Advances in Haematological Pharmacotherapy in 21st Century

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Abstract Last quarter of twentieth century and the first 10 years of 21st century has seen phenomenal development in haematological pharmacotherapy. Tailor made chemotherapeutic agents, vast array of monoclonal antibodies, epigenetic modifiers, growth factors for red cells white cells and platelets, peptidomimetics as growth factors, newer thrombin inhibitors, safer plasma derived protein molecules, recombinant molecules, newer immunomodulators, enzyme replacement therapy and above all a plethora of targetted molecules targeting innumerable pathways involved in cell division, growth, proliferation and apoptosis has given immense number of clinically usable molecules in the hand of modern haematologists to treat diverse hitherto untreatable haematological disorders effectively. In addition many old molecules are finding newer uses in diverse fields, thalidomide as an antiangiogenic molecule is a prime example of this genre. Present overview has tried to capture this rapidly evolving area in a broad canvas without going into details of indications and contraindications of the use of various drugs.

Keywords Pharmacotherapy · Haematology · Haematooncology

Introduction

Development in haematological pharmacotherapy was rather slow in the first half of twentieth century. Iron, Vitamin B₁₂ and folic acid was demonstrated as important

nutrients for haemopoietic system during this period. Though synthesis of corticosteroid hormone was accomplished around this time, its effect on various haematological disorders were explored mainly during second half of the twentieth century. Cytotoxic drugs, anti metabolites, other immunosuppressive drugs, antimalarials were all used extensively during second half of twentieth century. However during the last quarter of twentieth century certain important discoveries changed everything in the arena of haematological pharmacotherapy for many incurable disorders. These discoveries were (a) Development of innumerable antimetabolite and cytotoxic drugs. (b) Tremendous advances in blood product and supportive therapy allowing more intensive use of cytotoxic drug alone or in combination (c) Development of algorithms for preemptive antimicrobial treatment in immuno suppressed host before microbiology laboratory results were available (d) Development of monoclonal antibodies for management of various malignant and non malignant conditions (e) Recombinant proteins and better understanding of basic processes of cell division, differentiation, growth, migration and cellular death. More than 100 different cellular biochemical pathways involving innumerable stimulatory and inhibitory protein kinases, phosphatases and intracellular communication by various kinases and inter cellular communication by various lymphokines, cytokines, chemokines, monokines fractal kines along with identification of innumerable adhesion molecules paved the way for development of innumerable targetted therapies, growth factors etc. which we have just started to realise. The present review is on those chemical entities which we are regularly using in day to day treatment of haematological disorders. One of the author reviewed the subject in the pages of the same journal about 20 years back and correctly predicted an era of explosive development in this arena [1].

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Recombinant Proteins

Though recombinant human insulin was the first recombinant product to be used in medicine, recombinant human erythropoietins underwent clinical trial for anemia in chronic renal failure [2, 3]. With spectacular result way back in 1987–1988. This condition is still the major indication of use of this hormone but over the years the product was found to be useful for anemia of prematurity [4], in certain subsets of MDS where anemia is associated with <500 IU/ml of serum erythropoietin levels [5], in improving the quality of life in patients with cancer complicated by severe anemia [6] and the product has also been used for collecting more blood units for autologous red cell transfusion to avoid allogeneic exposure of blood [7] for preventing rare possibility of HIV and other viral transmission during transfusion. Immediately following discovery of recombinant erythropoietin other therapeutic recombinant proteins like G-CSF, GM-CSF were introduced to prevent chemotherapy induced neutropenia [8] and to reduce the neutropenic period following allogeneic or autologous stem cell transplantation [9]. G-CSF is also regularly used nowadays for mobilisation of haemopoietic stem cell in peripheral blood [10]. The product has also been found to be useful in treating cyclic neutropenia, Kostmann syndrome and agranulocytosis due to various causes [11–13]. When used locally it was found to improve chemotherapy induced stomatitis, mucositis [14] and expedited wound healing. The drug also improved the transport of chemotherapeutic agent like cytosine arabinoside into the leukaemic cell and is used for this purpose in certain chemotherapeutic combination for treatment of AML (FLAG-Ida) [15]. Several recombinant proteins like recombinant factor VIII, recombinant factor IX and recombinant active factor VII (novoseven) changed the lives of patients with bleeding disorders like severe haemophilia A, haemophilia B and haemophilia A patients with inhibitor [16–18]. Recombinant active factor VII has also found its use in innumerable congenital bleeding diathesis involving platelet dysfunction [19]. The product has been used with success for many acquired bleeding conditions. Developing a recombinant growth factor for treatment of chemotherapy associated thrombocytopenia or other causes of thrombocytopenia initially met with mixed success when recombinant IL6 [20] and recombinant IL11 [21] were used. But soon it was found out that these two growth factors are not the real thrombopoietin molecule investigators were looking for. However, thrombopoietin molecule was cloned soon enough [22–24] and a recombinant thrombopoietin molecule (oprelvekin^R) was available in the market. However this product was not found to be useful for chemotherapy associated thrombocytopenia moreover this protein lead to the development of antibodies

to thrombopoietin and aggravated the condition in a large number of patients. Hence the product was quickly withdrawn from the market [25]. However, this protein gave substantial leads to develop smaller thrombopoietic peptide (Rhomiplostim) and a peptidomimetic which can be given orally (Eltrombopag), both the drugs were found to be effective for supporting continuous treatment with interferon and ribavarin for hepatitis C, where interferon induced thrombocytopenia was found to be one of the major cause of treatment withdrawal [26, 27]. The two drugs have also been used successfully in immune thrombocytopenic purpura [28, 29]. Several cytokines like interferon α , interferon γ , interleukin 2 has now been cloned and the recombinant protein is now available to treat various malignant and non malignant disorders. Some of the disorders where interferon α had a brief period of application and success were hairy cell leukemia, multiple myeloma, chronic myeloid leukemia. As better products are now available to treat these conditions, interferon α is no longer used for treating these patients. However, interferon α is still used mainly for treatment of hepatitis C in combination with another anti viral drug, ribavarin and is now the standard of care [30]. Interferon α used in this condition could be simple interferon α or its long acting variant e.g. pegylated interferon alpha. Several recombinant proteins developed during this time are routinely used for treatment of congenital coagulation disorders e.g. recombinant factor VIII, recombinant factor IX, recombinant activated factor VII. Of this recombinant factor VII (novoseven) is increasingly used for various acquired and congenital bleeding disorder as broad spectrum haemostatic agent [31]. This product will not be active when bleeding is due to factor X, factor V, factor II and fibrinogen deficiency. However, the product was found to be quite safe even when used in very high dosage of 270 $\mu\text{g/kg}$ for haemophilia A patients with inhibitor formation [32]. Recombinant clotting protein has found extensive use in replacing missing clotting protein in Haemophilia A and Haemophilia B mainly because (i) The product can be obtained in extremely pure form (ii) unlimited amount of this product can be produced e.g. not limited by amount of plasma which can be collected from human donors (iii) many serious viral infections which is transmitted from man to man via plasma product can be eliminated. Recombinant proteins are also extensively used to lyse blood clots (Recombinant TPA, Alteplase, Tenecteplase etc.) in myocardial infarctions, acute pulmonary embolism, VOD following allogeneic BMT [33–35].

Moreover in certain situations where heparin cannot be used as an anti coagulant due to life threatening complications like heparin induced thrombocytopenia (HIT), recombinant proteins like recombinant hirudin, bivalirudin has been used with success [37].

The area of recombinant therapeutic proteins from human and other animal sources (Tick salivary protein as an anticoagulant) are going to expand day by day and these products are likely to continue to enrich a haematologist's pharmacological armamentarium in years to come.

Monoclonal Antibodies

Potentialities of monoclonal antibodies as important therapeutic agent for haematological pharmacotherapy was anticipated since the discovery of the process of making these antibodies by Kohler and Milstein [38]. However, initial experiment with mouse monoclonal antibodies for treatment of various leukaemias and lymphomas failed because of development of anti mouse antibodies in human and the targets for therapeutic action was not properly chosen, so that many receptor protein on malignant cells for which monoclonal antibodies were used started producing alternately spliced soluble targets which were not on cell membrane or else the cells did not produce these target at all! Moreover, if monoclonal antibodies were produced against intracellular targets then these antibodies were not capable of penetrating the intact cell membrane to reach its intended sites of action. Even then rat monoclonal antibody Campath-1 was regularly used invitro to reduce the T cell load from the marrows from unrelated donor and then the T cell depleted narrow was used for transplantation [39]. However advances in technique allowed us to marry monoclonal antibody technology with the techniques of recombinant protein production leading to humanised or human monoclonal antibodies suitable for use in haematological pharmacotherapy. Complement fixing anti CD-20 monoclonal antibody (Rituximab) is extensively used now a days for treating various types of lymphoid neoplasms expressing CD-20 and its result in intermediate and diffuse large B cell lymphoma as a part of CHOP-Rituximab are impressive [40, 41].

Rituximab can now be tagged with various suitable radio isotopes (^{131}I , ^{90}Yt) for effective therapy in cases of NHL where a small clone may have down regulated its CD20 production and may be involved in future relapse. Rituximab is increasingly being used with success for resistant ITP [42], Autoimmune haemolytic anaemia (AIHA) [43] and also in some patients with acquired inhibitor formation against factor VIII where classical therapies with steroid and/or cyclophosphamide were ineffective or could not be given [44]. Anti CD 33 in combination with calicheamicin (ozogomicin) is successfully used to induce remission in acute myeloid leukemia [45] particularly AML in the elderly where many cytotoxic drugs could not be used without the price of long term myelo suppression with its attendant complication. Anti CD3 monoclonal antibodies is used in

resistant acute Graft Versus host disease and humanised anti CD52 (Campath-1H) is used for both T cell as well as chronic B cell neoplasm as well as an agent to deplete T cell load when peripheral blood stem cell is obtained from unrelated HLA matched donor [46, 47] or for haploidentical marrow transplantation [48].

Monoclonal antibody directed against complement component C_{5a} (eculizumab) is regarded as a single most important advance in treating severe anemia associated with paroxysmal nocturnal haemoglobinuria [49] and the results are spectacular to say the least. Similarly anti VEGF receptor antibody Bevacizumab [50] has been used successfully in some of the patients with extensive hereditary haemorrhagic telangiectasia (HHT) threatening to cause liver failure. One of the monoclonal antibodies directed against GPIIb of platelet glycoprotein, abciximab (7E3) is now extensively used to prevent thrombosis following percutaneous transluminal angioplasty in coronary artery disease [51].

The area of use of monoclonal antibodies for the management of various haematological disorder is an evolving field and new antibodies with various new application and older antibodies with novel applications are likely to develop continuously.

Fractionated Plasma Proteins and Other Proteins

War lead to the development of plasma protein fractionation technology (Cohn's Fractionation), this technology continues to evolve through gel exclusion column chromatography, cation–anion exchange chromatography, affinity chromatography, immunoaffinity chromatography to the present level techniques of nanofiltration. All these technologies in various combination coupled with various virus inactivating procedures have given us replacement therapy for a host of coagulation disorders [52] specific immunoglobulins for prevention of certain diseases, antithrombin III, Protein C concentrates for use in thrombophilic conditions and intravenous immunoglobulins for its use in various autoimmune haematological disorders (e.g. ITP, acquired factor VIII deficiency) and in case of humoral immunodeficiencies either congenital and acquired as in case of chronic lymphatic leukaemia or for certain periods after allogeneic haematopoietic stem cell transplantation [53].

Antithymocytic globulin (ATG) or Antilymphocytic Globulin (ALG) produced in horses or in rabbits is extensively use in haematology for treating severe aplastic anaemia [54] or severe anaemia in myelodysplastic syndrome particularly those who are positive for DR15 [55, 56] and for conditioning the marrow transplantation for various disorders [57]. Similarly bacterial protein product like streptokinase is one of the most extensively used

orthrombolytic known to mankind. Apart from its extensive use for recanalisation the coronary artery to establish reperfusion in acute myocardial infarction, this product has also been used with success in severe pulmonary thromboembolism, in extensive venous thrombosis where quick development of patency is an urgency and for Hickman's line thrombosis [58].

Enzymes purified from placenta has been used with success for enzyme replacement therapy and has changed the lives of many patients with severe Gaucher's Disease. Tissue sealants are now a days produced by mixing fibrinogen and thrombin at the site of bleeding. In congenital bleeding disorder as well as in extensive operations where a big raw area remains, spraying tissue sealants in those areas can achieve effective haemostasis.

Cytotoxic Chemotherapy [58]

Cytotoxic Chemotherapy had its origin in the first world war when sulphur mustard was extensively used as wargas. Nitrogen Mustard (mustine hydrochloride) was the first cytotoxic chemotherapy of a class called alkylating agents. These agents work by polyfunctionally alkylating DNA molecules leading to cell death. It functions like ionizing irradiation in a cell cycle non specific manner and hence also called radiomimetic agents. Various alkylating agents in addition to nitrogen mustard was discovered in the latter half of twentieth century and found extensive application in malignant and non malignant haematological disorder e.g. Chlorambucil for CLL, lowgrade NHL, cold antibody mediated haemolytic anaemia and as combination chemotherapy in Hodgkin Disease (LOPP-EVAP), Melphalan alone or in combination with other agents are used in multiple myeloma. High dose melphalan was used for treating multiple myeloma and is used for conditioning before autologous or allogeneic haemopoietic stem cell transplantation. Procarbazine is mainly used in the form of combination chemotherapy in Hodgkin's disease. Busulphan was used for controlling leucocyte count in chronic myeloid leukaemia in chronic phase but is now superseded by Imatinib, a tyrosine kinase inhibitor about which we will have a detailed discussion in the following pages of this paper. Cyclophosphamide was developed as a broad spectrum alkylating agent which needed activation in the liver or inside the leukaemia/tumour cells before being an active alkylating agent. This agent is extensively used for many malignant and non malignant haematological disorders like various lymphomas, multiple myeloma, acute lymphoblastic leukaemia, steroid resistant ITP, multiple myeloma with renal disease to name a few. High dose cyclophosphamide with or without Busulphan is regularly used for conditioning before allogeneic haemopoietic stem

cell transplantation. High dose cyclophosphamide has also been tried as a definitive therapy for severe aplastic anaemia but high mortality rate prevented its further use inspite of its efficacy in 50% of patients [59]. Most recent addition in the alkylating agent family is Benda mustine. This drug underwent extensive trial and is now recommended for low grade NHL and CLL [60, 61].

A series of anti metabolites were developed for cytotoxic chemotherapy for different haematological malignancies. Methotrexate is the oldest and most extensively used anti metabolite. This is followed by cytosine arabinoside, Fludarabine Cladribine and 5'Azacytidine. They are also extensively used with great success in treatment of AML, Hairy Cell Leukaemia. In fact discovery of cytosine arabinoside and intercalating agents like Daunorubicin and its derivatives and Mitoxantrone started a new era in AML chemotherapy. Before introduction of 3 + 7 regime (Daunorubicin 3 days + Cytosine Arabinoside 7 days) AML was a virtually untreatable condition. With this regime almost 80% of the patient could be induced a first remission.

Many other combination chemotherapy developed around Intercalating agent + cytosine arabinoside with equal success. These combination chemotherapies i.e. DAT or TAD (with -6-thioguanine), ADE (with etoposide) FLAG-Ida (with Fludarabine, Idarubicin and G-CSF), HAM (High dose Ara-C with mitoxantrone) are extensively used for AML therapy.

Adenosine Deaminase inhibitor 2 deoxycoryformycin (Pentostatin) was found to have striking effect in curing Hairy Cell Leukaemia [62]. This drug replaced the previous interferon therapy for the disease. However, it had an extensive array of severe side effects hence a much milder drug cladribine is now a days used in this condition without any loss of efficacy [63].

Fludarabine, Cladribine are new class of antimetabolites. These drugs produces long term immunosuppression with potential for many opportunistic infections to emerge. There ability to produce long term immunosuppression have lead to the use of Fludarabine for minimally conditioned allogeneic haemopoietic stem cell transplantation [64].

This method of haemopoietic stem cell transplantation is applicable to much older patients and graft versus leukaemia effect is maintained well in this type of conditioning regime hence it is suitable for treating leukaemias where chemotherapy has failed and it is risky to use ablative marrow conditioning before transplantation.

Mitotic spindle poison as a cytotoxic drug was introduced in 1960s in the form of Vincristine and Vinblastine. These drugs are extensively used today for treatment of ALL and in various non Hodgkin's lymphomas. Vincristine has also been used in ITP to improve the platelet count.

Presently Vincristine analogue Vinorelbine is undergoing trial for many haematological malignancies [65].

Topoisomerases are important targets for cancer chemotherapy. Many intercalating agents like Daunorubicin, Mitroxastrone, Amsacrine acts as topoisomerase inhibitor, Etoposide (VP-16) and Vindesine (VM-4) are derivatives of podophyllum resin and are specific inhibitor of topoisomerase II. Etoposide is presently one of the most extensively used chemotherapeutic agent used for various haematological malignancies including Haemophagocytosis syndrome. However Topoisomerase II inhibitors also produce a specific sub category of AML involving specific cytogenetic changes in a small subset of patients receiving this drug [66].

One of the oldest antileukaemic drug for ALL i.e. L-asparaginase, has a unique mechanism of action. Discovered in mid 1950, this drug depleted L-asparagine from the surroundings of acute lymphoblastic leukaemia cells which needs this compound for its growth. L-asparaginase is presently obtained from bacterial sources like *E. Coli*, *Erwinia* etc. a recombinant L-asparaginase will also be available shortly.

L-Asparaginase inhibits protein synthesis by liver and produce a thrombophilic condition.

Inhibitors of Angiogenesis

Concept of inhibiting angiogenesis or manipulation of the microenvironment to make it hostile or inimical for tumor cell growth is a new paradigm for treatment of haematological and other malignant neoplasms and other clonal haematological disorders. The proof of this concept started with striking results obtained with Thalidomide, a known antiemetic agent with proven teratogenic effect (due to inhibition of angiogenesis) for treatment of refractory multiple myeloma [67]. Subsequently this drug in various combination with Melphalan, Dexamethasone and Bortezomib was developed as a frontline therapy for multiple myeloma [68]. Thalidomide has hypnotic side effects hence surrogates were produced some of which is 100 times more potent as antiangiogenic with reduction of its hypnotic effect. Lenalidomide is one such compound which is being extensively used in a subset of myelodysplastic syndrome associated with 5q-cytogenetic change [69]. In addition it can also be used in multiple myeloma like thalidomide. Lenalidomide can produce dose depended marrow suppression and both thalidomide and Lenalidomide can produce venous thrombosis, other side effects of thalidomide like constipation and peripheral neuropathy is also well known. Many more chemical entities in this area are expected to be developed in future.

Bevacizumab is a monoclonal antibody directed against VEGF-Receptor and prevent angiogenesis by preventing

the binding of its ligand. Its usefulness in hereditary haemorrhagic telangiectasia has already been referred to [70].

Targetted Therapies

With the demonstration of oncogenic activation in various haematological malignancies and finding various transcriptional deregulation due production of hybrid oncoproteins, concept of interfering with these protein's action to treat neoplastic conditions were gaining ground. Serendipitous demonstration of striking effect of All Trans Retinoic Acid (ATRA) in acute promyelocytic leukaemia [71] and subsequent demonstration of its target as PML-RARA hybrid oncogene [72] clearly showed that many such molecule which are dysregulated in various neoplastic disorder could be targetted to produce specific targeted therapies with salutary effect.

Chronic myeloid leukaemia (CML) is an interesting neoplasm for which a specific cytogenetic abnormality was historically known as Ph chromosome. Later on Ph chromosome was found to harbour a hybrid tyrosine kinase called BCR-Abl oncogene. Successful development of an inhibitor to this tyrosine kinase in the form of Imatinib and its spectacular success in causing major cytogenetic remission in this condition in a large number of CML patients for the first time [73] resulted in virtual explosion in the development of various tyrosine kinase inhibitors targeting various oncogenes.

So spectacular was the results of Imatinib therapy in CML in chronic phase that the usual frontline therapy for CML with alpha interferon was relegated to the pages of history like use of Busulphan, Hydroxyurea for this condition and Imatinib became the frontline therapy for this condition [74, 75]. Imatinib therapy for CML also relegated allogeneic haemopoietic Stem cell transplantation to secondary place for management of this condition [76].

Meanwhile innumerable cellular path ways with individual molecules in serine kinase, threonine kinases, Tyrosine kinases and specific phosphatases associated with these kinases have been delineated to explain cell division, apoptosis, cell migration, stimulation of angiogenesis, signal transduction, cytokine secretion, communication inside the cell and communication with other cells and the process of differentiation of a primitive cell to different lineages are being worked out in greater details providing the drug developers and unprecedented opportunity to develop specific targeted therapies for years to come.

After almost 7–8 years use of imatinib, imatinib resistance is challenging the drug developers and to overcome much resistance already a series of second generation tyrosine kinase inhibitors like Dasatinib and Nilotinib [77]

are already in the market and is in current use. Tyrosine kinase inhibitors for other pathways like JAK-stat pathway are on trial for treatment of other myeloproliferative disorders and even for other solid tissue tumours like Sorafenib for hypernephroma. Imatinib has found good use in certain subsets hypereosinophilic syndrome too [78] where hypereosinophilia is the result of activation of hybrid PDGF oncogene.

Imatinib has also been used successfully in gastrointestinal stromal cell tumour (GIST) and in tumours involving basophils and mast cells where c-Kit oncogene is activated. Many of the signal transduction mechanism starts from association of ligands with its cognate receptors on cell membrane, some of these receptors are tyrosine kinase enzyme themselves. The enzyme activates following dimerisation of the receptor after the ligand engages the receptor. Some of these activated receptors engage RAS oncoproteins (>50 members of the family). Many of the RAS oncoproteins are GTP binding/activating/translocating proteins and they themselves are important therapeutic targets for future development.

These proteins needs to be farnesylated, geranylated, behenoylated or myristoylated for associating with components of cell membrane. Each of these processes are also therapeutic targets which remains to be exploited. Many patient with ANLL and aggressive MDS has mutation of RAS oncoproteins or they acquire such mutations when the disease progresses [79]. First generation farnesyl transferase inhibitor is another class of compound (ZarnestraR, Tipifarnib) had limited success with AML [80]. Similarly drugs targeting protein kinase-C, aurora kinases, PI-3K/AKT are being developed and will be tested in various haematological disorders [81].

Apoptosis is another important cellular process that is defective in many haematological malignancies like CLL. Apoptosis is an elaborate process with innumerable molecules involved both in its afferent and efferent limb [82]. Bcl group of proteins are important member of the family one of the molecule targeting Bcl-2 called Oblimersen is already in the market and have shown limited activity against CLL [83]. As already discussed with more than 100 biochemical pathways in a cell having innumerable players in each pathway [84] will keep drug developers busy for years to come. Though all of them may not find its use in haematological pharmacotherapy, some of them will definitely be useful to haematologists.

Epigenetic Modifiers

One of the cornerstone of genetic thought was that what somebody has inherited cannot be changed and the information there in is there for the lifespan of the organism.

However, studies in molecular biology proved that genes are dynamic entities, not set in stone, they can be activated as well as silenced through proper manipulation with different chemicals. Silencing of several oncogenes were found to be associated with ALL [84]. Studies in baboons in 1980s [85] have shown that chemotherapeutic drugs like 5'Azacytidine can activate foetal haemoglobin production in baboons. Detailed investigations showed that 5'Azacytidine cause nonspecific demethylation of cytosine across the genome and activates the foetal haemoglobin gene along with other genes.

The subject of epigenetics was thus born. It was found that cytosine methylation and histone deacetylation are two important mechanism for epigenetic silencing of various parts of human genome. One of the 5'Azacytidine derivative decitabine is already in use to improve haemoglobin in MDS by epigenetic activation [86]. Histone deacetylase inhibitors are simple compounds like hydroxamic acid derivatives, staurosporin. They have been used under trial in MDS and in acute myeloid leukaemia [87].

Unfortunately epigenetic activation of HbF gene and other specific anti-oncogenes is still not specific and HDAC inhibitors and cytosine demethylators target the whole of the genome which is not desirable.

Inhibition of Protein Degradation Through Ubiquitination of Oncoproteins

Many oncoproteins (products of cellular oncogenes) and anti oncoproteins are degraded inside the cell after their desired action is over. The methods and biochemical reactions by which these proteins are marked and taken to different subcellular regions/organelles for degradation are still being worked out and large part of it is yet to be discovered. However one of the pathways marks the proteins towards lysosomal degradation and another pathway is through ubiquitination of proteins directing its degradation in proteasomes.

Proteins marked for degradation in proteasomes are ubiquitinated and then transferred to proteasome for degradation. Bortezomib a modified dipeptide boronic compound which binds reversibly with 26S proteasome subunit with high affinity leads to accumulation and dysregulation of key cellular proteins including CDK inhibitor p21, p27, cyclins and caspases, tumour suppressor p53, Bcl2 and the oncogenes; C myc, cfos, and c Jun, the transcriptional factors E2A, E2F, STAT. The compound also inhibits the degradation of IKB which prevents the activation of NFKB. Disruption of these multiple cellular process leads to G2M cell cycle arrest and apoptosis [88]. Bortezomib has anabolic effect on bone and prevents adhesion of myeloma cells to its microenvironment. Bortezomib alone

or in association with other drugs like Thalidomide or Lenalidomide has been found to be an important agent to treat multiple myeloma [88–90].

Heparins and Heparinoids

Heparin is one of the oldest and widely used fast acting antithrombotic. However heparin has many draw backs for regular use. The product needs to be injected and regular control by APTT is required. Long continued use of this product can cause osteoporosis and fracture. Moreover in a subset of patients it can cause life threatening thrombocytopenia (HIT) along with DIC [91]. Moreover the action of intravenous heparin is short lasting.

Hence products were needed with better therapeutic window and better safety profile and better duration of action. Main mechanism of action of standard heparin is via antithrombin in plasma. In hereditary and acquired antithrombin deficiency there is heparin resistance. However it was found that when heparin is broken down to <5000 kd molecules or <5000 kd molecule is separated from higher molecular weight species by various means then this low molecular heparins (LMW Heparins) develops different biological activity. They no longer bind to antithrombin but directly inhibit active factor X (Xa). The half life of the product increase and the chances of heparin induced thrombocytopenia is reduced. In addition low molecular weight heparin has fibrinolytic activity which could be helpful under certain circumstances. Innumerable brands of low molecular heparin are now available in the market (Fluxum, Indeparin etc.).

Synthetic heparinoids like sulphated penta saccharide (Fondaparinum) with improved anti Xa activity and safety from heparin induced thrombocytopenia are also available. The area of synthetic heparinoids with improved activity and function is a rapidly developing area for antithrombotic therapy [92].

Synthetic Thrombin Inhibitor

Developing a direct thrombin or factor Xa inhibitor, particularly for oral use has been of abiding interest to haematologist practicing antithrombotic therapy. First generation of such compounds like Gabexate, Argatroban, Nafamostat were not found to be useful. Ximelargatran had a transient use and had to be withdrawn from the market because of hepatotoxicity [93].

Recently two compounds Rivaroxaban (Anti Xa) and Dabigatran (Antithrombin) both oral drugs have completed several clinical trials and have been shown to have better safety and superior anti thrombotic effect [94, 95]. It is

expected that these two compound may replace heparin and Warfarin in many situations particularly in many situation where thrombocytopenis complicates thrombophilia e.g. liver disease. Heparin and Heparinoids have one advantage in that, neither do they cross the placenta nor jeopardise the fetus to either teratogenesis or to increased haemorrhagic tendency.

Iron Chelators [96]

Many patients particularly those having severe congenital haemolytic anaemia need life long red cell transfusion as a result they develop substantial iron overload with innumerable health consequences. One of the first iron chelator which was used successfully in β thalassaemia major and intermedia patients with iron over load was a hydroxamic acid derivative called Desferrioxamine. Desferrioxamine is an efficient iron chelator but needs to be given by 10–12 h subcutaneous injection for at least 5 days a week. The injection should start after 50 units of red cell concentrate has been transfused and needs to be continued life long.

As continuous subcutaneous infusion is uncomfortable, there were always an effort to develop orally active iron chelator. Deferiprone (L1) was first met orally active iron chelator. Its use in β thalassaemia patients was mired in controversy and was never used extensively in developed countries. However the product underwent clinical trial in India [97] and in India and in other developing countries it is still one of the major oral iron chelator used. It was shown that combined 2 days in a week of desferrioxamine with oral deferiprone may provide better removal of iron from intracellular stores where desferrioxamine cannot reach and this concept is popularly known as iron shuttle hypothesis [98].

Because of the controversy around deferiprone search for superior iron chelator continued and this lead to development of another oral iron chelator called Deferasirox [99]. This product can be given orally and the iron is excreted via biliary tree in the G.I. tract like desferrioxamine. Deferasirox is slowly replacing Deferiprone as the oral iron chelator. However search for better iron chelator which can be given orally continues.

Old Wines in New Bottles

Over the time many drugs which were used for some other purposes in medicine or which were withdrawn from the market because of various toxicities have found newer uses in haematological pharmacotherapy. The names of some of these drugs and its haematological applications are as follows:

- i) Arsenic Trioxide is now used with great efficacy for treating acute promyelocytic leukaemia [100].
- ii) Thalidomide is finding multiple use in haematological therapy, but its main use is in the field of multiple myeloma.
- iii) All Trans Retinoic Acid (ATRA) which was originally used in dermatological practice is now the mainstay of treatment for APML (AML-M3).
- iv) Hydroxyurea has found a new use in treating sickle cell anaemia, where it reduced various complications of the disease [101].
- v) Hydroxyurea is finding limited application in improving the quality of life in β thal intermedia patients or reducing the transfusion requirement in a proportion of β thal major patient [102].
- vi) Dapsone, an anti leprosy compound is now used to treat a subset of patient with ITP [103].
- vii) Clarithromycin with Dexamethasone has been used to treat Waldenstrom's macroglobulinaemia [104].
- viii) Injectable rifampicin and tetracycline locally has been found useful in treating haemophilic chronic arthropathy [105].
- ix) Psoralen compounds which has been used traditionally for vitiligo is now used for extra corporeal phototherapy in treating chronic lymphatic leukaemia or for graft versus host disease [106, 107]. Similar compound has now been used to make wet blood product safer from transmission of unknown infecting organism.
- x) Diflunisal: For removing amyloid.

Miscellaneous Compound Used in Haematological Pharmacotherapy

In addition to large classes of compounds and proteins described above there are miscellaneous compounds or products that are used for various purposes by haematologist. The list below is illustrative but not exhaustive.

- i) Substrate reduction therapy for Gaucher's disease where one cannot afford costly enzyme replacement therapy: Meglustat [108].
- ii) Therapy for Amyloidosis—Eprodinate [109].
- iii) Reducing fibrosis in myelofibrosis: Defibrotide.
- iv) Reducing Platelet count in essential thrombocythemia: Anagrelide
- v) Reducing Hypercalcaemia: Various Bisphosphonates
- vi) Protection against chemotherapy associated toxicity—Amifostine
- vii) Corynebacterium Parvum: For malignant pleural effusion.

New Formulation of Old Products

Several technical advances have allowed haematologist to use certain chemotherapy or anti infective drugs at a much higher concentration and with greater efficacy and reduced toxicity. The technical advance involves either pegylation of the parent compound or covering the parent compound in liposomes. These formulations are costly. However pegylated compounds have much longer half lives and is much less immunogenic than the parent compound. Liposomal formulation reduces the toxicity of the compound. Examples Pegylated interferons, Pegylated L-Asparaginase, Pegylated alpha interferon, Liposomal doxorubicin. Liposomal amphotericin are such examples. In future many more chemotherapeutic agents may be available in liposomal or pegylated formulation.

Primary prophylaxis in children with severe haemophilia is now gaining momentum as the preferred mode of management and this requires multiple intravenous injection of factor concentrates over many years (three times a week for factor VIII and two times a week for factor IX) hence demand for developing long acting factor concentrate is gaining ground and a few product is already in the horizon and will have clinical trial shortly.

Conclusion

The above overview clearly showed how haematological pharmacotherapy have progressed by leaps and bounds over the last 3 decades. With a mere handful of minerals and vitamins a haematologist of the bygone days must have felt helpless about innumerable conditions about which little was known and little could be done. Today haematologist is now flooded with drugs and compounds which can be used for different conditions. Understanding of the basic processes in the cell and unprecedented advances in molecular biology, combinatorial chemistry, drug designing, insilico bioinformatics is paving the way for a revolution in haematotherapy in days to come. In this brief over view I have not discussed the advances in procedures, cell based therapies and in advances in antimicrobial compounds which has improved haematological care substantially.

In this brief review we have tried to draw a landscape placing major developments in this field without going into too much details on individual drugs because that was not our objective. Our objective was to show the beautiful garden of haematological pharmacotherapy but not the details of individual flower in the garden as that would have enchanted the viewer and would have taken the beauty of garden away.

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