
WHAT'S NEW IN...

Haematology

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ABSTRACT

This article examines some of the recent advances in haematology in both the malignant and non-malignant areas of the speciality. Improvements in survival rates after effective chemotherapy now present the haematologist with the challenges of how to minimise therapeutic side effects without affecting outcome and the role of stratification as well as specific monitoring of enzyme activity are discussed. Many treatments for haematological malignancy have significant late effects which are only now becoming a problem - what these are, how to identify them and how they can be limited are examined. The increased knowledge of the altered pathways that lead to malignancy has allowed a whole slew of new therapies to be developed often with excellent results. The role of new iron chelation agents and the so called 'universal haemostatic agent' activated factor VII are also discussed.

INTRODUCTION

The speciality of haematology has two distinct branches dealing with malignancies such as leukaemia, lymphoma and myeloproliferative disorders and the non-malignant side which manages patients with haemoglobinopathies and inherited anaemia, coagulation abnormalities as well as running the National Blood Service. This article looks in detail at some of the major recent advances in both aspects of haematology.

MALIGNANT HAEMATOLOGY

Background

The presentation of haematological disorders has not changed. Haematological malignancies can be divided broadly into leukaemia and lymphoma. Their classification is now well structured and based on morphology, expression of specific surface proteins and specific genetic changes (1). Leukaemia can be classified as either acute or chronic, based on the clinical presentation which reflects differences in the characteristics of the underlying malignant cell. Further classification into myeloid or lymphoid depends on the characteristics of the cell type affected. Leukaemia usually presents with bone

marrow failure due to replacement of the normal marrow with malignant cells. This leads to anaemia, low platelets and bleeding and reduced white cells and infection. Lymphomas are a heterogeneous group of disorders that usually present with a mass of disease in either isolated sites or can be widely disseminated to many different areas. The presenting symptoms will vary much more widely depending on the sites involved. Myeloma, a disorder of plasma cells in the bone marrow, can be associated with considerable morbidity compared with other haematological malignancies because of the wide ranging effects on other tissues and organs such as renal and bone disease. For all malignant conditions the extent of the disease must be assessed by accurate staging to allow the correct treatment to be given. This will usually involve assessment of the disease at its source by bone marrow or lymph node biopsy, and radiological imaging to assess wider dissemination. Increased knowledge of the mechanisms underlying these disorders is allowing improvements in diagnosis and success of therapy.

Success of therapy

Success at treating haematological malignancies continues to improve in nearly all disorders and this is in part due to new therapies being developed (vide infra), but more importantly due to improvements in supportive care for patients of all ages. These improvements are mostly in the areas of improved detection and management of bacterial, viral and fungal infections and in blood product support with red cells and platelets (2).

Table 1 shows the most recent long term survival data for a selection of haematological malignancies. References for complete remission and survival rates are review articles and ranges are maximum and minimum rates cited therein.

The increased survival in ALL over time is due to several factors. The use of higher doses of methotrexate and cytarabine in consolidation phases and participation in clinical trials by more centres has improved care of adult and paediatric patients. In adults the use of stem cell transplantation in certain patients and earlier in treatment has improved outcome. Stratification of individuals into different risk groups has also allowed more intensive therapy to be

Condition	Complete Remission Rate	Survival Rates	References
ALL (Adult)	84%*	35% mean overall survival	(3)
ALL (Childhood)	>96%	63-83% 5yr event free survival	(4)
AML (Adult <60 yr)	72-82%	34-39% overall survival	(5)
AML (Adult >60yr with non-complex karyotype)	56%	7.5% overall survival	(6)
AML (Adult >60 yr with complex karyotype)	25%	0% overall survival	(6)
APL	>87%	>69% overall survival	(7)
DLBCL		90%	(8)
Burkitt lymphoma	65-100%	50-70%	(9)
Hodgkin lymphoma		>94% overall survival	(10)

Table 1 – Complete remission (CR) rates and survival rates for some of the common haematological malignancies. ALL – Acute Lymphoblastic Leukaemia; AML – Acute Myeloid Leukaemia; APL – Acute Promyelocytic Leukaemia; DLBCL – Diffuse Large B-Cell Leukaemia. (* mean CR rate)

given to those with poorer prognosis. Factors used to help stratify adult patients into poor risk groups include: age >50 years, WBC >30, T-cell subtypes, presence of the Philadelphia chromosome t(9;22), time to complete remission exceeding 3–4 weeks and measurable minimal residual disease (MRD) (3).

Success in treating other haematological malignancies is due to improved stratification of patients into risk groups and earlier aggressive therapy. This success has led to a shift in emphasis in some conditions to find ways of minimising exposure to harmful drugs or to determine what adverse effects may ensue and develop treatments or screening methods for them.

Preventing treatment toxicity

In some areas, particularly paediatric leukaemia, survival rates have reached very high levels and some emphasis is now moving towards determining the long term effects of therapy and identifying ways of reducing or tailoring therapy to individuals. This is achieved by determining activity of pathways involved in drug absorption, distribution, metabolism and excretion (11). Genetic variation between individuals and their metabolism of specific drugs was first

shown almost 40 years ago (12). In the 1980s variation in the single gene control of the metabolic pathway dealing with 6-mercaptopurine, one of the main drugs used in maintenance therapy in acute lymphoblastic leukaemia, was demonstrated (13). Those with reduced or absent thiopurine S-methyl transferase (TPMT) activity are more likely to suffer from prolonged bone marrow suppression and its consequences (infection and haemorrhage) and were more likely to suffer second malignancies as a consequence of therapy. (14–16). From this protocols for the treatment of paediatric ALL incorporate dose adjustments based on the level of enzyme activity and individual's blood counts through therapy. This approach has been shown to have no detrimental effect on relapse rates (17). Unfortunately the measurement of TPMT is not universal practice in all paediatric patients and even less so in the treatment of adult patients. In addition, it is only one enzyme that deals with a small proportion of drugs used in the treatment of ALL so tailor-made dosing regimens for all drugs in all patients is still some time away (11).

Long term effects of therapy

Direct effects of malignancy can have lasting effects on individuals, for example the damage to the kidneys caused by plasma cell disorders such as myeloma. However, these effects are often reversible once the underlying disease is successfully treated. The treatment itself can cause acute and chronic problems and it is only with the continuing improvement in success rates and long term survival of patients that we are now seeing unanticipated late effects of therapy. Recognition of these emerging late effects is leading to attempts to minimise therapy to prevent these problems with individual tailor made therapy being the ultimate goal.

The main long term effects of therapy that concern both patients and clinicians are those of second malignancies, organ dysfunction (cardiac, pulmonary and endocrine), reduced fertility and reduced quality of life. These effects are variable depending on the initial diagnosis, site of disease and therapies employed in treatment. For example patients with Hodgkin lymphoma treated with mantle radiotherapy are at increased risk of cardiovascular disease and breast cancer. Use of anthracycline containing chemotherapy regimens or use of radiotherapy increases the risk of treatment related AML. Alkylating agents and cyclophosphamide are known to lead to gonadal dysfunction. Corticosteroids used in many haematological malignancy regimens are the main culprits in causing osteopenia and osteoporosis.

The best documented second cancers

following therapy for haematological malignancy are breast cancer and acute myeloid leukaemia. Breast cancer is the most common second cancer in female survivors of Hodgkin lymphoma treated with mantle field radiation. The five year survival following therapy for Hodgkin lymphoma is now above 90% (10, 18). This gives a unique population that has been the focus for several cohorts of patients with many years of follow-up data - they have a relative risk of developing secondary breast cancer between 1.4 and 75 (19). The wide range for these relative risks is due to the heterogeneity of the patient groups studied. The age at treatment is very important. Treatment at a younger age increases risk, with the highest risk being between 10 and 16 years of age (20-22), due to the breast tissue being most susceptible to DNA damage during puberty (19). The increasing dose of radiotherapy has a linear relationship with relative risk with doses ≤ 5 Gy (23-27), but larger doses ≥ 20 Gy (20) (and even beyond 38Gy (30)) further increase the relative risk. The tumourigenic effect of radiation alone is well documented but the effects of combination with chemotherapy are inconclusive. The majority of studies show a reduced risk of secondary breast cancer (28-31), possibly due to a chemotherapy-induced reduction in ovarian function altering the sensitivity of breast tissue to the effects of radiation (30). Other authors disagree (31, 32).

The first trial combining chemotherapy with adjuvant radiotherapy was carried out in limited stage lymphomatous disease and showed no advantage in adding radiotherapy and no increased risk of long term adverse events (33). Further studies have also shown there is no advantage to combined modality therapy and that the risk of second malignancies outweighs any benefits of radiotherapy. (34). Therapy for all stages of disease is stratified according to patient stage, Hodgkin's International Prognostic Factor Project score, presence of B-symptoms and bulkiness of disease (34), ensuring continuing high cure rates and minimisation of long term consequences.

Myelodysplastic syndromes (MDS) and acute myeloid leukaemia can both follow radiotherapy and chemotherapy. The drugs implicated most often are alkylating agents and topoisomerase II inhibitors, such as etoposide and doxorubicin (35-37). The risk for developing these therapy related disorders are proportional to the cumulative dose and occur 5 to 6 years following exposure (35, 38). Most cases of secondary MDS or AML have a significant dysplastic phase before the criterion for AML ($\geq 20\%$ blasts) is met, and most cases succumb to complications of bone marrow failure before overt leukaemia occurs. Secondary AML has an extremely poor prognosis, even when caused by topoisomerase II inhibitors, with a recent

study giving an overall survival of only $14 \pm 8\%$. (39)

Cardiotoxicity, like second malignancies, is also multifactorial. The anthracycline chemotherapy agents, daunorubicin and doxorubicin, are the best known cause of late onset cardiomyopathy and heart failure. The risk is dose dependant with greater than 30% of patients receiving $>600\text{mg/m}^2$ developing cardiomyopathy and those receiving $>300\text{mg/m}^2$ having an 11-fold increased risk of clinical cardiac failure (40). The risk of sub-clinical cardiac failure is far higher depending on the definition of the outcome measured. One study has shown reduced left ventricular contractility in almost 57% of patients. However, there is significant variation as discussed in a recent review of 25 studies (41, 42). The combination of anthracyclines and radiotherapy further increases the risk of late cardiac effects. Radiation exposure causes pericardial effusions and pericarditis. The risks of these adverse effects are dose dependent, lifelong and increases with time from exposure. (43, 44) Within 10 to 30 years after radiation exposure 2-10% of patients will have developed symptomatic pericarditis (45). There are also other biological risk factors that increase treatment related cardiac toxicity including: female gender, age <5 years at exposure, underlying cardiovascular problems such as hypertension or cardiac anomalies and electrolyte disturbances, especially hypokalaemia and hypomagnesaemia (46-50). Alterations in the delivery of cardiotoxic drugs such as continuous infusions or reduction of dose frequency are hoped to reduce these long term effects. Currently there are no new drugs with significant anti-tumour activity or reduced cardiac effects to replace the widely used anthracyclines so patients are regularly monitored prior to, during and after therapy for these effects.

Preservation of fertility has become increasingly important in patients treated during childhood. The success of childhood cancer treatment has led to many patients surviving into adulthood and wanting to lead normal lives and raise families of their own. Toxic effects of chemotherapy are increased in males because of the higher sensitivity of the testes. Radiotherapy to the gonads of either sex leads to significant reduction in function. The effect on the ovary is age and dose dependant. Amenorrhoea affects 68% of prepubertal girls treated with abdominal radiotherapy for Hodgkin lymphoma, with doses from 12 to 15 Gy, and 100% of women over 40 years, even when low doses between 4 and 7 Gy are used (51-53). Doses needed to reduce testicular function are lower than those causing ovarian dysfunction (54, 55). However, fertility preservation is much easier for males. Sperm banking prior to therapy is now widely available in patients emotionally, physically and mentally able to perform and

successful pregnancies have been reported (56). Storage of ovarian tissue is more difficult as it requires laparoscopy or laparotomy. Reimplantation of this ovarian tissue following therapy is difficult and success is rare (57, 58). Unfortunately there is often poor utilisation of frozen sperm with recent studies revealing <5% of patients using their stored spermatozoa (59, 60). There are now series of successful pregnancies following long term cryopreservation of sperm (61).

Corticosteroids, although not conventional chemotherapeutic agents, have long been used in the treatment of haematological malignancies. They are used extensively in the treatment of ALL and many lymphomas. They have also been used for many years in the treatment of rheumatological conditions where their long term effects are well documented. Adverse effects of corticosteroid use include cataracts, osteopenia, osteoporosis, fractures and avascular necrosis. The dose and duration of corticosteroid therapy required to cause long term effects are very small. A dose of 5mg prednisone each day and a minimum period of therapy of three months have been shown to increase risk of fractures and osteoporosis respectively (62, 63). Effects are increased with younger

age at exposure (64). There are several medical ways to reduce, treat or prevent these bone changes including calcium and vitamin D supplements and bisphosphonates (62, 65), which allow remineralisation of bone, preventing and reversing the effects of corticosteroids and improve bone density (66). This class of drug have also been used with increasing frequency in the management of bone disease caused by myeloma. (67)

With increasing knowledge of specific conditions and their direct consequences, and increasing success of therapy, late effects of treatment can be assessed and then hopefully be prevented, treated or screened for.

Detecting haematological malignancies and directing therapy against them

Recently there have been significant advances in the understanding of mechanisms underlying the development of many malignancies - this is especially true in haematology. Many haematological disorders are caused by a change at the genetic level that leads to the alteration of pathways controlling cell differentiation, cell division and apoptosis. These pathways are being studied in great detail with the hope of understanding disease aetiology and for the development of potential cures.

Condition	Translocation	Comment	Prognosis
Acute Lymphoblastic Leukaemia	t(9;22)	Philadelphia chromosome - 33% of adult ALL	Unfavourable
	t(12;21) (p13;q22)	25% of child ALL	Favourable
	11q23	MLL gene rearrangement - Infant ALL	Unfavourable
	t(1;14)/t(10;14)/t(11;14)	T-cell ALL	
Acute Myeloid Leukaemia	t(8;21) (q22;q22)	AML-ETO	Favourable
	inv(16)(p13q22)/t(16;16)(p13;q22)	CBF β -MYH11	Favourable
	t(15;17)	Acute Promyelocytic Leukaemia – PML-RARA	Favourable
	11q23	MLL gene rearrangements	Intermediate
CML	t(9;22)(q34;q11.2)	Philadelphia chromosome	Favourable
Burkitt Lymphoma	t(8;14)	MYC – immunoglobulin heavy chain	
Mantle cell lymphoma	t(11;14)(q13;q32)	BCL-1 – immunoglobulin heavy chain	
Follicular lymphoma	t(14;18)(q32;q21)	BCL-2 – immunoglobulin heavy chain	

Table 2. Haematological malignancies with specific cytogenetic changes, and where more than one change exists within a condition its effect on prognosis. This is not an exhaustive list.

There are a number of haematological diseases that have now had specific genetic changes identified (Table 2). These are diagnostic in certain conditions like Burkitt lymphoma and seen in up to 60% of cases with other conditions like the acute leukaemias (68). These can be used in initial diagnosis and can be used to monitor response to treatment with fluorescent in situ hybridization (FISH) or reverse-transcriptase quantitative polymerase chain reaction (RQ-PCR). Certain cytogenetic changes can be used to stratify patients into different risk groups (6, 69). This is most important in the acute leukaemias where therapy is stratified depending on risk at initial diagnosis and response to therapy.

Some translocations such as t(9;22) and t(15;17) for CML and APL respectively code for specific proteins for which we now have specific targeted therapy. The B-cell lymphoproliferative disorders share common alterations in certain genes including immunoglobulin heavy chain (IgH) gene rearrangements. These are brought into close proximity of oncogenes and the IgH gene promotes their over expression leading to tumourgenicity.

There are an increasing number of mutations and translocations that are found in several different conditions. Table 3 shows these mutations. These changes, like larger chromosomal changes can be used to stratify patients into different risk groups.

Mutation	Conditions	Reference
FLT3 (fms-like tyrosine kinase)	AML and ALL	(70)
JAK2V617F (Janus kinase)	Myeloproliferative disorders	(71)
p53	CLL, Cancers of breast, brain, colon	(72)

Table 3 – Mutations seen in more than one haematological condition

The chromosomal changes found in haematological malignancies lead to tumour development by a number of mechanisms:

- 1) The repositioning of an oncogene close to another functionally active gene leading to increased activity of the active gene. For example the positioning of the ABL gene close to the BCR gene in CML.
- 2) The deletion or alteration of a tumour suppressor gene causing it to cease working or its activity to be reduced. The best example of this the TP53 gene which encodes the p53 protein. Mutations in this gene are found in up to 50% of spontaneous cancers and 14% of haematological malignancies, especially lymphoproliferative disorders (73, 74).
- 3) Alterations in DNA repair mechanisms leading to increased mutations and genetic rearrangements.

All these changes cause disruption in the normal control of cellular development. Either cell differentiation is blocked; kinase enzyme cascades are activated leading to uncontrolled cell proliferation; cell cycle progression controlled by cyclins is disrupted; or the normal pathways of apoptosis are disabled. Genetic changes affecting tyrosine kinases involved in the control of the cell cycle include the Philadelphia chromosome, a translocation between chromosomes 9 and 22 t(9;22)(q34;q11) (75), giving rise to the novel fusion gene, BCR-ABL, which encodes a constitutively active protein, tyrosine kinase. (76-78) seen in CML and ALL and the FLT3 mutations seen in AML and ALL. Mutations of FLT3 fall into two groups: a point mutation and an internal tandem duplication (ITD). The ITD causes spontaneous activation of the fms-like tyrosine kinase leading to uncontrolled proliferation of cells (79). The presence of ITD mutations have been shown to reduce remission induction rates and overall survival in paediatric and adult AML patients (80, 81). Other tyrosine kinases act on receptors involved in cell signalling and these are affected in the chronic myeloproliferative disorders (cMPD) such as polycythaemia vera (PV), essential

thrombocytosis (ET) and myelofibrosis (MF). These conditions have traditionally been grouped together but it has only recently been discovered they share a significant genetic link. Four separate research groups in 2005 all reported mutations of JAK2 in patients with cMPD (71, 82-84). JAK2V617F mutations can now be detected in 90-95% of patients with PV, 50-70% of patients with ET and 40-50% of patients with MF (71).

Recent interest has focused on the ubiquitin proteasome system. This is the intracellular protein degradation system and involved in removal and recycling of all proteins within eukaryotic cells. It is involved in many cellular functions including cell-cycle progression, signal transduction and apoptosis. The polypeptide ubiquitin binds to proteins destined for degradation allowing proteasome activation breaking proteins into small peptides and preserving ubiquitin for further use. Proteins that are known substrates for proteasome degradation include: cell growth inhibitory proteins (I κ B), cyclin-dependent kinase inhibitors (p21 and p27), tumour suppressors and oncogenes. Proteasomes are abnormally expressed in large numbers in dividing cells such as in the embryo and malignant cells. This increase in proteasome activity leads to increased removal of cell growth control proteins causing increased cell proliferation. Proteasome inhibition is a potential target for therapy in disorders where there is high proteasome activity such as myeloma and other B-cell non-Hodgkin lymphomas (85).

Another tool for the detection of haematological malignancy and their potential pre-malignant stages is immunophenotyping. This uses monoclonal antibodies (MAbs) to detect and quantify membrane, cytoplasmic or nuclear antigens by flow cytometry. Immunophenotyping can be used in initial diagnosis, planning targeted therapy and monitoring disease response.

Myeloid	CD 13, CD 33, CD 117, cytoplasmic myeloperoxidase
B-cell	CD19, CD10, cytoplasmic CD 22 and 79a
T-cell	CD2, CD7, cytoplasmic CD3
Progenitor	CD34, HLA-Dr, TdT

Table 4 – Immunophenotype markers for myeloid, B- and T-cell lymphoid and progenitor cells.

Different groups of MAbs (or markers) to specific antigens are used to distinguish different conditions. The groups of markers used are determined by recommendations for the British Society of Haematology and European Group for Immunologic Classification of Leukaemias (EGIL) (86, 87). Table 4 shows the common markers to myeloid, lymphoid and progenitor cells.

There are three surface antigens that are increasingly important when detected by immunophenotyping. These are CD 20, a B-cell antigen; CD33 a myeloid antigen and CD52 a lymphocyte antigen. Each of these antigens can be targeted by specific antibody-based therapies (*vide infra*) that are improving the outcome in many different haematological disorders. These 'high tech' laboratory processes to aid diagnosis and disease monitoring have in some respects taken over from traditional methods of investigation such as assessment of cell morphology by microscopy. However, the processes can be combined to ensure correct diagnoses are being made and treated. If a malignant cell looks like acute lymphoblastic leukaemia, has a corresponding immunophenotype as determined by flow cytometry and has a genetic change commonly found or specific to ALL then the diagnosis is certain. Incongruity between results raises doubts as to the true diagnosis.

With the availability of specific markers for different conditions methods can be employed to detect minimal residual disease at different points in treatment as the presence of any amount of MRD is a poor prognostic factor, especially in the acute leukaemias and certain B-cell lymphoproliferative disorders. Conventional morphology defines complete remission as <5% malignant cells visible by microscopy. This is not sensitive and better tests are now available including immunophenotyping, cytogenetics, FISH and PCR amplification. Immunophenotyping is the least sensitive, only being able to detect abnormal cells down to 0.01-0.1%. The immunophenotype of some disorders such as AML can change as the disease progresses giving false negatives (88, 89). Cytogenetics can detect non-random chromosomal translocations in up to 70% of ALL and 50% of CLL. However it requires an adequate number of suitable metaphases for analysis and is slow and labour intensive. Its sensitivity, similar to morphology, is 5%. FISH is superior to conventional cytogenetics at detecting small chromosome changes. Fluorescent probes are bound to specific areas of chromosomes and used to determine if translocations have occurred. Sensitivity is 1% so and can be used in follow-up samples for reassessment. PCR

amplification is the most sensitive test currently available to detect chromosomal changes from single gene disorders to large chromosomal translocations. It is a simple process, with a sensitivity of 0.001% (90).

New Therapies

In certain instances therapy can now target the specific genetically-induced enzyme or protein alterations or the cell surface immunophenotype of a condition. The best example of this is the targeting of an overactive tyrosine kinase in chronic myeloid leukaemia. This myeloproliferative disorder is characterized by the expansion of a clone of hematopoietic cells that carry the Philadelphia chromosome (Ph). Imatinib (Gleevec, Novartis; formerly called STI571) is a relatively specific inhibitor of the BCR-ABL tyrosine kinase (91, 92). Before the introduction of imatinib the standard treatment for CML was interferon-alpha and cytarabine (93, 94). The initial study that demonstrated superiority of imatinib over the combination therapy has continued to follow up patients showing continuing significant long term improved disease free survival. At five years of follow up 98% of patients have complete haematological response and 87% have complete cytogenetic response (95, 96). This study has also demonstrated a possible plateau in long-term survival and almost complete reduction in imatinib treatment failure. The next step is to determine who will not respond to imatinib and the development of new tyrosine kinase inhibitors that can be used in this small patient group. Dastinib and nilotinib are the two new drugs being used in imatinib resistant patients either alone or in combination to restore therapy success (97).

Other conditions with abnormal activation of a tyrosine kinase include AML and ALL with FLT3 mutations. FLT3 mutations give rise to an abnormal receptor tyrosine kinase expression that causes proliferation of leukaemia cell lines. FLT3 mutations are found in up to 30% of adult patients with AML (70) and 5-22% of patients with ALL with MLL gene rearrangements (98). There are several well characterised FLT3 inhibitors that have high activity at very low concentrations and have high activity at killing leukaemia cell lines (70). As single agents these drugs have limited long-term effects but seem to have synergistic effects when combined with conventional agents (99-102). Ongoing trials to assess FLT3 inhibitors in both AML and ALL will give more information about specific indications for these new drugs both as single agents and in combination.

Bortezomib (Velcade) is a new drug that is currently under some media scrutiny. It

has been approved for use in Scotland by the Scottish Intercollegiate Guidelines Network (SIGN) but not by the National Institute for Clinical Excellence (NICE) in England and Wales. It is a drug that has been shown to have activity in a number of malignancies including myeloma, non-Hodgkin lymphoma and cancers of the prostate, kidney, head and neck, and lung. It has been especially useful in myeloma where multiple cellular control pathways are deranged. Bortezomib is a small molecule proteasome inhibitor by binding to its active site. This gives rise to its anti-neoplastic effect by inhibition of cell growth signalling pathways, induction of apoptosis and inhibition of cell adhesion molecule expression. It has a significant effect on cells over expressing Bcl-2.

Preclinical studies showed Bortezomib had activity in myeloma cell lines as it directly inhibited cell proliferation and induced apoptosis independent of p21, p27 and p53 expression (103, 104). Good clinical activity was shown in phase I studies and activity was shown to be increased with the addition of dexamethasone (105-107). The Assessment of Proteasome Inhibition for Extending Remissions (APEX) study was the phase III trial that showed bortezomib gave a significant advantage over dexamethasone in prolonging time to progression and overall survival (108). There are significant side effects associated with the use of bortezomib that must be weighed against its benefit. At least 30% of patients suffer significant weakness, fatigue, gastrointestinal upset or neuropathy and 10-30% of patients suffer significant neutropenia and the risk of infection is high. The APEX data is the basis for the use of bortezomib internationally. Unfortunately, the single technology appraisal of bortezomib for relapsed and refractory multiple myeloma conducted by NICE concluded the APEX study data to lack clarity and detail and some factors about the efficacy of bortezomib were exaggerated. The preliminary guidance also states that there are other therapies such as thalidomide, conventional chemotherapy and stem cell transplantation that had not been compared to bortezomib and have potentially equal activity. In addition, the cost benefit calculations were considered to be inaccurate (109). The positive guidance was that bortezomib should be used only in the setting of clinical trials where the exact role and timing of its use can be determined. This is currently the draft guidance and should not preclude the use of bortezomib. It is one of a number of guidance documents from NICE that has caused concern to public and health professionals. However, with its significant cost, adverse effects and potentially equally effective other therapies, this might be the

right course of action within an overstretched health system.

The conditions with specific surface immunophenotype changes for which there are targeted therapy include mature B-cell lymphomas, chronic lymphocytic leukaemia and some acute myeloid leukaemias. CD20 is expressed on the surface of 95% of B-cell non-Hodgkin lymphoma (NHL) cells including Burkitt lymphoma, diffuse large B-cell lymphoma and follicular lymphoma (FL). The anti-CD20 antibody Rituximab is the most widely used and successful of the current monoclonal antibodies, causing cell death through antibody-dependent cell-mediated cytotoxicity, complement dependent cytotoxicity and direct induction of apoptosis (110). Conventional chemotherapy for DLBCL was cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP), which achieved complete response rates of 40-50% and overall survival rates of 35-40% (111). In 1998 a single agent study using rituximab in a number of patients with B-cell NHL showed Rituximab to have a response rate of 37% and combination with conventional therapy was investigated (112). The first phase II trial of Rituximab with CHOP chemotherapy had a complete response rate of 61% (113) followed by the Groupe d'Etude des Lymphomes de l'Adulte (GELA) randomised-controlled study of CHOP alone vs. CHOP-Rituximab. The addition of rituximab improved the event free and overall survival by 19% and 13% respectively (114). Five year follow-up of these patients in the GELA study continues to show significant advantage of the addition of rituximab (115). Other studies have since corroborated these results and the significant advantage the addition of a MAb to conventional therapy offers (116, 117). Current trials are attempting to determine the best dose frequency and intensity to further improve the benefits provided by targeted therapy.

Following the success of Rituximab in DLBCL it has been used in other B-cell NHLs including FL and mantle cell lymphoma. These chronic disorders often have a relapsing and remitting course. As a single agent Rituximab had a 40-48% response rate and 13-17 month mean time to progression period (118, 119). Combination of Rituximab with CHOP in FL increased long-term complete response rates to 55%, which increased to 80% with a Fludarabine containing regimen (120, 121). Further studies adding more agents have improved on these response rates and shown significant disease free advantages compared with pre-Rituximab chemotherapy in patients with all stages of disease. These studies have been incorporated in a recent meta-analysis concluding rituximab has a significant

response and survival benefit, with an 18-month survival of 85% (122).

Rituximab's success has led to other anti-CD20 MAbs to be investigated including two new drugs that are hybrids of an anti-CD20 antibody with a radio-isotope. Zevalin is Yttrium-90 (90Y) labelled Ibritumomab tiuxetan and Bexxar is Iodine-131 (131I) labelled Tositumomab. These radio-immunotherapy (RIT) conjugates combine tumour targeting of the antibody with potent therapeutic radioisotopes. In relapsed or refractory patients with FL single agent RIT produces response rates of 60-80% and complete response in 15-30% (123-128). In a comparison with Rituximab complete response to Zevalin was superior (30 vs. 16%). However, no difference in progression free survival was found (129). Use of RIT is more effective in treatment naïve patients (130). The significant drawback of RIT is the myelosuppression occurring 6-10 weeks after therapy. This limits its use to patients with <25% bone marrow disease or to the setting of autologous stem cell transplantation. Combination RIT and high dose therapy with stem cell rescue has been successful with one study reporting a 5-year overall survival of 67% compared with 53% without RIT. A further phase I/II study has shown an overall survival and relapse free survival of 92% and 78% respectively (131, 132).

Chronic lymphocytic leukaemia (CLL) is a common B-cell malignancy that is better termed a lymphoproliferative disorder than a leukaemia. Being a B-cell disorder it would be considered a good target for Rituximab therapy. Unfortunately results of single agent Rituximab in CLL have been poor (118, 133, 134). Combination with conventional therapies has been better, especially when combined with fludarabine. Complete response rates are almost doubled by the combination of Rituximab and Fludarabine (135, 136). The most successful MAb in treatment of CLL has been Alemtuzumab. This is an anti-CD52 monoclonal antibody expressed on peripheral blood lymphocytes and monocytes and macrophages. Alemtuzumab causes cell lysis via complement fixation, antibody-dependent cell-mediated cytotoxicity and direct induction of apoptosis (137), with an overall response rate of 33% (138). Alemtuzumab is now first line therapy for CLL in selected patients, but because CD52 is found on peripheral blood lymphocytes Alemtuzumab is more effective on blood and bone marrow disease than solid lymph node disease.

The lymphoproliferative disorders are now not the only group of diseases in which antibody therapy is used. Gemtuzumab ozogamicin (Mylotarg) is a humanised anti-

CD33 monoclonal antibody linked to calicheamicin, an antibiotic with potent anti-tumour activity (139). CD33 is found on leukaemia cells in 90% of patients with AML (140). The Gemtuzumab antibody binds to the surface CD33 and is internalised within the cell, releasing the calicheamicin, leading to apoptosis through DNA breakages (141). This has been shown to achieve complete remission in 16% of patients with relapsed disease in the pooled data from phase II studies (142). Combination of Gemtuzumab with conventional AML therapies has been of little benefit in relapsed patients with complete response rates between 10-20% (143-145). However, in previously untreated patients results are more promising. Response rates between 35-85% are reported with median survival approaching 12 months in one report (146, 147). Further assessment is needed to ensure the benefit of Gemtuzumab in AML and clinical trials are ongoing.

Antibody-directed therapy and drugs aimed at specific pathways controlling cell growth, division and differentiation are likely to come to the fore over the coming decades allowing improvements in outcome, reduction in side effects and limitations of long term effects of therapy.

NON-MALIGNANT HAEMATOLOGY

Iron overload

The majority of the haematology workload deals with malignancy and as such it is unsurprising that this has been where most research focuses. There have however been significant recent advances in the field of non-malignant haematology for specific groups of patients with demonstrable health needs. On a global scale the most important is the development of new iron chelation agents for those requiring long term transfusion therapy because of haemoglobinopathies (thalassaemia and sickle cell disease), myelodysplastic syndromes or other disorders requiring regular transfusion.

The human body has no effective mechanism for removal of excess iron. Body iron levels are regulated at the point of absorption in the duodenal enterocytes. Thus parenterally administered iron bypasses this control mechanism and any excess will be retained causing secondary iron overload (148) in the liver, heart and endocrine glands leading to hepatic impairment, cardiac failure and arrhythmias, infertility, poor growth and diabetes (149-152). Each unit of transfused blood contains 200-250mg of iron, so a typical adult patient requiring a three unit transfusion each month will gain 7-9g of iron annually that cannot be excreted (153).

Iron chelating agents aid the removal of excess iron. Desferrioxamine has been the mainstay of iron chelation therapy for 40 years. It is licensed for use in the treatment of any transfusion dependent anaemia and acute iron intoxication. It has been shown in large continuing cohorts to improve long term survival and minimise the long term effects of iron overload (154). Compliance is poor, especially amongst young people as it must be administered subcutaneously or intravenously over long periods with frequent local reactions. Poor compliance leads to gradual iron deposition and death through cardiac disease (155, 156); it also requires close monitoring for toxicities affecting the eyes, ears and skeletal system. (157).

In the 1990s a new thrice daily oral iron chelation agent called deferiprone (Ferriprox) was investigated. It is as effective as desferrioxamine at standard doses except in cases of mild iron overload where higher doses are required. Combination of this newer drug with desferrioxamine is highly effective at reducing iron stores within hepatic and cardiac tissue (158). Deferiprone is now the iron chelator of choice in significant iron-related cardiac disease either singly or in combination with a statistically significant improvement in both left ventricular function and overall survival (159, 160). The major concern with deferiprone, other than it not having ever completed phase III trials due to disagreements between researchers and the producing drug company (161), is its significant risk of neutropenia. This occurs in 8% of patients with complete agranulocytosis in 1% - although deaths have been rare regular weekly monitoring of blood count is required (162). Because of this significant risk and milder effects on hepatic function, deferiprone had only been licensed in patients with thalassaemia major when desferrioxamine therapy is contraindicated or inadequate.

Within the last year a once daily oral preparation called deferasirox has been licensed for treatment of iron overload due to transfusion in thalassaemia major and other transfusion dependent anaemias where other therapy has failed, including in children. These two factors make it more tolerable and useful than the other two preparations in the majority of patients. This new drug is as effective as desferrioxamine in patients with severe iron overload but less effective in mild cases (163). This is likely to reflect a fault in the phase III study design in which patients with less iron overload were continued on relatively high doses of desferrioxamine when the new drug was started (157). Adverse effects are common with this drug with gastrointestinal problems in 15%, skin

rash in 11% and 38% of patients having an isolated raised creatinine, but no evidence of further renal disease. These problems rarely cause cessation of therapy. The main problem that does lead to deferasirox being stopped is that of raised liver enzymes, usually ALT, and this must be monitored closely - it occurs more commonly in patients with hepatitis C (157).

Studies are now underway to determine which drug is best suited for a specific indication. For those starting on iron chelation deferasirox is probably the treatment of choice but long term sequelae are as yet unknown. Deferiprone remains the most useful in cases of severe cardiac disease, either on its own or in combination.

Coagulation

Recombinant Activated Factor VII

The use of recombinant activated factor VII (rFVIIa) has increased dramatically following largely anecdotal evidence. This expensive product was first developed in the 1990s to bypass factor VIII in patients with haemophilia who had developed inhibitors (antibodies) to their prophylactic factor VIII rendering it ineffective. Its licensed uses have been expanded to include rarer bleeding disorders where the use of blood products such as platelets or plasma may put patients at risk of sensitisation or infection including Glanzmann's thrombasthenia, congenital factor VII deficiency and acquired haemophilia. It is interesting then that this drug is one of the most requested agents for patients who are bleeding due to trauma, surgery or childbirth, despite the minimal amount of evidence to support its wider usage.

More than 1000 reports of rFVIIa use have been published, and more than half of these describe off-label (non-licensed) uses (164), largely due to the lack of randomised trials to prove its efficacy and the notion that a universal haemostatic agent is needed.

The physiology of factor VII must be understood to some degree before the success or failure of its use can be assessed. Factor VII combining with tissue factor (TF) is the first step of the coagulation cascade which is now known to be far more complex than the traditional intrinsic and extrinsic pathways. The binding of FVII and TF to form activated FVII (FVIIa) subsequently activates factors IX and X and consequently thrombin is formed. The formation of thrombin provides positive feedback on factors V, VIII and XI and activating platelets. These activated factors stimulate the cascade to produce more thrombin and ultimately a fibrin clot. This cascading of factor activation and amplification requires the presence of its constituent parts including the platelets that the cascade ultimately works on, and certain

physiological conditions. The patient's core temperature needs to be adequate for FVII to work. Coagulation factor and platelet activity decreases as the temperature falls (165-168). Acidosis also has a profound effect – as pH falls from 7.4 to 7.0 coagulation activity starts to fall and prothrombin activation falls by 70% (165). Hypothermia and acidosis together have an even greater detrimental effect (166). Anaemia also affects coagulopathy as erythrocytes have important mechanical and biochemical functions (169-171) aside from oxygen delivery. Treatment of anaemia with red cell transfusion is essential to improve bleeding time because of the reduced platelet aggregation seen if the haematocrit falls below 20% (172). Without a patient in a reasonable physiological state with the correct amount of blood products for factor VII to work on, there is little point in giving any, highlighting a recurring, fundamental problem with requests for the 'universal haemostatic agent'. Patients are often cold, acidotic and anaemic from their trauma, and the other required factors and

platelets are absent. Aggressive assessment and replacement of these is essential before reaching for the rFVIIa (164, 173).

The dose of rFVIIa needed to achieve haemostasis is difficult to determine. In most cases the dose is based on the experience with haemophilia patients and results of the early series (173). The recommended initial dose for treatment ranges from 90-120µg/kg with subsequent doses at the lower end of the range. Factor VII has a half life of less than 2 hours so repeat doses may need to be given at intervals of two to three hours. Because of the cost of rFVIIa (almost £1 per µg, with a dose for a 70kg patient costing almost £7000) lower doses are being used in trials to assess outcome in different situations.

There have been few trials assessing the effect of rFVIIa in clinical situations and most data has been collected in small series and often retrospectively. The end points in these trials are usually a reduction in blood product support rather than improved survival because of lack of statistical power. Table 5 details some recent trials and their

Patient type	Dose g/kg rFVIIa	Number	Outcome	Reference
Prostatectomy	Placebo vs. 20 vs. 40	36	Reduced packed red cell volume replacement required post operatively from 2500ml to 1000ml and 800ml for each treatment arm respectively	(174)
Blunt and penetrating trauma patients	Placebo vs. three sequential doses of 200, 100 and 100	277	Half as many blunt trauma victims required >20 unit blood transfusion in treatment arm. No significant difference was found in penetrating trauma victims	(175)
Intracerebral haemorrhage	Placebo vs. 40, 80 and 160	399	Significant reduction in 90 day mortality in two higher dose groups. Haematoma volume was significantly reduced in the higher dose group and neurological function was improved at 90 days if treatment occurred within 4 hours of documented haemorrhage.	(176)
Liver biopsy	5, 20, 80 and 120	71	Prophylaxis achieved adequate haemostasis during and following procedure	(177)
Upper GI bleeding in liver cirrhosis	Placebo vs. 8 doses of 100	245	Reduced bleeding in patients with severe liver disease only	(178)
Partial hepatectomy	Placebo vs. 20 and 80	204	Reduced peri-operative blood loss in higher dose group	(179)

Table 5 – Recent trials of off label rFVIIa use, patient numbers and outcome.

outcome. The published studies are generally positive about the effect of rFVIIa; but the risk of thrombotic events must also be remembered. In one study a greater than three fold increase in such adverse events (7% v 2%) was seen in patients treated for intracerebral haemorrhage (ICH) with rFVIIa compared to placebo (176). The reason for this effect is unclear but may be related to the localised nature of the ICH as opposed to a global coagulopathy - certainly other trials have not demonstrated such a large increase in thrombotic events except in situations where rFVIIa is used in conjunction with, or shortly after, other procoagulant products such as prothrombin complex concentrate (164). A 5-year review by the Federal Drug Administration reveals the actual incidence of thrombotic events following rVIIa use to be very low. There was an almost equal split between arterial (54%) and venous (46%) thrombotic events (180).

Although patients requiring rVIIa are rarely those of the haematologist themselves, the haematologists are often the gate-keeper from whom advice is sought and who release the product for use. Patients that may gain benefit for administration of rFVIIa are often those suffering major trauma and a number of guidelines and algorithms now exist to aid their management (164, 181). Requests for advice and help should be met with respect and common sense. A practical approach to these often complicated patients may be to let the haematologist liaise with the laboratories to assess coagulation and provide advice and blood product support; let the anaesthetist manage the airway and fluid balance; let the surgeon or trauma team manage obvious points of bleeding. This approach will reduce individual stress and through a multidisciplinary approach improve outcomes.

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