy of Pain Medicine. Guideline for the use of chronic opioid therapy in chronic noncancer pain: evidence review. Glenview, IL; 2009.

31. Christie JM, Simmonds M, Patt R, Coluzzi P, Busch MA, Nordbrock E, Portenoy RK. Dose-titration, multicenter study of oral transmucosal fentanyl citrate for the treatment of breakthrough pain in cancer patients using transdermal fentanyl for persistent pain. J Clin Oncol. 1998;16(10):3238–45

32. Moulin, DE. Methadone - An old analgesic with new tricks. Pain Research & Management (PRM), Autumn 2005;10(3):131

33. Ware MA, Gamsa A, Persson J, Fitzcharles MA. Cannabis for chronic pain: case series and implications for clinicians. Pain Res Manag. 2002 Summer;7(2):95-9.

34. [No authors listed], Cannabis-based medicines--GW pharmaceuticals: high CBD, high THC, medicinal cannabis--GW pharmaceuticals, THC :CBD. Drugs R D. 2003;4(5):306-9.

35. Vervoort T, et al. Catastrophic thinking about pain is independently associated with pain severity, disability, and somatic complaints in school children and children with chronic pain. Journal of Pediatric Psychology. 2006;31(7):674–83.

36. Sullivan MJ, et al. Theoretical perspectives on the relation between catastrophizing and pain. The Clinical Journal of Pain. 2001;17(1):52–64.

37. Syrjala KL, Donaldson GW, Davies MW, Kippes ME, & Carr JE. Relaxation and imagery and cognitive-behavioral training reduce pain during cancer treatment: A controlled clinical trial. Pain. 1995;63(2):189-98.

38. Garcia MK, McQuade J, Haddad R, Patel S, Lee R, Yang P, Palmer JL, Cohen L. Systematic review of acupuncture in cancer care: a synthesis of the evidence. J Clin Oncol. 2013;31(7):952-60.

39. Westrom KK, Maiers MJ, Evans RL, Bronfort G. Individualized chiropractic and integrative care for low back pain: the design of a randomized clinical trial using a mixed-methods approach. Trials. 2010;11:24.

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TREATMENT OF HIV-ASSOCIATED BURKITT LYMPHOMA

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ABSTRACT

Burkitt lymphoma (BL) is a highly aggressive B-cell malignancy, occurring with increased frequency among patients infected with HIV. For several years, the immunocompromised state of HIV-positive patients was advocated as a sufficient reason to avoid the intensive chemotherapeutic regimens used in HIV-negative BL. However, with the introduction of the highly active antiretroviral therapy (HAART), the subsequent improvement of the immunological state of HIV-positive patients, and the disappointing results of less intensive schedules, investigators began to apply the same chemotherapeutic regimens used as a gold standard in HIV-negative non-Hodgkin lymphoma (NHL), including the use of rituximab. Despite promising results of different schedules in early-phase studies, agreement on the treatment of HIV-positive BL is still lacking, and further trials are needed to define a standard of care. Moreover, new treatment frontiers need to focus on improving the outcome for patients with advanced immunosuppression, unfavourable prognostic features- such as advanced stages and high International Prognostic Index (IPI) scores - and for those with adverse tumour biology.

This paper aims to revise the main epidemiological and physiopathological features of HIV-positive BL, to summarise the most relevant steps in the treatment of affected patients, and to elucidate the role of HAART in allowing HIV-positive patients to be managed with the therapeutic strategies currently used in HIV-negative patients with BL.

Keywords: Burkitt lymphoma, AIDS, HIV, rituximab, chemoimmunotherapy, myc.

INTRODUCTION

Burkitt lymphoma (BL) is an aggressive B-cell non-Hodgkin lymphoma (NHL) with a high cellular turnover and a cell-doubling time of 24–48 hours. The World Health Organization's (WHO) classification of haematological malignancies describes three clinical and epidemiologic variants of BL: endemic, sporadic and HIV-associated BL (HIV-BL).[1] BL accounts for up to 20% of lymphoproliferative malignancies diagnosed in HIV-positive subjects. In Western countries, symptoms related to BL are often the first clinical clues of AIDS onset.[2] The untamed replication kinetics of cancer cells translates into the clinical presentation of the lymphoma, with an extensive extranodal involvement, and high mortality rate when treated with conventional chemotherapy regimens used in patients with diffuse large B-cell lymphoma (DLBCL). For this reason, several chemotherapeutic regimens for patients with HIV-BL have been developed in the last few years. These combinations often include the anti-CD20 monoclonal antibody rituximab, resulting in combinations with excellent efficacy, but important limitations in feasibility and tolerability.

This paper is focused on the different chemotherapeutic regimens used to treat HIV-BL, the impact of the introduction of the highly active antiretroviral therapy (HAART), the role of rituximab and other important drugs, and the rapport between toxicity and efficacy of each chemoimmunotherapy

regimen. Evidence from different trials concerning the introduction of the HAART and the use of rituximab in the chemotherapeutic regimens, which still lack unanimous consent, will be presented as well.

EPIDEMIOLOGY AND RISK FACTORS

HAART, widely used in clinical practice from 1996, has dramatically changed the natural history of HIV infection and AIDS development and has significantly improved the outcomes of HIV-positive patients in terms of host immune response, reduction in opportunistic infections, and incidence of HIV-related NHL (HIV-NHL). Before HAART, the incidence of NHL in HIV-positive patients was 60-200 times higher than in HIV-negative patients;[3] the incidence of HIV-NHL decreased from 13.6 per 100,000 person-years before 1996 to only 1.8 per 100,000 person-years between 2002 and 2006.[4] Nevertheless, the incidence HIV-BL has not been affected by HAART with progressive rates increasing.[5]

The risk of development of BL in HIV-positive patients is 15-fold higher than HIV-negative ones.[6] The mean age of onset is 38 years old, and males are more likely to be affected. Conversely to the incidence of other HIV-NHL, which increases with age, the incidence of HIV-BL shows three different age-related peaks: infancy, adulthood and old age. This trimodal trend has been reported also in the HIV-negative population,[7] and underlies the possibility that age itself, instead of age-related level of immunosuppression, could play a role in the development of HIV-BL. A different relative risk for the development of HIV-BL as related to the way of HIV transmission, has failed to be demonstrated.

PATHOGENESIS

BL development is strongly related to the overexpression of *c-myc* oncogene. The gene encoding for MYC, a cell-cycle regulator, is located on the long arm of chromosome 8. There are three types of balanced translocation, common to all the clinical variants of BL, involving the *c-myc* locus: the t(8;14)(q24;q32), which involves immunoglobulin heavy chain (IGH) genes located in 14q32 and is detected in 70-80% of BL patients; the t(2;8)(p12;q24), which involves IG kappa-light chain locus in the 2p12, and is detected in 15% of BL; and the t(8;22)(q24;q11), which involves IG lambda-light chain genes in the 22q11 and is detected in 5% of BL patients. In all cases, MYC locus is downstream to IG gene enhancers, causing gene overexpression.[8] Expression of MYC in normal B-lymphocytes induces cell apoptosis by a p53-dependent pathway. In malignant B-cells, apoptosis is prevented by mutations in TP53 tumour suppressor gene. Moreover, p53-independent pathways, such as overexpression in B-cell lymphoma 2 (Bcl-2), due to down-regulation of Bim protein, inhibits apoptosis.[9] However, in HIV-positive patients who are not diagnosed with HIV-BL translocation in *c-myc* locus are frequent, which suggests that this mechanism alone is not enough to induce neoplastic proliferation of B-lymphocytes. The translocation seems to be enhanced when the enzyme activation-induced cytidine deaminase (AID) is over-expressed. AID is involved in the IGH class switch.[7] Enzyme induction is favoured by chronic antigenic stimulation of B-lymphocytes, or by signalling, which enhances enzyme over-expression itself.

As in other lymphomas, relative risk increases with high HIV viral load and with the duration of exposure to such elevated viral load.[10,11] These features suggest a pathogenic role of HIV in BL, even though molecular mechanisms of the potential oncogenic effect of HIV have never been demonstrated.[12] Putative mechanisms regard the effect of elevated viral loads inducing a chronic antigenic stimulus for B-cells and cytokines production that may enhance the activity of the AID, promoting *c-myc* translocation and survival signalling to mutated B-lymphocytes.[10] Moreover, one of the HIV envelope proteins, the CD40 ligand, is able to activate AID itself,[13] and Trans-Activator of Transcription (TAT) protein is able to promote B-lymphocytes cell cycle with two different mechanisms. On one hand, TAT enhances the activation of cellular genes, such as IL-6 and IL-10; on the other, TAT inhibits Rb2/p130 protein, a tumour suppressor gene involved in halting cell cycle from G0/G1.[14,15]

Risk for the development of HIV-BL increases with CD4-positive cell count: the majority of patients have ≥250/μL CD4, while the risk lowers when CD4 are <50/μL, with only 15% of cases in patients showing CD4 <100/μL. This is in contrast with other HIV-NHL where the relative risk increases with lowering of CD4.[16] An adequate number of functional CD4 cells seems to be necessary for HIV-BL development and, paradoxically, a competent immune system may have a causative role.[17] CD4+ lymphocytes may be involved in survival signalling to B-lymphocytes of germinal centres, carrying *c-myc* translocation, and in preventing cell death. On the contrary, low CD4 counts may fail to protect aberrant B-lymphocytes, promoting the initiation

Table 1. Diagnosis and staging of HIV-BL.

DIAGNOSIS		
HISTOLOGY	The cells seem to be molded and the cytoplasm is deeply basophilic with squared-off cytoplasmic margins. A 'starry sky' appearance is due to scattered tangible body-laden macrophages that contain apoptotic tumour cells.[79] There are three histologic variants, all of them having very high mitotic rates, with a Ki67 proliferation index close to 100%, and with a high cellular turnover suggested by increased apoptosis.	
	BL with plasmacytoid differentiation	- Most common in HIV-BL - It is associated with EBV in 50% to 70% of cases - It is characterised by medium-sized cells with abundant basophilic cytoplasm and eccentric nuclei
	BL classic	- Accounts for 30% of HIV-BL and it resembles endemic BL - It is associated with EBV in 30% of cases - It is characterised by intermediate-size cells containing coarse chromatin and prominent basophilic nucleoli
	Atypical BL	- It is characterised by cells with greater nuclear pleomorphism and fewer but more prominent nuclei
IMMUNOPHENOTYPE	- Mature B-cell with germinal centre cell differentiation and expression of surface immunoglobulins (sIgs) with light chain restriction - Positive for: CD19, CD20, CD22, CD79a, CD10, Bcl-6 - Negative for: CD5, CD23, terminal deoxynucleotidyl transferase (TdT) - Bcl-2: usually negative; however, Bcl-2 can be expressed in 10% to 20% of cases[80]	
CYTOGENETICS	Karyotypic analysis of neoplastic cells are aimed to identify *c-myc* translocation, by fluorescence in situ hybridisation. Three patterns can be observed.	
	Balanced translocation involving c-myc locus (80-95%)	- *C-myc* translocations are not exclusive of BL, but may be found in other types of NHL, such as in 10-15% of cases of DLBCL, and identifies worse prognosis
	Normal karyotype (10%)	- Molecular mechanisms leading to MYC deregulation are still under investigation[81]
	Complex karyotype (rare)	- Worse prognostic factor[82]
STAGING	- For adult patients Cotswold-modified An Arbor Staging system is used[83] - For paediatric patients St Jude/Murphy Staging system is used (stage IV defined by CNS or bone-marrow involvement)[84]	
PHYSICAL EXAMINATION	- Evaluation of performance status are mandatory	
BLOOD CHEMISTRY	- Full blood count (CD4 count), complete biochemical profile, serum lactate dehydrogenase and uric acid (to assess tumour turnover), viral infections assessment (EBV, HIV viral load, hepatitis B/C viruses)	
INSTRUMENTAL EXAMS	- ^{18}F-Fluorodeoxyglucose (^{18}F-FDG) positron emission tomography/computed tomography (PET/CT),[85,86] enhanced total body CT scan - Bone marrow aspirate and biopsy (including flow cytometry and cytogenetic evaluations) - Cerebrospinal fluid sampling by lumbar puncture, with cytologic examination, flow cytometry, physico-chemical examination - Brain MRI (if CNS involvement is suspected or confirmed) - Echocardiography - Some exams should be indicated in the case of clinical or biochemical suspicion of organ involvement, for instance endoscopic studies	

of cell death programs. Moreover, with a lower CD4 number, fewer B-lymphocytes are activated and *c-myc* translocation rate lowers.[7]

Epstein-Barr virus (EBV) was detected in <40% of cases of HIV-BL and in ~100% of cases of endemic BL.[18] This peculiarity seems to lessen the causative role of this virus in this malignancy. One possible explanation is that neoplastic changes occur in highly proliferating B-lymphocytes in the germinal centre in EBV-negative BL, while in EBV-positive cases, they involve memory B-cells, whose survival is promoted by viral infection itself.[19] EBV is able to promote lymphocytes oncogenic transformation, not only with a chronic antigenic stimulation and the consequent cytokines deregulation, but also by means of specific protein production. Epstein-Barr nuclear antigen 1 (EBNA-1) protein allows EBV latent persistence in B-cells genome, increases genetic instability of B-lymphocytes, and could enhance those mutations involved in the selection of the neoplastic clone.[20] Moreover, production of latent membrane protein 1 (LMP-1) promotes both proliferative signalling, via the activation of NF-kB pathway, and anti-apoptotic stimulation via BCL-2 overexpression in B-cells carrying *c-myc* translocation.[21]

CLINICAL PRESENTATION, DIAGNOSIS, STAGING

HIV-BL is a highly aggressive disease, usually presenting with systemic symptoms, advanced stage disease, as well as extensive extranodal involvement. With respect to sporadic BL, HIV-BL shows more common bone marrow infiltration (46% versus 20%), bulky disease (54% versus 13%), less common abdominal lesions (46% versus 91%), rarer leukaemic dissemination, and more frequent meningeal involvement (38% versus 14%), the latter being asymptomatic in 25% of cases.[22]

In the absence of pathognomonic features, diagnosis is formulated on the basis of histologic, immunophenotypic and cytogenetic investigations on the excision biopsy of an involved organ, preferring a lymph node (Table 1). Once the diagnosis is obtained, following the WHO criteria,[1] staging must be performed (Table 1).

TREATMENT

General Background

BL is a highly chemosensitive malignancy, achieving high response rates with available chemotherapy combinations. However, patients who reach complete remission (CR) must be closely monitored, as most relapses occur within the first year[23] and are uncommon after 2 years.[24] While some studies had demonstrated the prognostic role of the International Prognostic Index (IPI), and CD4 cell count and complex karyotype,[25] a therapeutic decision is not usually based on these variables. Most patients are managed with intensified chemoimmunotherapy combinations independently of IPI risk and extension of disease. Only Central Nervous System (CNS) involvement can change therapeutic choice in HIV-BL. Patients with HIV-BL should be managed with modern chemotherapy (see below) in cancer centres with appropriate expertise. This is an important issue considering that failure to achieve complete remission (CR) after first-line chemotherapy is a definitively negative prognostic event.[25] Importantly, the management of these patients is complex, mostly due to the high rates of co-morbidity, co-infections and treatment-related events, as well as the necessity to prevent complications and to manage symptoms. Prevention and treatment of tumour lysis syndrome with alkaline infusions and allopurinole/rasburicase administration, and appropriate G-CSF use are strongly encouraged. While actively treated patients must undergo antifungal prophylaxis with fluconazole, antiviral prophylaxis with acyclovir and prophylaxis for *Pneumocystis jiroveci* with trimethoprim-sulfamethoxazole. During CT, nadir antibiotic prophylaxis with a quinolone is appropriated.

Due to the high frequency of CNS involvement, both at presentation and relapse, the use of intravenous drugs able to penetrate the blood brain barrier and to achieve therapeutic concentrations in the CNS, as well as drug delivery by intrathecal route, are required.

HAART is a relevant component of the treatment of HIV-NHL, and BL is no exception. Before 1996, no standardised treatment was available for HIV-BL, since all HIV-NHL types were managed with the same strategy. Low-dose and less-intensive combinations led to a poor outcome in patients with HIV-BL; CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) regimen was associated with a CR rate of 30-50% and a median survival of 6-9 months,[26,27] and 58% and 18 months, respectively, with the CDE infusion combination.[28] Near 60% of deaths were due to AIDS-related events and not to lymphoma. As a consequence, less intensive approaches have been proposed (m-BACOD), with a median survival

Table 2. Schedule of Hyper-CVAD, PETHEMA-LAL3/07-GMALL regimen (± Rituximab), CODOX-M/IVAC standard e/o modified, DA-EPOCH (± Rituximab).

Cyclophosphamide (CTX), vincristine (VCN), doxorubicin (DOXO), dexamethasone (DEX), methotrexate (MTX), cytarabine (Ara-C), Prednisone (PDN), ifosfamide (IFO), teniposide (VM26), rituximab (R), vindesine (VND), etoposide (VP16), therapy intra-thecal (TIT), intravenous (i.v.), continuous infusion (civ.), orally (o.), days (d), hours (h), patients (pts), chemotherapy (CT), absolute neutrophil count (ANC).

a) Ara-C to 1 g/m^2 if pts>60 y.
b) MTX 0.5 g/m^2 if pts>50 y.
c) Two additional doses of rituximab were administered 3 and 6 weeks after the last C cycle.
d) Depending on centre.
e) Maximum dose 750 mg/m^2.
f) VP16 reduce by 25% if ANC nadir<500/μL or platelets<25,000/μL (both for at least 3 d) If this occurs during a cycle in which no CTX was given or creatinine clearance<40 mL/min.
g) DOXO reduce by 25% if ANC nadir<500/μL or platelets<25,000/μL (both for at least 3 d) if this occurs during a cycle in which no CTX was given or direct bilirubin>2.5 mg/dl.
h) VCN reduce to 0.3 mg/m^2/day if constipation or unable to walk on heels; reduce to 25% if direct bilirubin>2.5 mg/dl or creatinine clearance<40 mL/min; discontinue if difficulty ambulating.
i) R discontinue if skin/mucositis attributed to R.

HYPER-CVAD REGIMEN[49]

- **8 courses** of alternating intensive CT every 3 weeks
- **TIT:** 12 mg of MTX d 2 and 100 mg of Ara-C d 7 every cycle
- **HAART** during CT is recommended

Odd-numbered Courses (1-3-5-7)				Even-numbered Courses (2-4-6-8)			
CTX	300 mg/m^2	i.v. twice	d 1-3	**MTX**	1000 mg/m^2	i.v. over 24 h	d 1
VCN	2 mg	i.v.	d 4, 11				
DOXO	50 mg/m^2	i.v.	d 4	**Ara-C[a]**	3000 mg/m^2	i.v. twice	d 2-3
DEX	40 mg/d	i.v. or o.	d 1-4 and d 11-14				

PETHEMA REGIMEN[53]

- **8 courses** of alternating intensive CT every 3 weeks
- **Pre-phase** (to prevent lysis syndrome) **CTX** 200 mg/m^2 i.v. and **PDN** 60 mg/m^2 i.v. d 1-5
- **TIT:** MTX 12 mg, Ara-C 30 mg, hydrocortisone 20 mg d 1 and 5 of each cycle
- **HAART** during CT is recommended

Odd-numbered A-cycles (induction and 2-4-6)				Even-numbered B-cycles (1-3-5-7)			
VCN	2 mg	i.v.	d 1	**VCN**	2 mg	1.v.	d 1
MTX[b]	3000 mg/m^2	i.v. over 24 h	d 1	**MTX**	3000 mg/m^2	i.v. over 24 h	d 1
IFO	800 mg/m^2	i.v.	d 1-5	**CTX**	200 mg/m^2	i.v.	d 1-5
DEX	10 mg/d	i.v. or o.	d 1-5	**DEX**	10 mg/d	i.v. or o.	d 1-5
VM26	100 mg/m^2	i.v.	d 4-5	**DOXO**	25 mg/m^2	i.v.	d 4-5
Ara-C	150 mg/m^2	i.v. twice	d 4-5				

PETHEMA REGIMEN + R[54]

- **6 courses** of alternating intensive CT every 4 weeks
- **Pre-phase** (to prevent lysis syndrome) **CTX** 200 mg/m^2 i.v. and **PDN** 60 mg/m^2 i.v. d 1-5
- **TIT:** MTX 15 mg, Ara-C 40 mg, DEX 20 mg d 2 and 6 of each A e B cycles
- **HAART** during CT is recommended

A-cycles (1-4)				B-cycles (2-5)				C-cycles (3-6)[c]			
R	375 mg/m^2	i.v.	d 1	**R**	375 mg/m^2	i.v.	d 1	**R**	375 mg/m^2	i.v.	d 1
VCN	2 mg	i.v.	d 2	**VCN**	2 mg	i.v.	d 2	**VND**	3 mg/m^2 (max. 5 mg)	i.v.	d 2

MTX	1500 mg/m²	i.v. over 24 h	d 2	**MTX**	1500 mg/m²	i.v. over 24 h	d 2	**MTX**	1500 mg/m²	i.v. over 24 h	d 2
IFO	800 mg/m²	i.v.	d 2-6	**CTX**	200 mg/m²	i.v.	d 2-6	**DEX**	10 mg/d	i.v.	d 2-6
VM26	100 mg/m²	i.v.	d 5-6	**DEX**	10 mg/d	i.v.	d 2-6	**VP16**	250 mg/m²	i.v.	d 5-6
Ara-C	150 mg/m²	i.v. twice	d 5-6	**DOXO**	25 mg/m²	i.v.	d 4-5	**Ara-C**	2000 mg/m²	i.v. twice	d 6

CODOX-M/IVAC REGIMEN[55]

- **low risk pts** received 3 cycles of CODOX-M
- **high risk pts** received alternating cycles of CODOX-M, IVAC, CODOX-M, IVAC
- **HAART** during CT is recommended

CODOX-M				IVAC			
CTX	800 mg/m²	i.v.	d 1	**IFO**	1500 mg/m²	i.v.	d 1-5
CTX	200 mg/m²	i.v.	d 2-5	**VP16**	60 mg/m²	i.v.	d 1-5
DOXO	40 mg/m²	i.v.	d 1	**Ara-C**	2000 mg/m²	i.v. twice	d 1-2
VCN	1.5 mg/m²	i.v.	d 1, 8, 15				
MTX	6720 mg/m²	i.v. over 24 h	d 10				
TIT Ara-C 70 mg d 1, 3 MTX 12 mg d 15				**TIT** MTX 12 mg d 5			

CODOX-M/IVAC REGIMEN Modified

Noy et al.[56]	**R** 375 mg/m² d 1, **CTX** 800 mg/m² twice d 1-2, **VCN** 2 mg cap, **MTX** 3000 mg/m²
Montoto et al.[58]	**VCN** 1.5 mg/m² (cap 2 mg), **MTX** 3000 mg/m² **for pts>65 y** MTX decreased to 1000 mg/m², IFO decreased to 1000 mg/m², Ara-C decreased to 1000 mg/m²
Barnes et al.[60]	**R** 375 mg/m² d 1, **CTX** 800 mg/m² twice d 1-2, **DOXO** 50 mg/m², **VCN** 2 mg cap, **MTX** 3000 mg/m², **TIT** Ara-C 50 mg
Rodrigo et al.[57]	**CTX** 800 mg/m² twice d1-2, **DOXO** 50 mg/m², **VCN** 2 mg cap, **R** 375 mg/m² d 8 (of CODOX-M) and d 4 (of IVAC), **MTX** 3000 mg/m² or standard[(d)], **TIT** Ara-C 50 mg (during CODOX-M) and MTX 12 mg (twice during IVAC) or standard[(d)]

DA-EPOCH REGIMEN[65]

- **6 courses** every 3 weeks
- **TIT** total 8 MTX 12 mg d 1,5 of cycles 3 through 6

Oral therapy d 1-5	**PDN** 60 mg/m²/d
Infused agents (civ. of 96 h) d 1-4	**VP16**[f] 50 mg/m²/d, **DOXO**[g] 10 mg/m²/d, **VCN**[h] 0.4 mg/m²/d
Bolus agents d 5	
CTX First cycle	After cycle 1 (DA-CTX[e])
if CD4cells≥100/μL CTX 375 mg/m²	if nadir ANC>500/μL or platelets>25,000/μL CTX ↑187 mg above previous cycle
If CD4cells<100/μL CTX 187 mg/m²	if nadir ANC<500/μL or platelets<25,000/μL CTX ↓187 mg above previous cycle

DA-EPOCH-R REGIMEN[47]

Same schedule DA-EPOCH with additional R 375[i] mg/m²

Arm A: before each EPOCH cycle	**Arm B:** weekly times 6 weeks after EPOCH completed

of 8 months, and ~10% of patients alive at 2 years.[29-31] The introduction of HAART has contributed to significant improvement of host immune response, reduced the risk of opportunistic infection,[32-35] and allowed HIV-positive patients to be treated with the same strategies used in HIV-negative patients,[36] often with similar results.[37] The risks and benefits of continuing HAART during chemotherapy have been variably interpreted. Some physicians are concerned by the uncontrolled HIV replication that may worsen immune function, whereas others are concerned by the risk of adverse effects of HAART on efficacy. Pharmacokinetic analyses have showed a 1.5-fold reduction in cyclophosphamide clearance in patients treated with CHOP plus HAART, while no changes in doxorubicin clearance.[38] CD4 increase during chemotherapy has raised the concern that HAART protects T-cells from chemotherapy. In HIV-BL patients treated with DA-EPOCH and SC-EPOCH-RR, HAART has been suspended for ~7 weeks in most cases,[39,40] without a relevant increase of infections, and with only a transient increase of HIV viral loads and decrease of CD4 cells. There is some concern over the use of protease inhibitors (PIs) during chemotherapy as these drugs induce cytochrome P450 isoenzymes 3A (CYP 3A) inhibition. However, data from clinical trials suggest similar clinical outcome when protease inhibitor-containing regimen are compared with non-PI-based regimen, but possibly at the expense of greater myelotoxicity.[41] Available evidence seems to suggest that HAART interruption during short-term chemotherapy is irrelevant from a clinical point of view, while longer treatments should request continued HAART assumption.

The Role of Rituximab

The advent of rituximab, a monoclonal antibody against the B-cell antigen CD20, has significantly improved outcomes in several B-cell lymphomas, including BL.[42] The intense expression of CD20 in tumour cells provides a strong rationale for the use of this antibody in Burkitt-oriented chemotherapies. However, the application of rituximab to HIV-BL is controversial, mostly due to its potential risk of additional immunosuppression and increased incidence of major infectious events, which was shown in a large randomised clinical trial.[43] Subsequent studies have not confirmed this excessive infectious risk,[44] and some groups have introduced rituximab to their prior protocols to treat HIV-NHL patients, with a high CR rate and without increased toxicities.[45,46] A recent trial from the Aids Malignancy Consortium (AMC) confirmed a good tolerance of the rituximab-chemotherapy combination, with or without HAART, though increased infectious deaths in patients with a CD4 count <50 cells/mL remained problematic.[47]

CHOP and Rituximab-CHOP Regimens

Among the first-generation schedule, CHOP and CHOP-like regimen were extensively studied in national cooperative-group trials and have been considered the standard approach for patients with aggressive NHL in the HIV setting. Full dose CHOP combined with HAART has been associated with 48% CRR, and a 5-year EFS of 40%.[38,32] As expected, dose reduction of CHOP drugs have been associated with better tolerability, while efficacy remained unchanged.[32] In HAART era, the wide use of rituximab-CHOP (R-CHOP) regimen has been associated with remarkably improved results in HIV-positive patients with DLBCL, while survival figures in BL have remained largely disappointing. In fact, median time to progression (TTP) was 22 weeks and 157 weeks for HIV-BL and other NHL, respectively,[43] and survival data were not statistically different among patients with HIV-BL and patients with other lymphoma categories, especially DLBCL. The response rate for DLBCL was 81% and the response rate for HIV-BL was 73%.[48]

Intensified Regimens

Hyper-CVAD was one of the first regimens assessed in HIV-BL[49] (Table 2); this combination was assessed in a series of HIV-associated aggressive lymphomas, with only six cases of HIV-BL. HAART has been taken by 64% of patients, with no evidence of increased toxicity.[50] Overall, tolerability and efficacy in HIV-BL are similar to those obtained in HIV-negative patients.[50,51] There are no data on addiction of rituximab to this schedule in HIV-BL. This combination has been associated with infectious events in 85% of cases, severe myelosuppression, neurotoxicity, a 15% treatment-related mortality (TRM), and only 23% of patients completed the planned treatment (eight courses) (Table 3).

A multidrug combination derived from the German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia GM-ALL B-ALL 05/93 protocol (Table 2) has been assessed in HIV-BL patients by PETHEMA Group.[52,53] More recently, this complex regimen was combined with rituximab and assessed in a prospective trial.[54] The addition of rituximab has been associated with improved tolerability and efficacy, but improvements should

Table 3. Feasibility, tolerability and toxicity of more commonly used chemotherapy regimens.

Patients (pts), number (N.°), days (d), chemotherapy (CT), treatment-related mortality (TRM), not reported (NR), rituximab (R).

a) Only 6 pts had HIV-BL.
b) Treatment termination due to reasons different from progression disease.
c) 14 pts with HIV-BL (other with L3ALL).
d) 16 pts with HIV-BL (other with L3ALL).
e) Toxicity expressed in number of courses.
f) 4 pts not received rituximab.
g) 7 pts with HIV-BL.
h) 8 pts with HIV-BL.
i) 16 pts with HIV-BL.
j) 11 pts with HIV-BL.
k) 10 pts with HIV-BL.

Regimen	Pts N°	Planned CT (days)	TRM (%)	Severe infections	Mycotic infections	Mucositis	Incompleted treatment[b] (%)
Hyper-CVAD[49] (prospective)	13[a]	168	15	85	23	NR	77
PETHEMA-LAL3/97-GMALL[53] (prospective)	19[c]	173	21	37	NR	32	72
PETHEMA-LAL3/97-GMALL+R[54] (prospective)	19[d]	173	16	26[e]	21	27	32
CODOX-M/IVAC[55] (retrospective)	8	84	13	88	NR	75	14
Modified CODOX-M/IVAC + R[56] (prospective)	22	84	0	32	5	9	36
Modified CODOX-M/IVAC(58) (retrospective)	30	84	10	20	NR	43	50
Modified CODOX-M/IVAC + R[60] (retrospective)	14[f]	84	10	20	NR	NR	NR
Modified CODOX-M/IVAC + R[57] (retrospective)	14[f]	84	0	50	NR	43	50
DA-EPOCH[65] (prospective)	39[g]	126	0	13	NR	3	23
DA-EPOCH + R[66] (prospective)	23[h]	126	0	16	NR	NR	NR
DA-EPOCH + R[47] Arm A (prospective)	51[i]	126	9.8	27	NR	2	6
DA-EPOCH + R[47] Arm B (prospective)	55[l]	189	7.3	29	NR	7	5
DA-EPOCH + R[67] (prospective)	29[m]	126	NR	NR	NR	NR	NR
Short-term chemo-immunotherapy[70] (retrospective)	15	90	6.7	35	NR	NR	13.4

not be attributed exclusively to rituximab considering that supportive care was also optimised and HAART was used in all patients. Overall, infection rates were not affected by the addition of rituximab.[53] Importantly, there were no differences in TRM or in CR rates between HIV-negative and HIV-positive patients, showing that this schedule can safely be applied to HIV-positive patients. Results were reported together for patients with BL-HIV or mature B-cell acute lymphoblastic leukaemia (L3ALL), and schedules for these subgroups of patients were similar, but not identical. Since toxicities and outcome were not reported separately, conclusions may be misleading (Tables 3 and 4). This regimen is very long (173 days) and exhibits a high toxicity rate, with a TRM of ~20%. The use of methotrexate in every cycle resulted in increased incidence of mucositis.

CODOX-M/IVAC is the most investigated chemotherapy combination in HIV-BL patients (Table 2). It is currently with different modifications of dosage and schedule according to patient's age, co-morbidity and risk.[55,24] Dose adjustments, mostly of methotrexate and vincristine, were followed by an evident reduction in adverse events, with only grade 1-2 mucositis, neurotoxicity and TRM (Tables 2 and 3), and with a progressive immunological recovery and CD4+ counts.[56-58] CODOX-M/IVAC, combined or not with rituximab, has been assessed in a few, small series of patients with HIV-BL (Table 3 and 4), resulting in a TRM of ~15%, severe infections in ~65% of cases, mycosis infections in ~5%, and mucositis in 75%.[56-60] Improved outcome in recent studies can be explained by a better management of patients and also by a positive patient selection. In fact, the study population had a remarkably better immune status, with high CD4 counts (median: 375/μL), lower HIV viral load (median <50 copies/mL) and more limited stage of disease (only 50% had stage IV).[56-58] Recent studies demonstrated that HAART can be used during CODOX-M/IVAC, without additional toxicities, resulting in an acceptable TRM, similar to those reported in HIV-negative patients. Although the addition of rituximab in recent series improved outcome and prevented relapses, without an increase in infectious events (Table 3), cytopaenias, myelosuppression and nephrotoxicity remain important concerns in HIV-BL patients treated with CODOX-M/IVAC.

Infusion Regimens

Infusion chemotherapy regimens exhibit a high activity in lymphoma patients, even when they were previously exposed to the cytostatics administered as an intravenous bolus, suggesting a schedule-dependent effect in favour of the infusional administration of certain cytotoxic agents in patients with lymphoid neoplasms.[61] This was the rationale for the use of infusional regimens in the treatment of HIV-NHL. CDE regimen, consisting of a 96-hour continuous intravenous infusion of cyclophosphamide (800 mg/m^2), doxorubicin (50 mg/m^2), and etoposide (240 mg/m^2) was the first assessed combination in HIV-BL, which was associated with a CRR of 45%, a 2-year FFS of 36% and 2-year OS of 43%, with significantly better tolerability and efficacy in patients treated in the HAART era.[62] Pooled results of three phase II trials addressing activity and tolerability of rituximab+CDE regimen have showed encouraging results, with a 70% CRR and a 2-year OS of 64%, but with increased risk for life-threatening infection (TRM= 8%).[63] These results were significantly poorer among patients with BL.

Another group has reported encouraging results with a 96-hour infusion regimen of doxorubicin, etoposide, and vincristine used in conjunction with intravenous bolus cyclophosphamide plus oral prednisone (EPOCH regimen) (Table 2). This regimen has been developed on the base of *in vitro* studies showing that tumour cells are relatively less resistant to prolonged low concentration exposure to the natural product–derived agents vincristine, doxorubicin, and etoposide, compared with brief higher concentration exposure.[64] In a first US National Cancer Institute (NCI) trial,[65] EPOCH therapy resulted in a 74% CRR and a 72% survival rate at a median follow-up of 53 months) in 39 patients with HIV-NHL (Table 3 and 4). The dose-adjustment strategy (DA-EPOCH) was implemented to reduce haematopoietic toxicity, and HAART was withheld during the study to avoid an increased risk of haematological toxicity with chemotherapy. HIV-BL patients enrolled were very few, and toxicities and subgroup analyses were not performed. Importantly, all deaths were related to CNS involvement, which was expected since DA-EPOCH does not include drugs that penetrate blood brain barrier, like methotrexate or cytarabine.

The addition of rituximab to DA-EPOCH (DA-EPOCH-R) has been associated with promising preliminary results in a prospective trial including 23 patients with BL (8 of them were HIV-positive), half of them advanced stage; at a median follow-up of 27 months, CR and OS rates were 100%, without toxic death.[66] A randomised phase II trial has demonstrated that concurrent rituximab plus infusion of EPOCH is

Table 4. Activity and efficacy of more commonly used chemotherapy regimens.

Rituximab (R), years (y), complete response (CR), 2-year-Progression Free Survival (2-y-PFS), 2-year-Overall-Survival (2-y-OS), Disease Free Survival (DFS), not reported (NR).

a) Percentuale relativa a pts in statio III e IV.
b) This is 1-year-OS.
c) This is 3-years-EFS.
d) This is 3-years-OS.
e) Dati generali riguardanti l' intera popolazione (80 pts) non solo i 14 HIV-BL.
f) This is 3-years-PFS.
g) Median HIV viral load log_{10}.
h) PFS and OS at 53 months.

Regimen	Median age y (range)	Stage IV (%)	Median CD4/μL	Median viral load copies/mL	Median follow-up (months)	CR (%)	2-y-PFS (%)	2-y-OS (%)
Hyper-CVAD[49] (prospective)	43 (32-55)	31	77	32,000	29	92	DFS:52	48
PETHEMA-LAL3/97-GMALL[53] (prospective)	41 (23-65)	57[a]	420	400,000	31	68	DFS:71	46
CODOX-M/IVAC[55] (retrospective)	41 (19-61)	88	149	6,357	34	63	EFS:60	NR
PETHEMA-LAL3/97-GMALL+R[54] (prospective)	39 (29-54)	42[a]	NR	NR	22	84	DFS:87	73
Modified CODOX-M/IVAC + R[56] (prospective)	40 (19-55)	NR	290	15,600	17	NR	NR	85.7[b]
Modified CODOX-M/IVAC[58] (retrospective)	38 (28-69)	70[a]	171	96,000	22	70	75[c]	52[d]
Modified CODOX-M/IVAC + R[60] (retrospective)	46 (17-78)	73[e]	237	22,604	NR	93	68[f]	68[d]
Modified CODOX-M/IVAC + R[57] (retrospective)	46 (32-56)	50	375	<50	12	86	ND	83
DA-EPOCH[65] (prospective)	40 (31-57)	67[a]	198	4.4[g]	53	74	73[h]	60[h]
DA-EPOCH + R[66] (prospective)	31 (18-66)	52	NR	NR	27	100	100	100
DA-EPOCH + R[47] Arm A (prospective)	44	84[a]	181	NR	30	63	66	70
DA-EPOCH + R[47] Arm B (prospective)	43	75[a]	194	NR	30	82	63	67
DA-EPOCH + R[67] (prospective)	35 (16-88)	59[a]	NR	NR	57	NR	NR	100
Short-term chemo-immunotherapy[70] (retrospective)	42 (27-63)	87[a]	248	23,640	25	80	73	73

Table 5. Short-term chemoimmunotherapy proposed by GICAT (Gruppo Italiano Cooperativo AIDS e Tumori).

Cyclophosphamide (CTX), methotrexate (MTX), cytarabine (ara-C), vincristine (VCN), doxorubicin (DOXO), rituximab (R), etoposide (VP16), metylprednisolone (MP), intravenous (i.v.), therapy intra-thecal (TIT), carmustine (BCNU), melphalan (M), autologous stem cell transplant (ASCT).

REGIMEN: Short-term chemoimmunotherapy[70]	
Schedule	**Therapeutic programme**
Induction -2; -1 **MP** 0.5 - 1 mg/Kg/d i.v. 0 **MP** 0.5 - 1 mg/Kg/d i.v. **CTX** 500 mg/m² over 1 h infusion **VCR** 2 mg total dose i.v. bolus 1 **MP** 0.5 - 1 mg/kg/d i.v. **CTX** 500 mg/m² over 1-h infusion 2 **R** 375 mg/m² 5 **MTX** 12 mg + **Ara-C** 50 mg + steroids, i.t. 7 **MTX** 3 g/m² i.v. over 6 h+ leucovorin rescue 14 **R** 375 mg/m2 15 **VP16** 250 mg/m² every 12 h 19 **MTX** 12 mg + Ara-C 50 mg + steroids, i.t. 21 **MTX** 3 g/m² i.v. over 6 h + leucovorin rescue 29 **R** 375 mg/m² **DOXO** 50 mg/m² i.v. bolus 33 **MTX** 12 mg + Ara-C 50 mg + steroids, i.t. 36 **R** 375 mg/m² **VCN** 2 mg total dose i.v. bolus **Consolidation** 50–51 **Ara-C** 2 g/m² in a 3-h infusion, twice a day (every 12 h) 52 **R** 375 mg/m² 60 **R** 375 mg/m²	**After induction:** If CR, high dose Ara-C and R based consolidation phase If PR, high dose Ara-C and R based consolidation phase followed by BEAM (BCNU, vp16, Ara-C, M) plus ASCT If SD/PD intensification phase, followed by BEAM plus ASCT **At the end of CT** If initial bulky disease or residual PET-positive single lesion were administered 36 Gy involved field irradiation

associated with improved outcome with respect to sequential administration of rituximab and EPOCH in 106 patients with HIV-NHL.[47] This trial included only 27 HIV-BL patients, but activity in these patients was encouraging with both combinations, with a CRR of 63% in concurrent arm and 82% in sequential arm. Recently, the efficacy of DA-EPOCH-R was assessed in patients with *myc*-related aggressive-B-cell lymphomas; preliminary results of 29 patients with BL (10 HIV-positive) showed a 100% OS at a median follow-up of 57 months[67] (Table 3 and 4).

Short-Term Chemoimmunotherapy

A dose-dense, short-term chemotherapy programme including seven active drugs and intrathecal drug delivery has showed excellent activity and safety profiles in HIV-negative patients with BL in the pre-rituximab era.[68] This regimen, proposed by the Gruppo Italiano Cooperativo AIDS e Tumori (GICAT), has been modified to be used with maintained efficacy and improved tolerability in HIV-BL. In particular, six doses of rituximab have been added and methotrexate dose has been reduced from 150 and 250 mg/Kg to 3 g/m², mostly to avoid mucositis, which constitutes an important route of access for infectious agents, and one of the main causes of death in these patients.[69] Treatment consists of a 36-day induction phase including sequential doses of fractionated cyclophosphamide, high doses of methotrexate and cytarabine, doxorubicin, vincristine, and etoposide, rituximab and intrathecal prophylaxis/treatment (Table 7). Subsequent treatment is tailored according to the objective response to induction phase: patients in CR are referred to high-dose cytarabine-based consolidation phase (Table 7); patients in partial response are referred to consolidation followed by BEAM plus autologous stem cell transplant; patients with stable or progressive disease are referred to intensification phase, followed by BEAM+ASCT. At the end of chemoimmunotherapy, patients with initial bulky disease or with a residual PET-positive

single lesion are evaluated for 36-Gy involved-field irradiation.

This modified chemoimmunotherapy regimen in 15 consecutive HIV-BL, with excellent safety profile and efficacy.[70] This intensive, short-term chemoimmunotherapy regimen is fast, safe, cost-effective, and active in HIV-BL, especially in patients responsive to HAART and with adequate CD4+ cell counts. It showed tolerability and efficacy similar to those reported with the original regimen in HIV-negative patients with BL,[68] and its activity and efficacy are similar to those attained with more demanding and resource consuming regimen in HIV-BL, with an apparently better tolerability profile (Tables 2 and 3). In fact, this program was delivered in a shorter period (median 90 days; range 52-143), without cases of mucositis, opportunistic infections and interruption due to toxicity, with manageable haematological toxicity, only mild infectious and a single toxic death. Autologous peripheral blood stem cell (APBSC) collection was successful in 9 out of 11 patients (median: $14{\cdot}10^6$ CD34+ cells/kg). There was a single case of G4 non-haematological toxicity (transient diarrhoea). Patients with Hepatitis B virus (HBV) or Hepatitis C virus (HCV) positivity completed the planned treatment (ASCT in three), and experienced only transient G3 increase of transaminase serum level, without significant chemotherapy delay. CRR at the end of the whole treatment was 80%; at a median follow-up of 25 months (range 9-33), 11 patients remained disease-free, with a 2-year PFS and OS of 73%. CD4+ cell count ≥200 cells/μL is a favourable prognostic factor.[70] These encouraging results will be confirmed in an ongoing multicentre prospective phase II trial called the CARMEN trial (ClinicalTrials.gov Identifier: NCT01516593).

Salvage Therapy and Role of Stem Cell Transplant

Standard salvage treatment for patients with relapsed or refractory HIV-BL remains to be defined as there are no studies focused on this issue, and recommendations are based on retrospective studies of HIV-associated lymphomas that include a few HIV-BL patients.[71-74] The first choice concerns the type of treatment, based upon patients' clinical conditions: supportive care, palliative chemotherapy or high-dose chemotherapy (HDC) plus autologous stem cell transplantation (ASCT).[75] The latter is considered the best curative option for HIV-negative patients with relapsed disease.[76] In the absence of significant differences, in terms of OS and PFS between HIV negative and HIV positive patients, this schedule is considered to be feasible even in HIV-related lymphomas.[72] In most patients, data is based on the outcome after HDC/ASCT of the entire HIV-positive population, not exclusively on HIV-BL.[71-74] In other case series, HDC/ASCT led to very poor outcome in HIV-BL, sometimes due to inefficiency of induction chemotherapy,[75] or to early deaths after ASCT.[77] Ferreri et al. demonstrated a very promising outcome with BEAM + ASCT after induction therapy in patients who achieved CR, resulting in 5 CR beyond the 6 CR obtained with induction therapy alone.[70] There are no data on allo-SCT in relapsed HIV BL.[78]

CONCLUSIONS

The introduction of HAART allowed the treatment of HIV-BL patients with the same intensive schedules proved to be curative in HIV-negative BL. Since there are no randomised trials comparing different first-line schedules (Table 4), gold standard regimen for HIV-BL is still debated. Available literature is mostly constituted by small retrospective and prospective studies considering patients with different lymphoma categories, other than HIV-BL. Thus, analysis and comparison of outcomes and toxicities is very difficult, potentially leading to unreliable conclusions. Adding rituximab on various schedules demonstrated good efficacy and tolerability compared to chemotherapy alone. The use of improved supportive therapy and antimicrobial prophylaxis significantly reduced adverse events, improving outcome. New de-escalated regimens could produce the same positive results obtained by more intensive and resource consuming combinations, with a lower risk of severe toxicity.[70] These innovative regimens should be assessed in prospective trials aimed also to identify prognostic markers to establish a risk-tailored overall strategy.

REFERENCES

1. Jaffe ES. The 2008 WHO classification of lymphomas: implications for clinical practice and translational research. Hematology Am Soc Hematol Educ Program. 2009;(1):523-31.

2. Blinder VS, Chadburn A, Furman RR, Mathew S, Leonard JP. Improving outcomes for patients with Burkitt lymphoma and HIV. AIDS Patient Care STDS. 2008;22(3):175-87.

3. Stebbing J, Gazzard B, Mandalia S, Teague A, Waterston A, Marvin V et al. Antiretroviral treatment regimens and immune parameters in the prevention of systemic AIDS-related non-Hodgkin's lymphoma. J Clin Oncol. 2004;22(11):2177-83.

4. Polesel J, Clifford GM, Rickenbach M, Dal Maso L, Battegay M, Bouchardy C et al. Non-Hodgkin lymphoma incidence in the Swiss HIV Cohort Study before and after highly active antiretroviral therapy. AIDS. 2008;22(2):301-6.

5. Diamond C, Taylor TH, Aboumrad T, Anton-Culver H. Changes in acquired immunodeficiency syndrome-related non-Hodgkin lymphoma in the era of highly active antiretroviral therapy: incidence, presentation, treatment, and survival. Cancer. 2006;106(1):128-35.

6. Engels EA, Biggar RJ, Hall HI, Cross H, Crutchfield A, Finch JL et al. Cancer risk in people infected with human immunodeficiency virus in the United States. Int J Cancer. 2008;123(1):187-94.

7. Guech-Ongey M, Simard EP, Anderson WF, Engels EA, Bhatia K, Devesa SS et al. AIDS-related Burkitt lymphoma in the United States: what do age and CD4 lymphocyte patterns tell us about etiology and/or biology? Blood. 2010;116(25):5600-4.

8. Bertrand S, Berger R, Philip T, Bernheim A, Bryon PA, Bertoglio J et al. Variant translocation in a non endemic case of Burkitt's lymphoma: t (8;22) in an Epstein--Barr virus negative tumour and in a derived cell line. Eur J Cancer. 1981;17(5):577-84.

9. Egle A, Harris AW, Bouillet P, Cory S. Bim is a suppressor of Myc-induced mouse B cell leukemia. Proc Natl Acad Sci USA. 2004;101(16):6164-9.

10. Engels EA, Pfeiffer RM, Landgren O, Moore RD. Immunologic and virologic predictors of AIDS-related non-hodgkin lymphoma in the highly active antiretroviral therapy era. J Acquir Immune Defic Syndr. 2010;54(1):78-84.

11. Zoufaly A, Stellbrink HJ, Heiden MA, Kollan C, Hoffmann C, van Lunzen J et al. Cumulative HIV viremia during highly active antiretroviral therapy is a strong predictor of AIDS-related lymphoma. J Infect Dis. 2009;200(1):79-87.

12. Jarrett RF. Viruses and lymphoma/leukaemia. J Pathol. 2006;208(2):176-86.

13. Epeldegui M, Thapa DR, De la Cruz J, Kitchen S, Zack JA, Martinez-Maza O. CD40 ligand (CD154) incorporated into HIV virions induces activation-induced cytidine deaminase (AID) expression in human B-lymphocytes. PLoS One. 2010;5(7):e11448.

14. Herndier BG, Shiramizu BT, Jewett NE, Aldape KD, Reyes GR, McGrath MS. Acquired immunodeficiency syndrome-associated T-cell lymphoma: evidence for human immunodeficiency virus type 1-associated T-cell transformation. Blood. 1992;79(7):1768-74.

15. Bellan C, Lazzi S, De Falco G, Nyongo A, Giordano A, Leoncini L. Burkitt's lymphoma: new insights into molecular pathogenesis. J Clin Pathol. 2003;56(3):188-92.

16. Gabarre J, Raphael M, Lepage E, Martin A, Oksenhendler E, Xerri L et al. Human immunodeficiency virus-related lymphoma: relation between clinical features and histologic subtypes. Am J Med. 2001;111(9):704-11.

17. Smith SM. AIDS-related BL and CD4 count: a clue? Blood. 2010;116(25):5435-6.

18. Knowles DM. Etiology and pathogenesis of AIDS-related non-Hodgkin's lymphoma. Hematol Oncol Clin North Am. 2003;17(3):785-820.

19. Bellan C, Lazzi S, Hummel M, Palummo N, de Santi M, Amato T et al. Immunoglobulin gene analysis reveals 2 distinct cells of origin for EBV-positive and EBV-negative Burkitt lymphomas. Blood. 2005;106(3):1031-6.

20. Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. Nat Rev Cancer. 2004;4(10):757-68.

21. Gaidano G, Capello D, Carbone A. The molecular basis of acquired immunodeficiency syndrome-related lymphomagenesis. Semin Oncol. 2000;27(4):431-41.

22. Just PA, Fieschi C, Baillet G, Galicier L, Oksenhendler E, Moretti JL. 18F-fluorodeoxyglucose positron emission tomography/computed tomography in AIDS-related Burkitt lymphoma. AIDS Patient Care STDS. 2008;22(9):695-700.

23. Ostronoff M, Soussain C, Zambon E, Ibrahim A, Bosq J, Bayle C et al. Burkitt's lymphoma in adults: a retrospective study of 46 cases. Nouv Rev Fr Hematol. 1992;34(5):389-97.

24. Magrath I, Adde M, Shad A, Venzon D, Seibel N, Gootenberg J et al. Adults and children with small non-cleaved-cell lymphoma have a similar excellent outcome when treated with the same chemotherapy regimen. J Clin Oncol. 1996;14(3):925-34.

25. Bower M, Stebbing J. AIDS-associated malignancies. Cancer Chemother Biol Response Modif. 2005;22:687-706.

26. Levine AM. Acquired immunodeficiency syndrome-related lymphoma. Blood. 1992;80(1):8-20.

27. Sparano JA. Treatment of AIDS-related lymphomas. Curr Opin Oncol. 1995;7(5):442-9.

28. Sparano JA, Wiernik PH, Hu X, Sarta C, Schwartz EL, Soeiro R et al. Pilot trial of infusional cyclophosphamide, doxorubicin, and etoposide plus didanosine and filgrastim in patients with human immunodeficiency virus-associated non-Hodgkin's lymphoma. J Clin Oncol. 1996;14(11):3026-35.

29. Levine AM, Wernz JC, Kaplan L, Rodman N, Cohen P, Metroka C et al. Low-dose chemotherapy with central nervous system prophylaxis and zidovudine maintenance in AIDS-related lymphoma. A prospective multi-institutional trial. JAMA. 1991;266(1):84-8.

30. Kaplan LD, Straus DJ, Testa MA, Von Roenn J, Dezube BJ, Cooley TP et al. Low-dose compared with standard-dose m-BACOD chemotherapy for non-Hodgkin's lymphoma associated with human immunodeficiency virus infection. National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group. N Engl J Med. 1997;336(23):1641-8.

31. Gisselbrecht C, Oksenhendler E, Tirelli U, Lepage E, Gabarre J, Farcet JP et al. Human immunodeficiency virus-related lymphoma treatment with intensive combination chemotherapy. French-Italian Cooperative Group. Am J Med. 1993;95(2):188-96.

32. Mounier N, Spina M, Gabarre J, Raphael M, Rizzardini G, Golfier JB et al. AIDS-related non-Hodgkin lymphoma: final analysis of 485 patients treated with risk-adapted intensive chemotherapy. Blood. 2006;107(10):3832-40.

33. Bower M, Gazzard B, Mandalia S, Newsom-Davis T, Thirlwell C, Dhillon T et al. A prognostic index for systemic AIDS-related non-Hodgkin lymphoma treated in the era of highly active antiretroviral therapy. Ann Intern Med. 2005;143(4):265-73.

34. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry

using a tissue microarray. Blood. 2004;103(1):275-82.

35. Lim ST, Karim R, Tulpule A, Nathwani BN, Levine AM. Prognostic factors in HIV-related diffuse large-cell lymphoma: before versus after highly active antiretroviral therapy. J Clin Oncol. 2005;23(33):8477-82.

36. Bower M, Collins S, Cottrill C, Cwynarski K, Montoto S, Nelson M et al. British HIV Association guidelines for HIV-associated malignancies 2008. HIV Med. 2008;9(6):336-88.

37. Kaplan LD. HIV-associated lymphoma. Best Pract Res Clin Haematol. 2012;25(1):101-17.

38. Ratner L, Lee J, Tang S, Redden D, Hamzeh F, Herndier B et al. Chemotherapy for human immunodeficiency virus-associated non-Hodgkin's lymphoma in combination with highly active antiretroviral therapy. J Clin Oncol. 2001;19(8):2171-8.

39. Little RF, Pittaluga S, Grant N, Steinberg SM, Kavlick MF, Mitsuya H et al. Highly effective treatment of acquired immunodeficiency syndrome-related lymphoma with dose-adjusted EPOCH: impact of antiretroviral therapy suspension and tumor biology. Blood. 2003;101(12):4653-9.

40. Dunleavy K, Little RF, Pittaluga S, Grant N, Wayne AS, Carrasquillo JA et al. The role of tumor histogenesis, FDG-PET, and short-course EPOCH with dose-dense rituximab (SC-EPOCH-RR) in HIV-associated diffuse large B-cell lymphoma. Blood. 2010;115(15):3017-24.

41. Bower M, McCall-Peat N, Ryan N, Davies L, Young AM, Gupta S et al. Protease inhibitors potentiate chemotherapy-induced neutropenia. Blood. 2004;104(9):2943-6.

42. Thomas DA, Faderl S, O'Brien S, Bueso-Ramos C, Cortes J, Garcia-Manero G et al. Chemoimmunotherapy with hyper-CVAD plus rituximab for the treatment of adult Burkitt and Burkitt-type lymphoma or acute lymphoblastic leukemia. Cancer. 2006;106(7):1569-80.

43. Kaplan LD, Lee JY, Ambinder RF, Sparano JA, Cesarman E, Chadburn A et al. Rituximab does not improve clinical outcome in a randomized phase 3 trial of CHOP with or without rituximab in patients with HIV-associated non-Hodgkin lymphoma: AIDS-Malignancies Consortium Trial 010. Blood. 2005;106(5):1538-43.

44. Ribera JM, Oriol A, Morgades M, Gonzalez-Barca E, Miralles P, Lopez-Guillermo A et al. Safety and efficacy of cyclophosphamide, adriamycin, vincristine, prednisone and rituximab in patients with human immunodeficiency virus-associated diffuse large B-cell lymphoma: results of a phase II trial. Br J Haematol. 2008;140(4):411-9.

45. Boue F, Gabarre J, Gisselbrecht C, Reynes J, Cheret A, Bonnet F et al. Phase II trial of CHOP plus rituximab in patients with HIV-associated non-Hodgkin's lymphoma. J Clin Oncol. 2006;24(25):4123-8.

46. Spina M, Jaeger U, Sparano JA, Talamini R, Simonelli C, Michieli M et al. Rituximab plus infusional cyclophosphamide, doxorubicin, and etoposide in HIV-associated non-Hodgkin lymphoma: pooled results from 3 phase 2 trials. Blood. 2005;105(5):1891-7.

47. Sparano JA, Lee JY, Kaplan LD, Levine AM, Ramos JC, Ambinder RF et al. Rituximab plus concurrent infusional EPOCH chemotherapy is highly effective in HIV-associated B-cell non-Hodgkin lymphoma. Blood. 2010;115(15):3008-16.

48. Boue F, Gabarre J, Gisselbrecht C, Reynes J, Cheret A, Bonnet F et al. Phase II trial of CHOP plus rituximab in patients with HIV-associated non-Hodgkin's lymphoma. J Clin Oncol. 2006;24(25):4123-8.

49. Cortes J, Thomas D, Rios A, Koller C, O'Brien S, Jeha S et al. Hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone and highly active antiretroviral therapy for patients with acquired immunodeficiency syndrome-related Burkitt lymphoma/leukemia. Cancer. 2002;94(5):1492-9.

50. Thomas DA, Cortes J, O'Brien S, Pierce S, Faderl S, Albitar M et al. Hyper-CVAD program in Burkitt's-type adult acute lymphoblastic leukemia. J Clin Oncol. 1999;17(8):2461-70.

51. Bowman WP, Shuster JJ, Cook B, Griffin T, Behm F, Pullen J et al. Improved survival for children with B-cell acute lymphoblastic leukemia and stage IV small noncleaved-cell lymphoma: a pediatric oncology group study. J Clin Oncol. 1996;14(4):1252-61.

52. Oriol A, Ribera JM, Esteve J, Sanz MA, Brunet S, Garcia-Boyero R et al. Lack of influence of human immunodeficiency virus infection status in the response to therapy and survival of adult patients with mature B-cell lymphoma or leukemia. Results of the PETHEMA-LAL3/97 study. Haematologica. 2003;88(4):445-53.

53. Oriol A, Ribera JM, Brunet S, del Potro E, Abella E, Esteve J. Highly active antiretroviral therapy and outcome of AIDS-related Burkitt's lymphoma or leukemia. Results of the PETHEMA-LAL3/97 study. Haematologica. 2005;90(7):990-2.

54. Oriol A, Ribera JM, Bergua J, Gimenez Mesa E, Grande C, Esteve J et al. High-dose chemotherapy and immunotherapy in adult Burkitt lymphoma: comparison of results in human immunodeficiency virus-infected and noninfected patients. Cancer. 2008;113(1):117-25.

55. Wang ES, Straus DJ, Teruya-Feldstein J, Qin J, Portlock C, Moskowitz C et al. Intensive chemotherapy with cyclophosphamide, doxorubicin, high-dose methotrexate/ifosfamide, etoposide, and high-dose cytarabine (CODOX-M/IVAC) for human immunodeficiency virus-associated Burkitt lymphoma. Cancer. 2003;98(6):1196-205.

56. Noy A, Kaplan L, Lee J. Feasibility and toxicity of a modified dose intensive R-CODOX-M/IVAC for HIV-associated Burkitt and atypical Burkitt lymphoma (BL): preliminary results of a prospective multicenter phase II trial of the AIDS Malignancy Consortium (AMC). Blood. (ASH Annual Meeting Abstracts) 2009;114:3673.

57. Rodrigo JA, Hicks LK, Cheung MC, Song KW, Ezzat H, Leger CS et al. HIV-Associated Burkitt Lymphoma: Good Efficacy and Tolerance of Intensive Chemotherapy Including CODOX-M/IVAC with or without Rituximab in the HAART Era. Adv Hematol. 2012;2012:735392.

58. Montoto S, Wilson J, Shaw K, Heath M, Wilson A, McNamara C et al. Excellent immunological recovery following CODOX-M/IVAC, an effective intensive chemotherapy for HIV-associated Burkitt's lymphoma. AIDS. 2010;24(6):851-6.

59. Mead GM, Barrans SL, Qian W, Walewski J, Radford JA, Wolf M et al. A prospective clinicopathologic study of dose-modified CODOX-M/IVAC in patients with sporadic Burkitt lymphoma defined using cytogenetic and immunophenotypic criteria (MRC/NCRI LY10 trial). Blood. 2008;112(6):2248-60.

60. Barnes JA, Lacasce AS, Feng Y, Toomey CE, Neuberg D, Michaelson JS et al. Evaluation of the addition of rituximab to CODOX-M/IVAC for Burkitt's lymphoma: a retrospective analysis. Ann Oncol. 2011;22(8):1859-64.

61. Sparano JA, Wiernik PH, Strack M, Leaf A, Becker N, Valentine ES. Infusional cyclophosphamide, doxorubicin, and etoposide in human immunodeficiency virus- and human T-cell leukemia virus type I-related non-Hodgkin's lymphoma: a highly active regimen. Blood. 1993;81(10):2810-5.

62. Sparano JA, Lee S, Chen MG, Nazeer T, Einzig A, Ambinder RF et al. Phase II trial of infusional cyclophosphamide, doxorubicin, and etoposide in patients with HIV-associated non-Hodgkin's lymphoma: an Eastern Cooperative Oncology Group Trial (E1494). J Clin Oncol. 2004;22(8):1491-1500.

63. Spina M, Jaeger U, Sparano JA, Talamini R, Simonelli C, Michieli M et al. Rituximab plus infusional cyclophosphamide, doxorubicin, and etoposide in HIV-associated non-Hodgkin

lymphoma: pooled results from 3 phase 2 trials. Blood. 2005;105(5):1891-7.

64. Gutierrez M, Chabner BA, Pearson D, Steinberg SM, Jaffe ES, Cheson BD et al. Role of a doxorubicin-containing regimen in relapsed and resistant lymphomas: an 8-year follow-up study of EPOCH. J Clin Oncol. 2000;18(21):3633-42.

65. Little RF, Pittaluga S, Grant N, Steinberg SM, Kavlick MF, Mitsuya H et al. Highly effective treatment of acquired immunodeficiency syndrome-related lymphoma with dose-adjusted EPOCH: impact of antiretroviral therapy suspension and tumor biology. Blood. 2003;101(12):4653-9.

66. Dunleavy K, Little R, Pittaluga S, Grant N, Shovlin M, Steinberg S et al. A prospective study of dose-adjusted (DA) EPOCH with rituximab in adults with newly diagnosed Burkitt lymphoma: a regimen with high efficacy and low toxicity. Annals of Oncology. 2008;19(suppl.4):009.

67. Dunleavy K, Pittaluga S, Wayne S, Shovlin M, Johnson J, Little R et al. MYC+ aggressive lymphomas: novel therapy of untreated Burkitt lymphoma (BL)and MYC+ diffuse large B-cell lymphoma (DLBCL)with DA-EPOCH-R. Annals of Oncology. 2011;22:071.

68. Di Nicola M, Carlo-Stella C, Mariotti J, Devizzi L, Massimino M, Cabras A et al. High response rate and manageable toxicity with an intensive, short-term chemotherapy programme for Burkitt's lymphoma in adults. Br J Haematol. 2004;126(6):815-20.

69. Galicier L, Fieschi C, Borie R, Meignin V, Daniel MT, Gerard L et al. Intensive chemotherapy regimen (LMB86) for St Jude stage IV AIDS-related Burkitt lymphoma/leukemia: a prospective study. Blood. 2007;110(8):2846-54.

70. Ferreri AJ, Bruno Ventre M, Donadoni G, Cattaneo C, Fumagalli L, Foppoli M et al. Safety and activity of a new intensive short-term chemoimmunotherapy in HIV-positive patients with Burkitt lymphoma. Br J Haematol. 2012;159(2):252-5.

71. Balsalobre P, Diez-Martin JL, Re A, Michieli M, Ribera JM, Canals C et al. Autologous stem-cell transplantation in patients with HIV-related lymphoma. J Clin Oncol. 2009;27(13):2192-8.

72. Diez-Martin JL, Balsalobre P, Re A, Michieli M, Ribera JM, Canals C et al. Comparable survival between HIV+ and HIV- non-Hodgkin and Hodgkin lymphoma patients undergoing autologous peripheral blood stem cell transplantation. Blood. 2009;113(23):6011-4.

73. Krishnan A, Molina A, Zaia J, Smith D, Vasquez D, Kogut N et al. Durable remissions with autologous stem cell transplantation for high-risk HIV-associated lymphomas. Blood 2005;105(2):874-8.

74. Re A, Michieli M, Casari S, Allione B, Cattaneo C, Rupolo M et al. High-dose therapy and autologous peripheral blood stem cell transplantation as salvage treatment for AIDS-related lymphoma: long-term results of the Italian Cooperative Group on AIDS and Tumors (GICAT) study with analysis of prognostic factors. Blood. 2009;114(7):1306-13.

75. Bayraktar UD, Ramos JC, Petrich A, Gupta N, Lensing S, Moore PC et al. Outcome of patients with relapsed/refractory acquired immune deficiency syndrome-related lymphoma diagnosed 1999-2008 and treated with curative intent in the AIDS Malignancy Consortium. Leuk Lymphoma. 2012;53(12):2383-9.

76. Philip T, Guglielmi C, Hagenbeek A, Somers R, Van der Lelie H, Bron D et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med. 1995;333(23):1540-5.

77. Gabarre J, Marcelin AG, Azar N, Choquet S, Levy V, Levy Y et al. High-dose therapy plus autologous hematopoietic stem cell transplantation for human immunodeficiency virus (HIV)-related lymphoma: results and impact on HIV disease. Haematologica. 2004;89(9):1100-8.

78. Gupta V, Tomblyn M, Pedersen TL, Atkins HL, Battiwalla M, Gress RE et al. Allogeneic hematopoietic cell transplantation in human immunodeficiency virus-positive patients with hematologic disorders: a report from the center for international blood and marrow transplant research. Biol Blood Marrow Transplant. 2009;15(7):864-71.

79. Swerdlow SH, Campo E, Harris NL, Pileri S et al. WHO classification of tumors of Haematopoietic and Lymphoid Tissues. (2008), France: IARC Press.

80. Rodig SJ, Vergilio JA, Shahsafaei A, Dorfman DM. Characteristic expression patterns of TCL1, CD38, and CD44 identify aggressive lymphomas harboring a MYC translocation. Am J Surg Pathol. 2008;32(1):113-22.

81. Guikema JE, de Boer C, Haralambieva E, Smit LA, van Noesel CJ, Schuuring E et al. IGH switch breakpoints in Burkitt lymphoma: exclusive involvement of noncanonical class switch recombination. Genes Chromosomes Cancer. 2006;45(9):808-19.

82. Lovisa F, Mussolin L, Corral L, Pillon M, Cazzaniga G, Biondi A et al. IGH and IGK gene rearrangements as PCR targets for pediatric Burkitt's lymphoma and mature B-ALL MRD analysis. Lab Invest 2009;89(10):1182-6.

83. Moormeier JA, Williams SF, Golomb HM. The staging of non-Hodgkin's lymphomas. Semin Oncol. 1990;17(1):43-50.

84. Murphy SB. Classification, staging and end results of treatment of childhood non-Hodgkin's lymphomas: dissimilarities from lymphomas in adults. Semin Oncol. 1980;7(3):332-9.

85. Cheson BD. Role of functional imaging in the management of lymphoma. J Clin Oncol. 2011;29(14):1844-54.

86. Brust D, Polis M, Davey R, Hahn B, Bacharach S, Whatley M et al. Fluorodeoxyglucose imaging in healthy subjects with HIV infection: impact of disease stage and therapy on pattern of nodal activation. AIDS. 2006;20(7):985-93.

RNA INTERFERENCE (RNAi): AN EFFECTIVE WAY TO DEVELOP RATIONAL COMBINATION THERAPIES WITH HYPOMETHYLATING AGENTS IN ACUTE LEUKAEMIAS AND MYELODYSPLASTIC SYNDROME

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ABSTRACT

Therapeutic progress in aggressive myeloid malignancies such as acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS) and advanced myeloproliferative neoplasm (MPNs) has been slow. This is partially due to the heterogeneity of the diseases and the lack of molecular understanding, which delays effective drug development. There will be potentially three rather effective avenues to arrive at novel molecular vulnerabilities that can be therapeutically exploited. First, is identifying disease-specific mutations or other genomic aberrations (i.e. translocations, aberrant methylation) that will result in targeting affected and closely associated genes. Second, is tracing disease evolution over time, clonal evolution by various approaches, many of which will include current genomic tools and assays. Third, a more unbiased, broad discovery approach either with small molecule screens or, in our opinion, by identification of specific molecular vulnerabilities by RNA interference (RNAi). RNAi is a mechanistically rather agnostic high-throughput approach to find essential targets, alone or in combination with commonly used anti-cancer agents. In this paper we will briefly summarise some ideas and early results of RNAi screens, with a focus on hypomethylating agents and how RNAi can identify rational combination therapies with 5-azacytidine that can be rapidly translated into the clinical setting.

Keywords: Small interfering RNA, RNAi, AML, MDS, MPN, hypomethylating agents, 5-azacytidine, decitabine.

INTRODUCTION

The most common type of acute leukaemia in adults is acute myeloid leukaemia (AML), which is one of the malignancies with the highest mortality rate.[1] For most AML patients, treatment and outcome has not changed for decades; and especially for patients with relapsed and refractory disease, options are dismal. This is particularly true for the many elderly AML patients, that often do not qualify, or are poor candidates for cytotoxic chemotherapy, and for whom allogeneic transplantation therapeutic options are limited.[2] In addition, the biology of elderly AML is very distinct compared to younger patients (i.e. <40 years) and therefore the response rates are much less in elderly AML patients than for younger patients.[3] In contrast, multiple new therapies and agents have been developed for chronic leukaemias,[4] and outcomes for paediatric/young adult lymphoid leukaemias have remarkably improved.[5] Therefore, cytotoxic regimens do not benefit most elderly patients, and lower intensity therapies are similarly effective in AML patients with an age greater than 65-70 years.[6] The development and approval of hypomethylating agents and Janus kinase 2 (JAK2) inhibitors for myelodysplastic syndrome (MDS)[7] and myeloproliferative neoplasm (MPNs)[8] respectively, has improved the outcome for these myeloid malignancies. However, advanced aggressive forms of MDS have a low survival rate of 0.4-0.8 years, similar to AML.[9] Therefore, novel treatments and combinations are an urgent clinical need in relapsed

and refractory acute leukaemias as well as *de novo* treatment of AML, both for elderly AML patients and for aggressive forms of MDS and MPNs.[10]

The hypomethylating agents 5-azacytidine and decitabine have shown response and survival rates that are almost as good as cytotoxic chemotherapies in older patients with AML and MDS.[6] Still there remains a large number of patients in the range of 25-50% who do not respond to 5-azacytidine and decitabine, even in the upfront treatment. Ultimately, most patients become resistant and progress while being treated on 5-azacytidine.[11] Therefore, clinical-translational research goals are needed to improve upfront response rates to 5-azacytidine and decitabine and to overcome initial or developing resistance to hypomethylating agents. This article will provide an overview and examples of using RNA interference (RNAi) and give examples of practical translation of RNAi-derived genomic knowledge into clinical development.

In AML disease biology there is, to date, no single underlying genetic event that may be exploited as a sole drug target.[12] Cytotoxic combination regimens are still standard therapy options for patients curable by chemotherapy or those likely proceeding to allogeneic transplant. Nevertheless, cytotoxic regimens have reached their therapeutic limit[13] and new targeted agents directed at specific genomic aberrations will need to be developed in order to improve therapy outcomes while minimising side-effects. In AML, MDS and MPNs, genetic aberrations in myeloid transcription factors, mutations in kinases, and inactivation of 'growth controlling' genes such as tumour suppressor genes (i.e. p53 or apoptosis regulators[14]) act together in malignant transformation. Complementary structural molecular aberrations, such as mutations or translocations, co-occur together and operate on the basis of altered, epigenetic transcription. This is supported by the increasing number of mutations in 'epigenetic' genes.[14] Accordingly, single agent 5-azacytidine, decitabine or kinase inhibitors alone have limited single agent clinical activity. Therefore, several oncogenic aberrations should be inhibited simultaneously to achieve better clinical responses. To date however, the essential targets to interfere with, alone or in combination with hypomethyalting agents, are unknown.

5-Azacytidine Combinations

5-azacytidine and decitabine affects methylation, mostly by reducing global methylation, while differential methylation may occur at tumour suppressor (less methylation) versus on oncogenes (higher levels of methylation).[15] However, the true underlying mechanism of action of 5-azacytidine and decitabine is still poorly understood. Despite this undefined mechanism, 5-azacytidine and decitabine have been investigated clinically with a number of other drugs. Several trial reports combining hypomethylating agents with histone-deacetyltransferase (HDAC) inhibitors have been published, for example suberoylanilide hydroxamic acid (SAHA), valproic acid, or entinostat. However, response rates were only modestly improved in combination. The overall response rates (ORR) (often defined as complete remission (CR) or CR with incomplete count recovery (CRi), partial remission (PR), and sometimes stable disease (SD)) range from ~25 to ~50%[16-18] depending on the study, with possibly higher ORR in newly diagnosed untreated patients of up to 67% as reported in one publication.[19]

Another frequently studied combination is combined 5-azacytidine and lenalidomide. A rationale is provided based on clinical grounds, as both have single-agent activity in AML and MDS and rather few overlapping side-effects, except possibly myelosuppression. A consideration to their known mechanism is that 5-azacytidine affects cycling cells and lenalidomide inhibits cell cycle progression. Hence, the reported studies tested different schedules including sequentially versus concurrent dosing, as well as slightly different lenalidomide doses. Furthermore, the patient population in the reported studies varied, which may explain some of the differences in the reported responses. Generally lenalidomide at 10 mg was the best tolerated dose with full dose 75 mg/m^2 5-azacytidine; some studies escalated lenalidomide to 50 mg daily during the first cycles, which was generally found to be too toxic when combined with 5-azacytidine.[20] 5-azacytidine-lenalidomide combinations were tested in higher risk MDS and in AML patients. The ORR for most studies across MDS and AML are roughly in the range of ~41-75%.[20-23] The highest response rate was a 44% CR rate in one MDS study; however, the median survival of 13.6 months in that study was lower than the 24.5 months in Aza-001 study,[24] the landmark study that tested single-agent 5-azacyditinde in MDS. In untreated elderly AML, a similarly high CR/CRi rate of 44% (n=7/16) was observed, however this study was small.

Comparable CR/CRi rates for single agent 5-azacytidine and decitabine in AML are around ~17-

18% with an ORR of up to 71% and 48% respectively for MDS or AML.[24,25]

Limited clinical evidence that lenalidomide may truly sensitise to 5-azacytidine comes from observations of three patients: all three patients received the 5-azacytidine-lenalidomide combination and at some point were taken off lenalidomide and continued on single-agent 5-azacytidine. At disease progression, lenalidomide was again added to 5-azacytidine and all three patients responded, suggesting that lenalidomide may indeed clinically sensitise to 5-azacytidine.[26] However, no firm conclusions can be drawn from these observations in these three patients, and larger studies regarding the potential of combining 5-azacytidine with lenalidomide or HDAC inhibitors are needed. In fact, a randomised Phase II trial is ongoing to compare single agent 5-azacytidine versus combination treatment of 5-azacytidine and lenalidomide (10 mg) or 5-azacytidine and SAHA in intermediate and high risk MDS. Similar studies are ongoing in the US and in Europe.

Novel Hypomethylating Drugs

Few next-generation hypomethylating agents are under development; for example, the decitabine pro-drug SGI-110, which is a dinucleotide of decitabine and deoxyguanosine. SGI-110 is cleaved into decitabine *in vivo*. This pharmacokinetic change is expected to prolong decitabine exposure and thereby increasing the effect on deeper hypomethylation. Data from a Phase I study of AML and MDS patients treated with SGI-110 were presented at the American Society of Hematology (ASH) 2012,[27] with an update at the annual meeting of the European Hematology Association (EHA) 2013.[28] Results showed that SGI-110 has a longer *in vivo* exposure than decitabine and achieves deeper de-hypomethylation. Complete and incomplete responses (CR/CRi/CRp) were seen, and haematological improvements were also observed.[27] However, with the expected heterogeneity of a Phase I patient population, it is too early to estimate response rates in comparison to the currently approved and commercially available drugs. A Phase II expansion cohort for newly diagnosed AML and MDS patients is ongoing and accruing patients.

RNAi Approach to Rational Combinations with Hypomethylating Agents

One of the main obstacles to developing rational 5-azacytidine or decitabine combinations is a lack of understanding of their mechanistic underpinnings. A broader, high-throughput target approach using RNAi screening may be able to avoid some of these limitations. An RNAi approach is rather 'agnostic', mechanistically unbiased a priori, and mostly limited by how many genes and which small interfering RNA (siRNA) libraries are included in the initial RNAi screens.

In brief, RNAi is a naturally occurring phenomenon[29] in many eukaryotes, including humans, arising from short RNA molecules that complementary bind specific messenger RNA (mRNA) molecules transcribed in cells. Through various intra-cellular processes, ultimately mRNA is degraded and not translated into proteins, which amounts to silencing or inhibition of a specific gene. Experimentally this natural occurring phenomenon is exploited by the design of synthetic siRNA molecules.[30] Libraries covering each human gene have been synthesised and are commercially available. These short RNAi molecules are transfected into leukaemia cells *in vitro* under concurrent treatment with, for example, 5-azacytidine.[31,32] With this approach, hundreds to thousands of genes can be inhibited individually at the same time and assessed if their inhibition augments the anti-leukaemic activity of anti-cancer drugs. A detailed description of the siRNA platform that our laboratory uses was recently published for a Cytarabine siRNA kinome screen.[33]

Up to now, only few large RNAi screens have been presented in leukaemia cells. Many of these tested inhibition of genes alone without addition of a drug. These screens can identify synthetic lethal interactions when a specific gene is inhibited.[34] One of the first siRNA screens assessed the tyrosine kinome and few other selected genes (altogether >100 genes) in AML cells.[35] Specific kinases were found that inhibited leukaemia cell growth. The same group extended their findings experimentally into patients to attempt to find functional relevant targets in patients' leukaemia cells.[36] Another landmark study utilised an shRNA (viral/vector based RNAi molecules) approach to assess 40-45 genes situated on the commonly deleted regions (CDA) of chromosome 5 in MDS. The gene RPS14 was found as a key regulator of disturbed erythropoiesis in 5q deleted MDS.[37] This study inspired the RNAi translational research field and contributed to understanding the so-called 5q MDS Syndrome, although it has not led to concrete therapeutic approaches.

Only few RNAi sensitiser screens, i.e. treating leukaemia cells with a drug and inhibiting many genes with the siRNA library, have been conducted.

An all trans-retinoic acid (ATRA) sensitiser screen has been published,[38] without translational application to date.

So far, our laboratory has presented the only high-throughput 5-azacytidine sensitiser screen in leukaemias.[39] We tested 861 genes individually, including the entire human kinome (that is, all known kinase genes) to determine if any of these genes, when inhibited by siRNA, would enhance the activity of 5-azacytidine. Surprisingly, kinases did not potentiate the activity of 5-azacytidine. However, in these screens we found the B-cell lymphoma 2 (BCL-2) family proteins as important sensitisers to 5-azacytidine. We subsequently used drugs in a clinical development that targeted B-cell lymphoma-extra-large (BCL-XL)[40] or BCL-2 respectively[41,42] and demonstrated that ABT-737 is synergistic with 5-azacytidine *in vitro* as well as in short term culture of samples from AML, MDS and MPN patients. While targeting BCL-XL or other anti-apoptotic molecules may be an applicable concept for cytotoxic therapies, the selectivity of sensitisation and lack of kinase targets identified in our assays from a large gene set, has important clinical implications for the selection of an agent to be combined with 5-azacytidine in clinical trials.

Recently, we conducted a similar siRNA screen of 289 genes on CDR of chromosome 5 and 7 in combination with 5-azacytidine, and identified a pathway that is potentially targetable, with validation studies currently ongoing. A clinical trial with yet another novel drug is in development based on the RNAi results (R. Tibes, personal communication).

These examples highlight an exciting potential of RNAi screens to yield high-profile targets that can be further evaluated as to their potential value for clinical translation into novel combinations. Given the high-throughput nature of RNAi, more targets are discovered than drugs are available and the rate-limiting step of translating RNAi findings into the clinic is the access and availability of drugs that correspond to the identified targets. Consequently, RNAi screens are increasingly conducted with parallel small molecule compound screens to increase the chances of discovering novel agents for clinical application.

One such example is the identification of BRD4 as a vulnerable gene in leukaemia cells. BRD4 regulates Myc expression via epigenetic mechanism.[43,44] Subsequently the agent JQ1 was discovered inhibiting BRD4 and thus epigenetic regulation of Myc.

Finally, RNAi screens can be used in an isogenic cell line or mutation context, i.e. a recent paper used FLT-3 mutated cells and performed a whole genome RNAi screen to find synthetic lethal targets in a FLT-3 mutational background and identify pathway concepts.[45]

CONCLUSION

In conclusion, the treatment and outcome of patients with advanced stages of AML, MDS and MPNs needs to be improved and new rational combination regimens developed. For drugs with a yet unclear mechanism of action (i.e. hypomethylating agents) and drugs that act rather non-specific, i.e. cytoroxics, an RNAi approach offers an economical, rapid way to discover genes that may modify the activity of known cancer drugs like 5-azacytidine. Novel molecular interactions can be identified, and if agents that target the respective genes hit from RNAi screens are available, these can be evaluated rapidly as to their potential for translation into rational combination clinical studies.

REFERENCES

1. Ferrara F, Schiffer CA. Acute myeloid leukaemia in adults. Lancet. 2013;381:484-95.

2. Pollyea DA, Kohrt HE, Medeiros BC. Acute myeloid leukaemia in the elderly: a review. Br J Haematol. 2011;152:524-42.

3. Yanada M, Naoe T. Acute myeloid leukemia in older adults. Int J Hematol. 2012;96:186-93.

4. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2012 update on diagnosis, monitoring, and management. Am J Hematol. 2012;87:1037-45.

5. Ram R, Wolach O, Vidal L, Gafter-Gvili A, Shpilberg O, Raanani P. Adolescents and young adults with acute lymphoblastic leukemia have a better outcome when treated with pediatric-inspired regimens: systematic review and meta-analysis. Am J Hematol. 2012;87:472-8.

6. Quintas-Cardama A, Ravandi F, Liu-Dumlao T et al. Epigenetic therapy is associated with similar survival compared with intensive chemotherapy in older patients with newly diagnosed acute myeloid leukemia. Blood. 2012;120:4840-5.

7. Garcia-Manero G. Myelodysplastic syndromes: 2012 update on diagnosis, risk-stratification, and management. Am J Hematol. 2012;87:692-701.

8. Tibes R, Bogenberger JM, Geyer HL, Mesa RA. JAK2 inhibitors in the treatment of myeloproliferative neoplasms. Expert Opin Investig Drugs. 2012;21:1755-74.

9. Greenberg PL, Tuechler H, Schanz J et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120:2454-65.

10. Tibes R, Bogenberger JM, Mesa RA. JAK inhibition: the key to treating

myeloproliferative neoplasm? Expert Rev Hematol. 2012;5:583-5.

11. Prebet T, Gore SD, Esterni B, et al. Outcome of high-risk myelodysplastic syndrome after azacitidine treatment failure. J Clin Oncol. 2011;29:3322-7.

12. Marcucci G, Haferlach T, Dohner H. Molecular genetics of adult acute myeloid leukemia: prognostic and therapeutic implications. J Clin Oncol. 2011;29:475-86.

13. Ziogas DC, Voulgarelis M, Zintzaras E. A network meta-analysis of randomized controlled trials of induction treatments in acute myeloid leukemia in the elderly. Clin Ther. 2011;33:254-79.

14. Ley TJ, Miller C, Ding L, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368:2059-74

15. Ghoshal K, Bai S. DNA methyltransferases as targets for cancer therapy. Drugs Today (Barc). 2007;43:395-422.

16. Soriano AO, Yang H, Faderl S, et al. Safety and clinical activity of the combination of 5-azacytidine, valproic acid, and all-trans retinoic acid in acute myeloid leukemia and myelodysplastic syndrome. Blood. 2007;110:2302-8.

17. Garcia-Manero G, Kantarjian HM, Sanchez-Gonzalez B, et al. Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia. Blood. 2006;108:3271-9.

18. Blum W, Klisovic RB, Hackanson B, et al. Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia. J Clin Oncol. 2007;25:3884-91.

19. Kuendgen A, Bug G, Ottmann OG, et al. Treatment of poor-risk myelodysplastic syndromes and acute myeloid leukemia with a combination of 5-azacytidine and valproic acid. Clin Epigenetics. 2011;2:389-99.

20. Ramsingh G, Westervelt P, Cashen AF, et al. A phase 1 study of concomitant high-dose lenalidomide and 5-azacitidine induction in the treatment of AML. Leukemia. 2013;27:725-8.

21. Sekeres MA, List AF, Cuthbertson D, et al. Phase I combination trial of lenalidomide and azacitidine in patients with higher-risk myelodysplastic syndromes. J Clin Oncol. 2010;28:2253-8.

22. Sekeres MA, Tiu RV, Komrokji R, et al. Phase 2 study of the lenalidomide and azacitidine combination in patients with higher-risk myelodysplastic syndromes. Blood. 2012;120:4945-51.

23. Pollyea DA, Kohrt HE, Gallegos L, et al. Safety, efficacy and biological predictors of response to sequential azacitidine and lenalidomide for elderly patients with acute myeloid leukemia. Leukemia. 2012;26:893-901.

24. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. J Clin Oncol. 2010;28:562-9.

25. Kantarjian HM, Thomas XG, Dmoszynska A, et al. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. J Clin Oncol. 2012;30:2670-7.

26. Sekeres MA, O'Keefe C, List AF, et al. Demonstration of additional benefit in adding lenalidomide to azacitidine in patients with higher-risk myelodysplastic syndromes. Am J Hematol. 2011;86:102-3.

27. Kantarjian HM, Roboz GJ, Rizzieri DA, et al. Results From the Dose Escalation Phase of a Randomized Phase 1-2 First-in-Human (FIH) Study of SGI-110, a Novel Low Volume Stable Subcutaneous (SQ) Second Generation Hypomethylating Agent (HMA) in Patients with Relapsed/Refractory MDS and AML. ASH Annual Meeting Abstracts. 2012;120:414.

28. O'Connell C, Tibes R, Walsh K, et al. Outcomes of Intermediate or High Risk Myelodysplastic Syndromes (MDS) Patients Post Azacitidine and/or Decitabine Treatment Failures with SGI-110, A novel second Generation Hypomethylating Agent (HMA). European Hematology Association Meeting; 2013.

29. Hannon GJ, Rossi JJ. Unlocking the potential of the human genome with RNA interference. Nature. 2004;431:371-8.

30. Dorsett Y, Tuschl T. siRNAs: applications in functional genomics and potential as therapeutics. Nat Rev Drug Discov. 2004;3:318-29.

31. Tibes R, Bogenberger JM, Hagelstrom RT, Buechel ME, Azorsa DO. Abstract A202: RNAi as a tool to identify novel molecular vulnerabilities in myeloid leukemias. Molecular Cancer Therapeutics. 2009;8:15-19.

32. Bogenberger JM, Xin Shi C, Hagelstrom T, et al. Late-Breaking Poster Presentations - Functional Genomics. Abstract LB-128: Synthetic lethal RNAi screening identifies inhibition of Bcl-2 family members as sensitizers to 5-azacytidine in myeloid cells. Cancer Research. 2010;70:doi:10.1158/1538-7445.AM10-LB-128.

33. Tibes R, Bogenberger JM, Chaudhuri L, et al. RNAi screening of the kinome with cytarabine in leukemias. Blood. 2012;119:2863-72.

34. Chan DA, Giaccia AJ. Harnessing synthetic lethal interactions in anticancer drug discovery. Nat Rev Drug Discov. 2011;10:351-64.

35. Tyner JW, Walters DK, Willis SG, et al. RNAi screening of the tyrosine kinome identifies therapeutic targets in acute myeloid leukemia. Blood. 2008;111:2238-45.

36. Tyner JW, Deininger MW, Loriaux MM, et al. RNAi screen for rapid therapeutic target identification in leukemia patients. Proc Natl Acad Sci USA. 2009;106:8695-700.

37. Ebert BL, Pretz J, Bosco J, et al. Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. Nature. 2008;451:335-9.

38. Hattori H, Zhang X, Jia Y, et al. RNAi screen identifies UBE2D3 as a mediator of all-trans retinoic acid-induced cell growth arrest in human acute promyelocytic NB4 cells. Blood. 2007;110:640-50.

39. Bogenberger JM, Shi C-X, Gonzales I et al. RNAi Screening Identifies BCL-XL As An Erythroid Lineage-Specific 5-azacytidine Sensitizer While the BCL-2/BCL-XL/BCL-W Inhibitor ABT-737 Results in More Universal Sensitization in Leukemia Cells. ASH Annual Meeting Abstracts. 2011;118:3513.

40. Oltersdorf T, Elmore SW, Shoemaker AR, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature. 2005;435:677-81.

41. Souers AJ, Leverson JD, Boghaert ER, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013;19:202-8.

42. Roberts AW, Seymour JF, Brown JR, et al. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. J Clin Oncol. 2012;30:488-96.

43. Filippakopoulos P, Qi J, Picaud S, et al. Selective inhibition of BET bromodomains. Nature. 2010;468:1067-73.

44. Zuber J, Shi J, Wang E, et al. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature. 2011;478:524-8.

45. Caldarelli A, Muller JP, Paskowski-Rogacz M, et al. A genome-wide RNAi screen identifies proteins modulating aberrant FLT3-ITD signaling. Leukemia. 2013;doi:10.1038/leu.2013.83.[Epub ahead of print].

PREVENTING INFECTIONS IN HIGHER-RISK MYELODYSPLASTIC SYNDROME PATIENTS TREATED WITH HYPOMETHYLATION AGENTS

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ABSTRACT

Hypomethylation agents became the standard of care for patients with high-risk myelodysplastic syndrome (MDS). While long-term benefits of azacitidine (AZA) and decitabine (DEC) were demonstrated in multiple studies, methods to enhance patients' safety during therapy with those agents are pending. The causative correlations between drug administration and non-life threatening complications such as injection-site erythema or gastrointestinal (GI) discomfort are obvious. However, infections, which are the most common life-threatening complication among higher risk MDS patients, are frequent even in those receiving therapy other than hypomethylation agents, including supportive care solely. Therefore, the contribution of hypomethylation therapy to infection risk is difficult to determine. Herein, data regarding infectious complications, their prevalence, risk stratification, and methods of prevention will be reviewed.

Keywords: Myelodysplastic syndrome, azacitidine, infections, prophylaxis.

INTRODUCTION

Myelodysplastic syndrome (MDS) is often referred to as a preleukaemic condition. However, only in a minority of patients, MDS transforms into leukaemia. The natural history of MDS is best described by observational studies conducted prior to the introduction of hypomethylation agents. Indeed, among patients in whom causes of death were recorded, infection or bleeding resulting from bone marrow failure were reported to be the main causes of deaths, while leukaemic transformation was much less prevalent.[1,2] In the era of hypomethylation therapy, most patients with higher risk MDS receive prolonged therapy. 3 to 6 or even 12 cycles of therapy with azacitidine (AZA) or decitabine (DEC), are required to achieve the maximal beneficial effect.[3-6] Ensuring patients' safety during this prolonged therapy is essential. Therefore, development of protocols aiming to prevent potential serious infections and other complications while awaiting clinical response is desired. Although no prospective randomised trials have been conducted and no guidelines are available,[7] the data which are accumulating are enabling the identification of patients with the greatest risk for infection, and evaluating the efficacy of different potential methods for infection prevention.

HYPOMETHYLATION EFFECT AND INFECTION

Therapies available for higher risk MDS patients can be divided into disease modifying and palliative. Currently, apart from intensive chemotherapies and allogeneic stem cell transplantation (Allo-SCT), which are beyond the scope of this review, the disease modifiers that are widely in use in MDS are AZA and DEC. The most hazardous side effect of hypomethylation agents is their effect on haematopoiesis. Pancytopaenia, including neutropaenia, is often worsened following AZA or DEC initiation. In major prospective trials, the prevalence of neutropaenia increased from 76% to 91% in AZA-treated patients[8] and from 35% to 47% in patients receiving DEC.[9] Surprisingly, most studies

reported that the infection rate in patients treated with hypomethylation agents, although prominent, was not higher than the parallel prevalence in the untreated higher risk MDS patient population.[8-12] Yet, these statements describe the total infection rate throughout the whole follow-up period and should be further dissected. It is imperative to take into account that the timeline of infectious events differs between groups. Higher risk MDS patients, receiving none or palliative therapy, deteriorate over time and infections are more customary later in the disease course. In contrast, most patients on hypomethylation agents experience a transient decrease in blood counts following the first cycles of therapy, which may improve over time. Multiple studies confirmed that, while on hypomethylation therapy, infection risk is very high during the first two-three cycles of therapy and it substantially decreases thereafter.[13-15] Thus, although hypomethylation agents are not associated with an increase in total infection burden, during palliative therapy infections are often associated with progression of the underlying disease, while during hypomethylation therapy infections occur earlier and should be considered drug-related. Multiple mechanisms may be accountable for immune suppression during hypomethylation therapy. One mechanism is related to the decrease in neutrophil count yet, hypomethylation leads to changes in expression levels of genes that may alter immune function. The immunomodulating effect of hypomethylation agent was demonstrated both in mice[16] and in humans,[17] and an increase in T-cell regulatory activity was reported. DEC was shown to facilitate immunosuppression in the context of innate immune response.[18] Overall, hypomethylation therapy triggers complicated processes and its effect on the immune system is behind its direct effect on neutrophil count.

Identifying MDS Patients with the Highest Risk for Infection

Most studies reporting the outcome of AZA and DEC therapy elaborated on drug efficacy and their haematological effects in various patient populations, but only briefly described infectious complications. Even the response criteria in myelodysplasia, issued in the year 2000 and revised in 2006,[19,20] includes just a general statement that a neutrophil level lower than 1,000x10^9/L may serve as an acceptable cut-off for infection risk. This cut-off was suggested based on acceptable discrimination in leukaemia patients and not on solid evidence obtained in MDS patients. The first work to identify risk factors for infection during AZA therapy was a retrospective Israeli survey with a high national coverage. It included 97% of all higher risk MDS/acute myeloid leukaemia (AML) patients treated with AZA in Israel during a 3-year period.[15] Data of 928 treatment cycles prescribed to 184 patients were recorded. Infection rate was 16.5%, three-quarters of events required hospitalisation, and about one-fifth were fatal. In multivariate analysis, only low haemoglobin level, low platelet count, and unfavourable cytogenetics were found to predict infection. Prior to each cycle, poor cytogenetics and a platelet count below 20x10^9/L are most predictive of infection development. Although a neutrophil count below 500x10^9/L is also associated with infection, some patients may experience multiple infections even if their neutrophil counts are normal, while others live well suffering from no infections despite a prolonged neutropaenia. Infection risk is likely to be related to the reserves of the bone marrow (BM) and its ability to respond to early signs of microorganism invasion, which does not always correlate with peripheral neutrophil count. Poor cytogenetics and low platelet count may be associated with poorer BM reserves. It was reported that favourable cytogenetics and a rise in thrombocyte count during AZA therapy predict a good haematological response and a longer survival.[21] This is the other side of the same coin. Cytogenetics and platelet counts represent the BM potential for better (response) or for worse (infection).

Definition of Infection and Common Causative Germs

Studies in MDS patients reported different incidence of infection and a wide spectrum of outcomes. This may be explained by variation in MDS severity among participants in different studies and by diverse criteria of infection recognition. Table 1 summarises the studies reporting infection incidence, while most of them ignored the infection outcome. In addition, although many of the patients who progressed to AML during therapy succumbed to infection, it is difficult to reveal in some studies whether those patients were considered among infection-complicated patients or not. Data regarding types of microorganisms and syndromes affecting higher risk MDS patient during hypomethylation therapy are scarce. Available information suggests that bacterial infections are responsible for the vast majority of infectious events during hypomethylation therapy.[15] Clearly, MDS patients are immunosuppressed and prone to various types of opportunistic infections. Invasive fungal infections also deserve attention,

Table 1. Infection incidence during hypomethylation therapy.

Type of Study	Number of Participants	AZA or DEC	Incidence	Outcome (Death Rate)	Reference
Prospective	99	AZA	20%	NR	(34)
Prospective	179	AZA	0.6 per patient-year	NR	(8)
Prospective	66	DEC	27%	7%	(11)
Prospective	89	DEC	28%	NR	(35)
Prospective	95	DEC	1%	NR	(27)
Retrospective	38	AZA	29%	18%	(36)
Retrospective	184	AZA	16.5%	20%	(15)

yet, information regarding their incidence is scarce and this issue requires a well-designed, prospective follow-up study, outfitted with appropriate CT scans and galactomannan monitoring, similar to those performed in AML or post Allo-SCT patients.

Infection prevention should be focused on the most common and/or dangerous germs. Tailoring infection prevention methods requires a clear recognition of the nature of infectious events. In higher risk MDS patients treated with hypomethylation agents, risk factors (poor cytogenetics and low thrombocyte prior to therapy), the most vulnerable period of time (first two or three cycles), and the commonest infectious germs (common bacteria) should be the basic parameters to be taken into account while developing prophylaxis protocols.

Methods for Infection Prevention During Hypomethylation Therapy

General prevention methods

Higher risk MDS patients are prone to infection due to multiple defects in the immune system function. Not only are many of the patients neutropaenic, but defects in the normal function of neutrophils, B, T and NK cells, and iron overload, if present, may all alter response to microorganism invasion.[7] It is therefore highly important to educate patients and their families on standard precautions according to the customary local protocol. Hand hygiene, avoidance of close contact with people suffering from contagious diseases, and vaccination of family members should be encouraged. Patients should be urged to immediately contact their primary treating physician or local health care facilities in case of fever or early signs of infection, using an efficient communication channel.

Growth factors (G-CSF or GM-CSF)

The rationale for using myeloid growth factor in higher risk MDS patients is based on extrapolation of data obtained from other neutropaenic settings, which are often related to chemotherapy. There are no studies evaluating the potential benefits of simultaneous usage of hypomethylation agent and myeloid growth factors. The only data available are derived from studies in higher risk MDS patients treated with chemotherapy[22,23] or from observational studies;[24] none of them demonstrated a survival benefit. Thus, the major drawback from the usage of G-CSF or GM-CSF, in the context of MDS, is lack of evidence for its effectiveness. Physicians commonly express fear from facilitating leukaemic transformation by growth factors. However, such apprehension is also not supported by evidences. Moreover, safety of G-CSF or GM-CSF usage in AML was demonstrated by 18 controlled studies.[25] Until the firm benefits of reducing infection and hospitalisation or prolongation of survival can be demonstrated, the rise in neutrophil counts, resulting from growth factors administration, does not necessarily argue for its routine concomitant prophylaxis administration during hypomethylation therapy.

Antibiotic prophylaxis

Routine prophylaxis application of antibiotics in neutropaenic patients is debatable. Knowledge of the epidemiology and prevalence of different microorganisms generating infections in higher risk MDS patients is essential for determination of a preferred antibiotic prophylaxis protocol. Microbiology data is scarcely available, and in many studies patient microbiological evaluation was incomprehensive. With this limitation in mind, existing data suggest that bacterial infections are

most prevalent and responsible for the majority of infection-related deaths of higher risk MDS patients.[7,15] Usage of anti-mould or anti-viral agents cannot be routinely recommended outside of clinical trials but antibacterial prophylaxis may be justified.

Currently, fluoroquinolone prophylaxis is recommended for a limited group of cancer patients who become neutropaenic after chemotherapy. Duration and profoundness of neutropaenia are the main parameters used to justify antibiotic prophylaxis in these patients.[26,27] However, as discussed above, in higher risk MDS patients, neutropaenia is not the most powerful factor associated with infection risk. Yet, in a small retrospective study of 28 patients receiving DEC, prophylaxis with antibiotics and G-CSF was reported to decrease the rate of infections during therapy.[28] However, not only is this study small and retrospective, but even the antibiotic protocols vary among patients within this trial. Notably, the likelihood for emergence of resistant bacteria in patients' flora and within the institution environment increases with prolonged antibiotic prophylaxis. Thus, the use of prophylactic antibiotic protocols during prolonged hypomethylation therapy should be targeting local microbiological flora, restricted to the most fragile patients throughout the highest risk periods only.

Hypomethylation agent dose

Possible correlations between AZA or DEC doses and infection rates are difficult to reveal. In our large retrospective study, we reported that a reduced dose of 75 mg/m^2 for 5 days was prescribed in about one-third of the reported 928 AZA cycles. A history of previous infection, non-haematological co-morbidities, and advanced age are among the reasons considered by doctors when AZA therapy is decided upon. To untangle the potential connection between a previous infection and the following AZA dosage which may alter the ability to evaluate the dose effect of hypomethylation agents on infection risk, we limited the analysis to the data of the initial AZA cycle prescribed to our group of 184 patients. Interestingly, even though the dominant specific factor that drove physicians to decrease the AZA dose was not identified, characteristics of the patients receiving a full AZA dose of 75 mg/m^2 for 7 compared to 5 day cycles were similar; lowering the AZA dose significantly reduced infection risk.[29]

PRACTICAL RECOMMENDATIONS

Hypomethylation agents are spreading rapidly as the treatment of choice for higher-risk MDS patients. Infections are the leading cause of early mortality during therapy and therefore the importance of infection prevention could not be underestimated. In the absence of solid evidence, practical prophylaxis policy should rely on the following principles. First, prospective studies of prophylaxis protocols should be encouraged. Endeavours should be focused on patients with the highest risk for infection, such as those who present with low platelet counts and poor cytogenetics, especially during the first two cycles of hypomethylation therapy. Outside of clinical trials, all patients and families should receive detailed and comprehensive instructions regarding general prevention methods (e.g. hand hygiene, vaccines, isolation policy, etc). Patients should be monitored for early signs of infections and evaluated for comorbidities that may aggravate the infection risk (e.g. chronic lung disease, diabetes, peripheral vascular disease). Advanced age and a moderate elevation in creatinine level do not increase the risk for infection and do not justify prophylaxis or reduction of hypomethylation agent dose. Prophylaxis is advised for patients presenting with poor cytogenetics or with a platelet count lower than 20,000 cell/mcl prior to AZA administration, especially during the first two cycles of therapy. Fluoroquinolones, G-CSF and even a decrease in AZA dose may lower the infection rate.

Since no studies comparing the efficiency of these methods are available, recommendations are based on speculative estimation of benefits and adverse effects. Many physicians hesitate to use G-CSF in higher risk MDS patients due to its potential effect on blast proliferation, although even in leukaemic patients, G-CSF administration did not increase relapse rates.[30] The issue of whether reducing hypomethylation dose may alter the drug efficacy is still debatable,[31,32] and in a large study aiming for the establishment of AZA response predicting score, both reduced and standard AZA doses yielded identical response rates (41% versus 44%).[33] Yet, the initial two cycles of low-dose hypomethylation agents followed by full-dose cycles in patients recognised as prone to infection is a protocol that should be prospectively evaluated. Currently, it is likely that a time-limited antibiotic usage, restricted to patients at high risk, confined to the first two cycles of therapy, may lead to the highest benefits at the lower adverse cost.

REFERENCES

1. Sanz GF, Sanz MA, Vallespi T, Canizo MC, Torrabadella M, Garcia S, et al. Two regression models and a scoring system for predicting survival and planning treatment in myelodysplastic syndromes: a multivariate analysis of prognostic factors in 370 patients. Blood. 1989 Jul;74(1):395-408.

2. Malcovati L, Porta MG, Pascutto C, Invernizzi R, Boni M, Travaglino E, et al. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol. 2005 Oct 20;23(30):7594-603.

3. Silverman LR, Fenaux P, Mufti GJ, Santini V, Hellstrom-Lindberg E, Gattermann N, et al. Continued azacitidine therapy beyond time of first response improves quality of response in patients with higher-risk myelodysplastic syndromes. Cancer. 2011 Jun 15;117(12):2697-702.

4. Oki Y, Kondo Y, Yamamoto K, Ogura M, Kasai M, Kobayashi Y, et al. Phase I/II study of decitabine in patients with myelodysplastic syndrome: a multi-center study in Japan. Cancer Sci. 2012 Oct;103(10):1839-47.

5. Kantarjian HM, Thomas XG, Dmoszynska A, Wierzbowska A, Mazur G, Mayer J, et al. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. J Clin Oncol. 2012 Jul 20;30(21):2670-7.

6. Kornblith AB, Herndon JE, 2nd, Silverman LR, Demakos EP, Odchimar-Reissig R, Holland JF, et al. Impact of azacytidine on the quality of life of patients with myelodysplastic syndrome treated in a randomized phase III trial: a Cancer and Leukemia Group B study. J Clin Oncol. 2002 May 15;20(10):2441-52.

7. Toma A, Fenaux P, Dreyfus F, Cordonnier C. Infections in myelodysplastic syndromes. Haematologica. 2012 Oct;97(10):1459-70.

8. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009 Mar;10(3):223-32.

9. Lubbert M, Suciu S, Baila L, Ruter BH, Platzbecker U, Giagounidis A, et al. Low-dose decitabine versus best supportive care in elderly patients with intermediate- or high-risk myelodysplastic syndrome (MDS) ineligible for intensive chemotherapy: final results of the randomized phase III study of the European Organisation for Research and Treatment of Cancer Leukemia Group and the German MDS Study Group. J Clin Oncol. 2011 May 20;29(15):1987-96.

10. Silverman LR, McKenzie DR, Peterson BL, Holland JF, Backstrom JT, Beach CL, et al. Further analysis of trials with azacitidine in patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the Cancer and Leukemia Group B. J Clin Oncol. 2006 Aug 20;24(24):3895-903.

11. Wijermans P, Lubbert M, Verhoef G, Bosly A, Ravoet C, Andre M, et al. Low-dose 5-aza-2'-deoxycytidine, a DNA hypomethylating agent, for the treatment of high-risk myelodysplastic syndrome: a multicenter phase II study in elderly patients. J Clin Oncol. 2000 Mar;18(5):956-62.

12. Maurillo L, Venditti A, Spagnoli A, Gaidano G, Ferrero D, Oliva E, et al. Azacitidine for the treatment of patients with acute myeloid leukemia: report of 82 patients enrolled in an Italian Compassionate Program. Cancer. 2012 Feb 15;118(4):1014-22.

13. Lee JH, Jang JH, Park J, Park S, Joo YD, Kim YK, et al. A prospective multicenter observational study of decitabine treatment in Korean patients with myelodysplastic syndrome. Haematologica. 2011 Oct;96(10):1441-7.

14. Santini V, Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Silverman LR, List A, et al. Management and supportive care measures for adverse events in patients with myelodysplastic syndromes treated with azacitidine*. Eur J Haematol. 2010 Aug;85(2):130-8.

15. Merkel D, Filanovsky K, Gafter-Gvili A, Vidal L, Aviv A, Gatt ME, et al. Predicting infections in high-risk patients with myelodysplastic syndrome/acute myeloid leukemia treated with azacitidine: a retrospective multicenter study. Am J Hematol. 2013 Feb;88(2):130-4.

16. Jaebok Choi, Julie Ritchey, Julie L. Prior, Matthew Holt, William D. Shannon, Elena Deych, et al. *In vivo* administration of hypomethylating agents mitigate graft-versus-host disease without sacrificing graft-versus-leukemia. Blood. 2010;116(1):129-39.

17. Oliver C. Goodyear, Mike Dennis, Nadira Y. Jilani, Justin Loke, Shamyla Siddique, Gordon Rya et al. Azacitidine augments expansion of regulatory T cells after allogeneic stem cell transplantation in patients with acute myeloid leukemia (AML). Blood. 2012;119(14):3361-9.

18. Y Hu, Q Cui, Y Gu, L Sheng, K Wu, J Shi, et al. Decitabine facilitates the generation and immunosuppressive function of regulatory γδT cells derived from human peripheral blood mononuclear cells. Leukemia. 2013 Jul;27(7):1580-5.

19. Cheson BD, Bennett JM, Kantarjian H, Pinto A, Schiffer CA, Nimer SD, et al. Report of an international working group to standardize response criteria for myelodysplastic syndromes. Blood. 2000 Dec 1;96(12):3671-4.

20. Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood. 2006 Jul 15;108(2):419-25.

21. van der Helm LH, Alhan C, Wijermans PW, van Marwijk Kooy M, Schaafsma R, Biemond BJ, et al. Platelet doubling after the first azacitidine cycle is a promising predictor for response in myelodysplastic syndromes (MDS), chronic myelomonocytic leukaemia (CMML) and acute myeloid leukaemia (AML) patients in the Dutch azacitidine compassionate named patient programme. Br J Haematol. 2011 Dec;155(5):599-606.

22. Zwierzina H, Suciu S, Loeffler-Ragg J, Neuwirtova R, Fenaux P, Beksac M, et al. Low-dose cytosine arabinoside (LD-AraC) vs LD-AraC plus granulocyte/macrophage colony stimulating factor vs LD-AraC plus Interleukin-3 for myelodysplastic syndrome patients with a high risk of developing acute leukemia: final results of a randomized phase III study (06903) of the EORTC Leukemia Cooperative Group. Leukemia. 2005 Nov;19(11):1929-33.

23. Ossenkoppele GJ, van der Holt B, Verhoef GE, Daenen SM, Verdonck LF, Sonneveld P, et al. A randomized study of granulocyte colony-stimulating factor applied during and after chemotherapy in patients with poor risk myelodysplastic syndromes: a report from the HOVON Cooperative Group. Dutch-Belgian Hemato-Oncology Cooperative Group. Leukemia. 1999 Aug;13(8):1207-13.

24. Greenberg P, Taylor K, Larson R. Phase III randomized multicenter trial of recombinant human G-CSF in MDS. Blood. 1993;82((11)[suppl 1]):196a.

25. Jacob M Rowe. Interpreting Outcome Data in Hematological Malignancies: A Paradigm for Clinical Studies. RMMJ. 2013;4(1):e0004.

26. Gafter-Gvili A, Fraser A, Paul M, Leibovici L. Meta-analysis: antibiotic prophylaxis reduces mortality in neutropenic patients. Ann Intern Med.

2005 Jun 21;142(12 Pt 1):979-95.

27. Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2011 Feb 15;52(4):427-31.

28. Lee JH, Lee KH, Kim DY, Kim SH, Lim SN, Kim SD, et al. Decreased incidence of febrile episodes with antibiotic prophylaxis in the treatment of decitabine for myelodysplastic syndrome. Leuk Res. 2011 Apr;35(4):499-503.

29. Ofran Y, Filanovsky K, Gafter-Gvili A, Vidal L, Aviv A, Gatt ME, et al. P-287 Infectious events are much more prevalent following a seven compared to five days cycle of azacitidine regardless of patient's age. Leukemia Research. 2013;37(1):S151-S152.

30. Rowe JM, Neuberg D, Friedenberg W, Bennett JM, Paietta E, Makary AZ, et al. A phase 3 study of three induction regimens and of priming with GM-CSF in older adults with acute myeloid leukemia: a trial by the Eastern Cooperative Oncology Group. Blood. 2004 Jan 15;103(2):479-85.

31. Kantarjian H, Oki Y, Garcia-Manero G, Huang X, O'Brien S, Cortes J, et al. Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia. Blood. 2007 Jan 1;109(1):52-7.

32. Lyons RM, Cosgriff TM, Modi SS, Gersh RH, Hainsworth JD, Cohn AL, et al. Hematologic response to three alternative dosing schedules of azacitidine in patients with myelodysplastic syndromes. J Clin Oncol. 2009 Apr 10;27(11):1850-6.

33. Raphael Itzykson, Sylvain Thépot, Bruno Quesnel, Francois Dreyfus, Odile Beyne-Rauzy, Pascal Turlure, et al. Prognostic factors for response and overall survival in 282 patients with higher-risk myelodysplastic syndromes treated with azacitidine. Blood. 2011;117(2):403-411.

34. Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. J Clin Oncol. 2002 May 15;20(10):2429-40.

35. Kantarjian H, Issa JP, Rosenfeld CS, Bennett JM, Albitar M, DiPersio J, et al. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer. 2006 Apr 15;106(8):1794-803.

36. Breccia M, Loglisci G, Salaroli A, Serrao A, Petrucci L, Mancini M, et al. 5-Azacitidine efficacy and safety in patients aged >65 years with myelodysplastic syndromes outside clinical trials. Leuk Lymphoma. 2012 Aug;53(8):1558-60.

Sickle Cell Society calls for more minority blood donors

The NHS Blood and Transplant (NHSBT) have joined forces with the Sickle Cell Society. Together they hope to raise awareness for more blood donors from the black community. Around 12,500 people in the UK have sickle cell anaemia, and it is most common among black, Asian and mixed-race people.

NHS Blood and Transplant spokesperson, Theo Clarke said: "We hope the Sickle Cell Society's appeal will help inspire more people from the black community to give blood." It is crucial that people from the same ethnic backgrounds donate as a number of different blood types are more common and specific to people from certain ethnic backgrounds.

The current levels of both black, Asian, and mixed-race blood donors are very low; only 200 people from the same ethnic background give blood every day, but 7,000 donors are needed in order to keep up with demand. People from black, Asian and mixed-race backgrounds often share the same blood type, so recruiting donors from these ethnicities helps to collect enough of each blood group.

One family from Nottingham who greatly appreciates blood donations is the Udueni family. Their 9-year-old son Henry was diagnosed with sickle cell anaemia as a toddler and now receives transfusions every month.

> Only 200 people from black, Asian or mixed-race backgrounds give blood every day, but 7,000 donors are needed in order to keep up with the demand.
>
> *- Sickle Cell Society*

Henry's father, Anthony, argued that blood donors have changed his son's life, saying: "Before he received blood, he was at a very high risk of having a stroke, but since receiving transfusions, the brain scans showed he's at a much reduced risk."

He added: "My message to blood donors is that we're eternally grateful – the blood that you give directly adds value to Henry's life."

John James, CEO of Sickle Cell Society, commented: "We will continue to support NHS Blood and Transplant's work to meet the challenge of matching supply with demand... Blood is collected to meet patient need."

The Sickle Cell Society also helped to support National Blood Week target in recruiting more young donors. National Blood Week succeeded in their aim as by the end of the week, 7,516 donors, which made-up 65% of the total number of those who registered, aged between 17 and 34, listed themselves as new donors.

The campaign has also succeeded in attracting celebrity support. Vanessa Agyemang, an international model from Greenwich whose friends have been diagnosed with sickle cell disease themselves, has become an ambassador for the blood drive. She commented: "Because most people within our community don't give blood, encouraging people to do so is really important."

This was just one of many events which took place during National Blood Week, all of the events were aimed to encourage more young donors.

New child study is Bayer's new response to treating haemophilia

Children, aged up to 12 years old, are to be enrolled into the Bayer HealthCare group's new Phase III trial 'PROTECT VIII Kids' to evaluate the investigational compound BAY94-9027 for the treatment of haemophilia A.

Haemophilia A is the most common type of haemophilia, characterised by prolonged and/or spontaneous bleeding. PROTECT VIII Kids is a multicentre, multinational, partially-randomised, open-label trial which will enrol 50 previously treated patients worldwide who have severe haemophilia A and a documented history of at least 50 exposure days with any factor VIII (FVIII) product.

Bayer's trial investigating BAY94-9027 is to determine if it can extend the circulating half-life of rFVII, a recombinant human factor, by inserting a single cysteine (amino acid) site to its surface, which serves as an attachment site for a polyethylene glycol (PEG) polymer.

Dr George Lamm, Vice President of Global Clinical Development Hematology, Bayer HealthCare, said they "are happy that prophylaxis with this long-acting compound is now being studied in children younger than 12 years of age to complement our ongoing adult study."

Bayer's primary aim is to prolong the duration of protection from bleeds and allow for less frequent infusions when used prophylactically, alongside having the ability to treat acute bleeding. BAY94-9027 is dosed at least once weekly and on-demand, and as needed for acute bleeding.

A member of the Bayer HealthCare Executive Committee and Head of Global Development, Dr Kamal Malik, said; "The children with haemophilia and their parents, can find the frequent infusion associated with prophylactic treatment regimen as a significant burden. It's that understanding that drives us at Bayer to develop new and innovative molecules like BAY94-9027."

> Bayer's primary aim is to prolong the duration of protection from bleeds, and allow for less frequent infusions.
>
> *- PROTECT VIII Kids*

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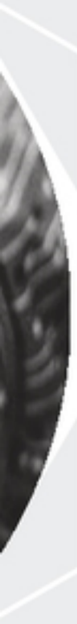


Roche have brought the very latest in technological advances to diabetics living in Ireland. Accu-Chek® features strip-free technology in the form of 50 tests on a continuous tape held on a cassette, thus there is no need to handle or dispose of single test strips.

It also provides six lancets in a drum with an integrated finger pricker, making the handling of single lancets obsolete, and includes a PC-ready report function. The function offers a comprehensive overview for both patients and caregivers to help them easily interpret blood glucose profiles, with a 99.5% accuracy rating.

The innovation of Accu-Chek® has a three-fold advantage. Firstly, it ensures that patients can monitor their blood glucose whenever and wherever, which has shown to improve patient adherence. It cuts handling steps by more than two-thirds, and it also provides substantial cost-saving for the Health Service Executive (HSE).

A diabetes specialist nurse at County Kildare's Naas General Hospital, Jackie McGrath, commented that she had received "positive feedback from my patients who are using the new Accu-Chek® Mobile system. A big advantage is the fact that there are no lancets or dirty strips to dispose of which makes it a good choice for people who are on the go."

The Accu-Chek® Mobile system has also been awarded the prestigious Red Dot Design Award in both 2011 and 2012.

NHS and Paramount Pictures join in blood hunt

In another attempt to raise the number of blood donors, a new strategy to encourage more blood donations has seen NHS Blood and Transplant (NHSBT) and Paramount Pictures partner together, through the release of the 2013 box-office movie World War Z.

Paramount has helped to persuade film enthusiasts to give blood by promoting donations through their UK website, by combining their social media channels and newsletters to help.

Over the last decade there has been a 23% drop in active donors, and new research has shown that the number of people donating may have fallen because there is a lack of knowledge about the process itself. Therefore, every aspect of donating blood, the facts, the figures, and the did-you-knows, will be opened up.

The NHSBT wants to utilise Paramount Picture to welcome both new and returning donors. As only 4% of the eligible population are active blood donors, the NHSBT needs 200,000 newly registered blood donors every year to ensure that levels remain stable. Furthermore, hospitals in England and North Wales need around 7,000 units of blood every day to treat patients with a range of health issues.

Jon Latham, Assistant Director of Marketing for NHS Blood and Transplant, said that although they can currently meet present demands for blood, an increase in the demand for blood over the next 10 years is expected, as a result of an ageing population requiring more complex surgical procedures, such as joint replacements and cancer therapies.

He said: "Over the last few years we have seen a decline in the number of younger people stepping forward to give blood and we are keen to find more creative ways to reach them. Recruiting new donors, in particular young adults, is important to ensuring a healthy donor base and blood supply for the future."

Bioengineered vein now a reality

On June 25th 2013, a 62-year-old man from Danville, Virginia, who was diagnosed with end-stage renal disease, received a bioengineered vein developed by doctors at Duke University Hospital, North Carolina. Completed in a mere 2 hours, the operation was the result of over a decade of work.

The new vein is an off-the-shelf, human-based product with no biological properties that would cause organ rejection, dubbed a "pioneering event in medicine," by Dr Jeffrey H. Lawson, a vascular surgeon and vascular biologist at Duke Medicine, who helped to develop the technology and perform the implantation.

The bioengineered vein is the product of a 15-year collaboration between Dr Lawson and Dr Laura Niklason, co-founder of Humacyte, a spin-off biomaterial engineering company. Dr Lawson and Dr Niklason began working together when they discovered a shared interest in engineering blood vessels. Dr Lawson remarked: "It is exciting to see something you've worked on for so long become reality. We talk about translational technology – developing ideas from the laboratory to clinical practice – and this only happens where there is the multi-disciplinary support and collaboration to cultivate it."

In the beginning, a person's own cell was used to seed the scaffolding, reducing the risk of rejection. However, this process took too much time and made mass production near impossible.

After many setbacks, the researchers made a number of advancements, creating a vein which is engineered by cultivating donated human cells on a tubular scaffold to form a vessel. The vessel is then cleansed of the qualities that might trigger an immune response.

A biodegradable mesh was also created and used as the scaffolding for the veins, which adjusts to any shape, and forms into a blood vessel of varying lengths and widths.

Another key improvement which strengthens the bioengineered tissue is a pulsing force introduced during the growth process. The nutrients are pumped through the tube in a heartbeat rhythm to build the physical properties that are similar to native blood vessels. A life-like vein then results in months.

Dr Lawson said: "This sets the groundwork for how these things can be grown, how they can incorporate into the host, and how they can avoid being rejected immunologically... A blood vessel is really an organ – its complex tissue. We start with this, and one day we may be able to engineer a liver of a kidney or an eye."

In pre-clinical tests, the veins have performed better than other synthetic and animal-based implants. The veins adopt the cellular properties of a blood vessel, but they don't just elude rejection; they actually become indistinguishable from living tissue as cells grow into the implant. This, on top of the fact that the bioengineered veins have been developed in such a way that they can be stored in the fridge for over a year, gives the technology potential to be successful both medically, and commercially.

"Reassuring" progress highlights a brighter outcome for young adults

A new study has released figures that have highlighted an increase in cure rates for teenagers and young adults who are diagnosed with acute myeloid leukaemia (AML), a typically aggressive form of leukaemia, compared to 30 years ago.

The research has shown that in 1975, only 8% of those who were diagnosed were cured, whereas presently the cure rates for patients aged between 15-24 who were diagnosed in 2006, stands at 48%. This is due to improvements in both treatment and care over the last three decades.

Pam Thornes, Trust Manager at the Laura Crane Youth Cancer Trust, said: "It's reassuring to see from the study that cure rates in young people with specific cancers are far greater than they were 30 years ago. This is a testament to the research, which charities such as the Laura Crane Youth Cancer Trust are helping to fund, to better understand cancer, which has led to the advancement in cancer care and treatment."

Thornes added: "Young people with cancer often get overlooked and usually get treated as a child or an adult, which in many cases isn't tailored to their age-specific needs."

The research in this study has highlighted that the various AML types in younger people is easier to treat with chemotherapy. They can generally be given more intense treatment, and the short-term side-effects can now be managed effectively. In order to assess effectiveness, clinical trials and the experimental treatments used are vital, although the number of people participating could improve.

Dr Anjali Shah a research scientist at the London School of Hygiene and Tropical Medicine emphasised: "The main reason for these improvements is the development of new treatments, combined with good levels of recruitment to UK clinical trials. These key issues have been effective in curing more people of AML. But levels of cure of this disease in England remain lower than those observed in other European countries, such as Sweden."

Patients aged between 15-24, diagnosed in 2006, now see a cure rate of 48%.

- Dr Anjali Shah, London School of Hygiene and Tropical Medicine

New monitoring technology to help acute lymphocytic leukaemia patients

A new study has highlighted that genetic technologies to monitor remaining cancer cells are particularly useful for patients with aggressive acute lymphocytic leukaemia (ALL). The technology will help to inform treatment decisions and can ultimately predict patient outcomes.

ALL is the second most common type of acute leukaemia; 20–30% of patients with ALL also develop Philadelphia chromosome positive (Ph+) ALL. This development is often caused by a genetic abnormality and a result of poor prognosis. As this is a very aggressive disease, clinicians do not have a reliable way to ensure the patients will not relapse.

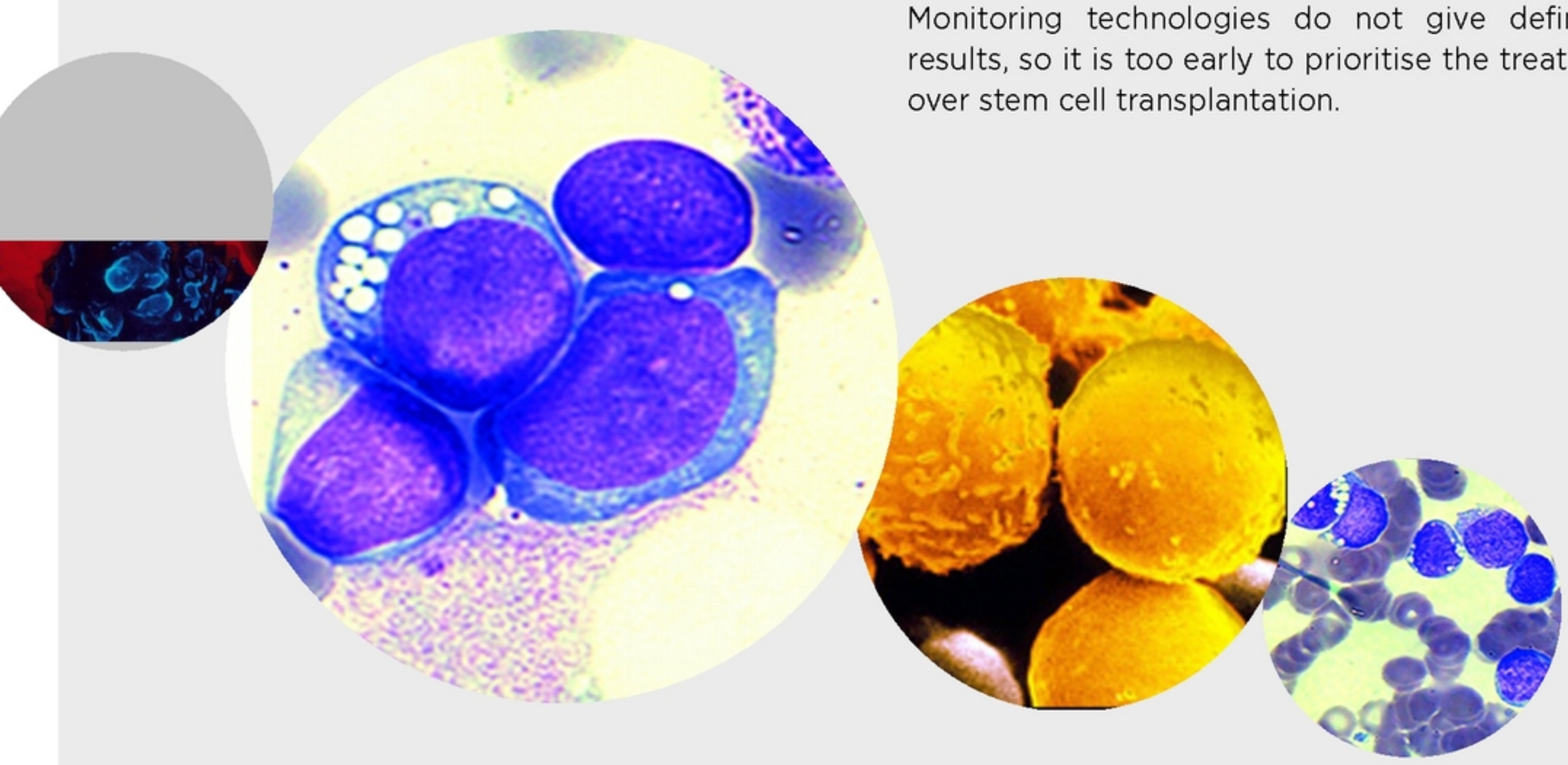
The common preventive treatment for patients with Ph+ ALL is an allogeneic stem cell transplant in which the patient receives matching stem cells. This procedure can have harsh side effects and is not always universally available. As an alternative, a new class of cancer drugs named tyrosine kinase inhibitors (TKIs) can be used. TKIs target abnormal, leukaemia-causing protein BCR-ABL. This treatment has shown improvements in survival rates, which could make stem cell transplantation unnecessary.

Lead study author, Dr Farhad Ravandi, of the University of Texas MD Anderson Cancer Center, said: "The arrival of tyrosine kinase inhibitors has marked the beginning of an exciting era in which we can begin considering alternative preventive cancer treatments and look to spare patients from the risk of toxicities that often accompany stem cell transplants."

Dr Ravandi continued: "Now that we know that these drugs are effective, we can take the next step and focus on studying lingering disease in Ph+ ALL patients to guide more effective treatments and ultimately predict and manage possible relapse."

After treatment, patient's cancer cells, known as minimal residual disease (MRD), may remain and can often cause a relapse. The primary outcome of Dr Ravandi's study was to monitor if a negative MRD reading was associated with prolonged survival. Revandi suggested that monitoring technology will search for genetic aberrations in a patient's blood and bone marrow, and will indicate if there is a presence of cancerous cells.

However, the MRD readings did not accurately predict all patient outcomes. Dr Ravandi commented: "The next step is to refine and standardise our approach to better define which patients are truly disease-free and who should be recommended for more aggressive treatment." Monitoring technologies do not give definitive results, so it is too early to prioritise the treatment over stem cell transplantation.



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International Conference on Haematological Disorders in the Elderly (ESH 2013)

6th-7th November 2013

Barcelona, Spain

The ESH conference aims to define ageing in biological terms, to address its consequences on the bone marrow, lymphoid tissue and on haemostasis, and to discuss resulting disorders. Treatment issues in the different type of haematological malignancies will be addressed with emphasis on the unique aspects of treatment related to ageing.

Hellenic Society of Hematology 24th National Congress 2013 (EAE 2013)

14th-16th November 2013

Athens, Greece

The 24th National Congress of the Hellenic Society of Hematology covers, in nine scientific departments, the entire spectrum of hematology. Their aim is to promote scientific knowledge through educational activities. It aims to support multi-centre clinical trials and epidemiological studies organised at the initiative of the Science Departments.

American Society of Hematology 55th Annual Meeting and Exposition (ASH 2013)

7th-10th December 2013

New Orleans, Louisiana, USA

ASH is the premier hematology event of the year, an invaluable educational experience for all. It aims to inform attendees about the latest clinical updates in research, therapies and practice strategies through educational and scientific programmes.

6th Annual T-Cell Lymphoma Forum 2014 (TCLF 2014)

23rd-25th January 2014

San Francisco, USA

This two day meeting is focused on providing the latest information on novel agents and treatment approaches. This forum provides a platform for attendees in TCL to gather and exchange ideas and information to improve outcomes for this patient's population. This forum is intended for all clinicians who are interested in TCL.

Clinical Updates in Haematology on Lymphoid Neoplasms and Myeloma (ESH 2014)

5th-6th February 2014

Paris, France

The ESH meeting aims to provides updates on patient management, offering a clinically and practically oriented programme that reflects state-of-the-art knowledge as well as innovation, in a range of different hematologic disorders. It also features an in-depth presentation which is followed by discussions, clinical cases as well as controversies.

10th European Congress on Hematologic Malignancies

7th-9th March 2014

Vienna, Austria

This Congress hopes to provide clinicians with new important clinical updates concerning the management of patients with haematologic malignancies. The aim of the Congress is to show how new technologies, new research and ultimately new discoveries have changed the way physicians treat their patients, and have now began to develop more individual approaches.

The 40th Annual Meeting of the European group for Blood and Marrow Transplantation (EBMT 2014)

30th March – 2nd April 2014

Milan, Italy

EBMT is devoted to the promotion of all aspects associated with the transplantation of haematopoietic stem cells from all donor sources and donor types including clinical research, education, standardisation, quality control, and accreditation for transplant procedures.

World Federation of Hemophilia World Congress 2014 (WFH 2014)

11th-15th May 2014

Melbourne, Australia

This Congress is the largest international meeting dedicated to haemophilia, von Willebrand disease, rare factor deficiencies and inherited platelet disorders. The congress will include a robust medical program, presented by leading experts who will discuss the latest scientific and clinical developments in diagnostics, disease managements and research. There will also be a multidisciplinary program that covers emerging topics related to bleeding disorders which are inherited.