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Myeloma: new insights

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In 1844, Solly described the first case of myeloma to be reported in the literature [1]. It occurred in a 39-year old housewife called Sarah Newberry, who presented with a 4-year history of fatigue and back pain and subsequently developed fracture of both femurs as her husband carried her to the bed. She was admitted on 15 April 1844 to St Thomas' Hospital where she was treated with orange infusions, rhubarb pills and opiates but died suddenly 5 days after admission. A post mortem revealed that the cancellous bone had been replaced by red matter consisting of clear cells with one or two well defined nucleus. Solly also noted that her urine contained a large amount of phosphate of lime and concluded the bane was being absorbed and excreted by the kidneys. It took over 100 years before the first chemotherapy drug, Melphalan, was shown to be of benefit for disease control but, as a single agent by mouth [2], only 50% of patients were induced to complete remission and overall survival remained unchanged at 2.5–3 years for almost a generation [3].

In 1987, Selby and colleagues [4] reported an improved complete response rate (CRR) to 30% by dose escalation of melphalan to 140 mp/m^2 and to 50% with further dose escalation to 180 mp/m^2 with haemopoietic stem-cell support. Although initially associated with a high morbidity, due primarily to GI toxicity, improved supportive care significantly reduced complications and the challenge now has become that of maintaining the complete responses (CR) that are induced. Autologous stem-cell transplantation has been shown in uncontrolled studies of patients with chemotherapy-sensitive disease to be relatively safe with a transplant-related mortality of 5–8% [5]. In addition, this approach is effective in improving the CRR and in prolonging progression-free and overall survival (OS) [6,7]. Several historical comparisons have shown superiority for high-dose therapy (HDT). The Intergroupe Francais du Myelome study (IFM90) [8,9] was the first randomised study to demonstrate a significantly improved CRR or very good partial response (VGPR) of 38% for HDT versus 14% ($P<0.001$) for conventional chemotherapy. This study has now matured with a median follow-up of 7 years and an updated analysis confirms the superior outcome for HDT for event-free survival (EFS) (median 28 months versus 18 months, 7-year EFS 16% versus 8%, $P=0.01$) and OS (median 57 months versus 44 months, 7-year OS 43% versus 25%, $P=0.03$). A further French trial (IFM 190) [10], however, did not demonstrate a survival advantage for the HDT arm and the results from a number of other studies are pending. Furthermore, it should be noted that the 7-year survival in IFM90 was low with no evidence of a plateau. A further note of caution has been injected by a paper from Blade and colleagues [11] showing that, in patients who are potential candidates for early HDT intensification and auto transplantation, but who were conventionally treated in a combination chemotherapy study, there was no difference in OS with historical controls.

Tandem autologous transplants have been advocated as a way forward to improve the results of autologous transplantation. At least four groups have reported results on randomised trials of tandem transplantation with a median follow-up of between 27 months and 5 years [12,13]. They show that, whilst this approach increases the clinical and molecular CRR, the impact on EFS and OS has been disappointing and only a small proportion of people are able to tolerate this approach and proceed to the second tandem transplant. Femand and colleagues 1998 [14] compared the strategy of autologous tandem transplantation as planned consolidation treatment versus patients who received one autograft but as a planned initial procedure with the second autograft performed as rescue therapy at the later stage of disease progression. They were unable to

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show a difference in OS between the two groups, although, overall, the time on chemotherapy was shorter in the tandem treatment group.

Allogeneic transplantation in multiple myeloma has still to be tested in a randomised clinical trial. However, registry data have been able to show a number of individuals with long-term disease-free survival, suggesting that this approach may work in selected individuals [15]. The improved relapse-free survival of 30–40% at 5 years is achieved at the cost of high treatment-related mortality [16]. Bjorkstrand [17] has reported data from the European Blood and Marrow Transplantation Registry showing a fall from 40–50% for patients reported between 1989 and 1991 to 30% for those transplanted between 1995 and 1999. Patient selection for allogeneic transplantation is probably critical, but this approach is likely to be appropriate for only a small minority of patients. No direct comparison or randomisation has been performed between allogeneic and autologous transplantation. European Blood and Bone Marrow Group Registry data show a complete response rate at 5 years of 9% in allogeneic transplantation and 4% in autologous transplants with a maximum duration of complete remission of 113 months for allogeneic versus 38 months for autologous transplantation. The continuing late relapses after CR up to 91 months in the allogeneic patients and 38 months in the autologous transplant patients. Thus, autologous transplantation appears to have better outcomes than chemotherapy in terms of response rate, EFS and OS and it is important to note that all major clinical trials report a median age for transplantation of between 49 and 52 years, which is well below the median age that is seen for all registries of multiple myeloma, suggesting that more than half the patients will not be eligible for this treatment approach.

Concepts of immunological tolerance and anergy in patients with cancer were developed with the identification of cellular immune responses to tumour-associated antigens (TAAs), some of which have now been characterised [18]. The inability of the cytotoxic T-cell (CTL) to recognise the tumour cell may arise as a result of defective intercellular adhesion, aberrant antigen presentation, absent co-stimulatory molecules or mediation of apoptosis or prevention of activation of immune mechanisms by soluble factors or cytokines. In an effort to improve the outcome of allogeneic stem-cell transplantation, non-myeloablative transplantation has been introduced with the intention of reducing toxicity by reducing the chemotherapy intensity of induction whilst retaining the immunological benefits of the graft versus tumours effect [19]. The benefits of this approach are currently being studied by the UK Myeloma Forum and the European Blood and Bone Marrow Transplant group in a phase II study using autologous stem-cell transplantation as early consolidation followed by a non-myeloablative transplant for patients with an HLA matched donor. The results of these studies are awaited with interest. Graft versus host disease has been reported in the autologous and syngeneic transplant setting by Hood and colleagues [20] and strategies to enhance this CTL effect have been attempted in a number of tumours including myeloma using cyclosporin A and interferon alpha with limited success. Whilst the role of CTL in cancer immunology has been investigated in some detail over the last 10 years [21], the role of NK cells has been less well developed. However, endogenously generated activated killer cells have been demonstrated after autologous and allogeneic marrow transplantation but not after chemotherapy [22,23]. We are currently looking at the role of NK cells in the control of this disease post transplantation.

As we enter a new millennium, the poor results seen with chemotherapy raise the issue of how to improve the results of treatment in multiple myeloma (Fig. 1). Recently, substantial insight has been achieved into the cellular signalling mechanisms that result from the application of external stimuli to a myeloma cell. Such stimuli arise from the interactions of the cell with soluble factors, such as cytokines [24,25], antibodies and small molecules or cell–cell contact [26,27] with lymphocyte, osteoblasts, osteoclasts or stromal cells and results in the proliferation, apoptosis or migration of the cell. Interruption of these signalling pathways may allow control of myeloma cell survival whilst limiting damage to normal haemopoietic progenitor cells. The importance of the myeloma micro-environment in the homeostasis of the myeloma cell is also becoming much clearer. Increasingly, there is evidence to support the model that certain cytokines in the bone marrow micro-environment and macromolecules in the extracellular matrix and supportive stromal cells are important for the survival and growth of myeloma cells (Fig. 2). Events that interfere with the micro-environment will potentially either inhibit myeloma cell growth or induce the cells to undergo apoptosis [28,29]. The development of strategies to interrupt this network will almost certainly lead to further novel therapies in the future [30].

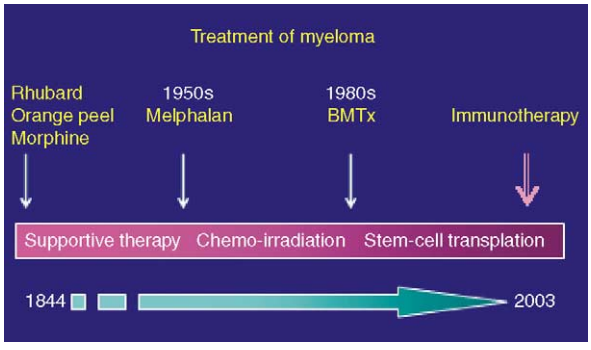

Fig. 1. The evolution of treatment strategies from 1844 to the present time.

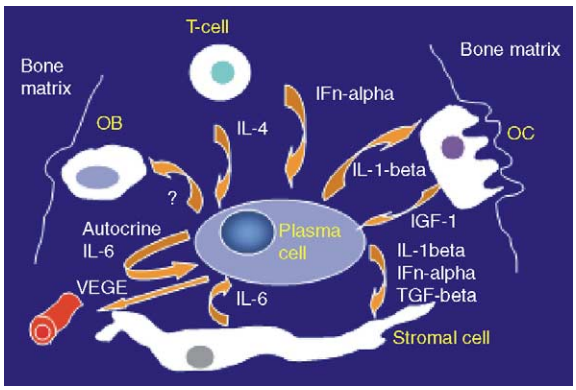

Fig. 2. The cytokine network demonstrating the interaction between the myeloma cell and the stromal cells composing the bone marrow micro-environment controlling cell proliferation and survival.

Interleukin (IL)-6 is known to be an important growth and survival factor for myeloma cells [31,32]. It has been shown to be secreted in an autocrine fashion from myeloma cells and in a contact-mediated paracrine manner from bone marrow stromal cells. Soluble IL-6 receptor (sIL-6R) is shed by myeloma cells and, at physiological concentrations, is capable of significantly sensitising myeloma cells to IL-6. Myeloma cells also produce transformed growth factor beta (TGF-beta), which is known to stimulate the production of IL-6. Insulin growth factor-1 (IGF-1) may also play an important role in the control of myeloma proliferation during the early stages of the disease when the cells are known to express the IGF-1 [33] receptor and may in addition act as a survival factor by preventing dexamethasone-induced apoptosis [34]. Other growth factors that have been shown to be synthesised by myeloma cells include monocyte colony stimulating factor (M-CSF) and hepatocyte colony stimulating factor (H-CSF) [35], both of which correlate with tumour burden and survival, respectively. A number of other cytokines working through a common GP130 membrane-bound receptor may play an important role in the early stages of disease such as IL11, insulin growth factor 1, TGF beta and Oncostatin-M [36,37]. Other positive growth factors include G-CSF, TNF alpha, hepatocyte growth factor, IL10 and IL7. Other cytokines and interleukins exert a negative effect by inhibiting survival through a number of newly identified transcriptional pathways [38]. These include interferon alpha, beta and gamma. The importance of the microenvironment is also being investigated, and the close relationship between plasma cell growth and the micro environment including the bone matrix, stromal cells and blood vessels is an area of fruitful research. Strategies to either upregulate apoptotic pathways or inhibit proliferation of the myeloma cell at the receptor or cytokine level are therefore being developed as innovative therapies in this disease [39]. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (βFGF)-2 are secreted by multiple myeloma and or BM stromal cells and may play a role both in tumour cell growth and survival, as well as BM angiogenesis [40–42]. Given its known anti-angiogenic activity, thalidomide may inhibit activity of VEGF, βFGF-2, and/or angiogenesis in multiple myeloma [43,44]. The availability of new genetic techniques have allowed investigation of translational changes at the DNA level and, more recently, the identification of a variety of transcriptional and posttranslational mechanisms, which ultimately dictate whether the cell will proliferate, undergo apoptosis or migrate. The MAPK and JAK/STAT pathways have been shown to be important molecular pathways responsible for proliferation of the myeloma cell. Binding of the IL-6 receptor to IL-6 causes activation of tyrosine kinases and transphosphorylation of the receptor and subsequent activation of signalling protein cascade (including STAT1, STAT3, Raf-1 and MAPK). Ultimately, these events promote the phosphorylation of oncogenes such as c-myc and c-fos that enhance proliferation [45]. However, upregulation of NFkB induces, through a series of phosphorylation steps, downregulation of apoptosis genes, which ultimately results in inhibition of apoptosis [46]. Other important apoptotic pathways include the caspase and RAFTK pathways through which a number of drugs, including dexamethasone, have been shown to induce cell death. Dexamethasone induced apoptosis is inhibited by IL-6 via activation of SHP2, which specifically blocks RAFTK activation, thereby protecting myeloma cells. Fas-induced apoptosis, however, is inhibited by upregulation of Bcl XL, which is induced by IL-6 via the JAK-STAT pathway and the availability of an endogenous inhibitor, FLIP-L, induced by the adhesion of the myeloma cell to fibronectin in the stromal microenvironment [47]. This latter has been identified as an important pathway conferring drug resistance in a number of other tumours in experimental cell lines.

A number of agents have been identified which impact upon myeloma cell growth at all these different levels. Thalidomide was originally used in myeloma because of its known anti-angiogenic affect [48,49]. In addition to this, it is also known to have a number of other activities [50]. These include modulation of the cytokine millieu primarily through an anti-TNF activity [51], alteration of the expression of cell-adhesion molecule (CAM) expression on bone marrow stromal cells [52] and an indirect effect through an effect on CD8-positive T-cells [53] and possibly an on NK cell activity [54]. A direct antitumour effect on the myeloma cell itself has also been reported [55]. The relative importance of these different mechanisms has yet to be resolved.

Phase 2 trials have shown that approximately 30% of patients who had been heavily pretreated with relapsed and/or refractory disease will respond to Thalidomide

as a single agent in doses ranging from 200 to 800 mg per day [56,57]. Side-effects of Thalidomide include sleepiness, peripheral neuropathy, constipation and skin rashes which appear to be dose-related. A potential advantage of Thalidomide is that it is marrow-sparing, an important property in patients who have been heavily previously treated. In an attempt to improve efficacy and reduce toxicity, a number of Thalidomide analogues have been developed [58]. Two of these are currently in clinical development, namely CC5013 and CC4047. These have shown very promising results in early clinical trials [59,60]. A number of other novel agents have been investigated for these anti-angiogenic effects. Thalidomide may also be acting against multiple myeloma via its immunomodulatory effects, such as induction of a Th1 T-cell response with secretion of interferon gamma (IFN-γ) and IL-2.

Monoclonal antibody therapy is emerging as a potentially safe and effective therapeutic strategy for a number of haematological therapies. Anti-CD20 antibody has been effective in non-Hodgkin's lymphoma but is only weakly expressed on 20% of myeloma cells, although anecdotal reports have shown some responses which may be enhanced with the use of interferon gamma which induces CD20 expression on myeloma cells. Early clinical trials have met with limited success in subgroups of patients and studies are ongoing [61]. Other potential surface antigens that may be suitable for antibody directed therapy include IL-6, Muc-1, HM1.24, CD38, CD40, CD54 (ICAM-1) and syndecan-1, either as the naked antibody or as a carrier for toxins or radioisotopes [62]. Antibodies to IL6, one of the major cytokines controlling myeloma cell proliferation and apoptosis, have been used in early phase II clinical trials, but again have had only limited effect on outcome [63]. HM1.24 antigen [64] is expressed on myeloma cells but not on normal tissues, including the peripheral blood, bone marrow, haemopoeitic progenitor cells, lymph node, liver, spleen or kidney. PBMCs of myeloma patients have NK and LAK activity but little is known about ADCC specific for myeloma cells. However, humanised anti-HM1.24 monoclonal Ab can mediate ADCC activity against myeloma cells in the presence of effector cells. Only chimeric and humanised forms of anti-CD38 have been previously reported to have ADCC activity against myeloma cells in the presence of effector cells of between 40 and 60% cytotoxic. However, CD38 is also expressed on normal tissues such as haematopoietic cells. Other antibodies are in development, but their role in the management of myeloma is yet to be defined. An interesting development in this area is the conjugation of antibodies to radioactive toxins that target treatment to the disease. Results of early clinical trials in lymphoma are awaited with interest as these agents may be applicable to multiple myeloma in the future. Posttranslational processing of cellular proteins has been a major focus of attention in molecular research over the last 20 years. Degradation of intracellular protein may be critical in defining the response of the cell to external stimuli by controlling intracellular protein expression. One mechanism by which this control is exerted is via a multicatalytic 26S proteasome [65]. This process is responsible for degradation of all short-lived proteins and 70–90% of long-lived proteins and potentially, therefore, the cell-cycle process, DNA repair and transcription, angiogenesis and regulation of signal transduction. PS 341 is a novel proteasome inhibitor that targets the myeloma cell and the bone micro-environment by inhibiting the degradation of ubiquinated proteins (Fig. 3). NFkB is present in the cell as a complex bound to an NFkB inhibitor [26]. PS 341 functions as an inhibitor of posttranslational protein degradation and thus blocks the dephosphorylation of the NF kappa B inhibitor complex, causing decreased levels of NF kappa B and down-regulation of anti-apoptotic gene translation in the nucleus [66]. A number of newer agents with proteasome inhibitor activity are also now in development.

A variety of tumour antigens have been identified in myeloma which may prove to be suitable targets for vaccine therapy to stimulate both cell-mediated and antibody-mediated immunity [67]. Potential tumour targets include membrane-bound antigen, such as the glycoprotein MUC-1, and intracellular targets, such as MHC Class 1 and II or mutated proto-oncogenes, e.g. p53. The monoclonal immunoglobulin expressed by the tumour is an obvious potential target as it is unique for each tumour. The complementarity determining regions (CDRs) are composed of the three hypervariable loops on the distal surfaces of the V domain of the heavy chain of the immunoglobulin molecule. The random assembly of small gene segments during ontogeny determines the unique antigen-binding site of the cell. This very uniqueness means that the V region may itself

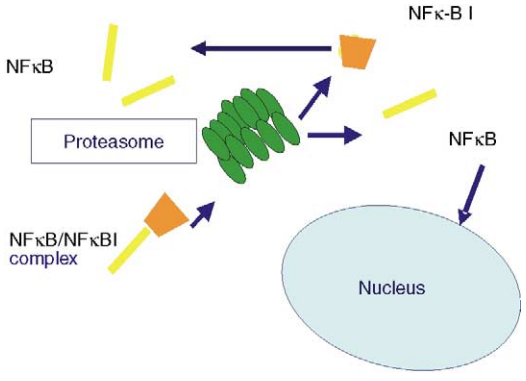

Fig. 3. The proteasome is composed of a 20S core unit plus two 19S regulatory units. The NFkappa-B/NFkappa-B inhibitor complex (NFκ-B/NFκ-BI) is labelled with ubiquitin and broken down in the proteasome into its component parts. The unbound NFκ-B returns to the nucleus whilst the inhibitor binds to free cytoplasmic NFκ-B.

be a target for anti-idiotypic antibodies. In human myeloma, the idiotypic immunoglobulin is present in the cytoplasm or is secreted and may be a target for CTLs. A number of phase I/II studies of vaccines using idiotypic protein with or without cytokine adjuvants have shown that this approach is capable of stimulating delayed hypersensitivity and low-level T-cell proliferation against the target. Kwak and co-workers [68,69] have adopted an innovative approach using this vaccine by immunising two healthy stem-cell donors with promising early results. A number of issues, however, remain outstanding before this approach can be used with confidence in myeloma. These include problems of immunising against self-antigens, the formulation of the vaccine, the induction of cellular or antibody responses and the timing of the vaccination in the disease process to ensure that the patients' immune capacity is not irrevocably impaired.

The interaction of the myeloma cell with the microenvironment has been extensively investigated in recent years [70–72]. Bisphosphonates have been used to inhibit osteoclast activity and reduce bone fractures and hypercalcaemia [73]. With the new generation bisphosphonates that are now available, it has become clearer that these drugs may also have an antitumour effect [74]. This benefit may be induced via either a cytokine-mediated effect on the bone marrow stromal cell, a direct apoptotic effect on the myeloma cell or more intriguingly via an NK cell mediated effect [75,76]. Clinical trials are in progress to evaluate the effects of these drugs on the tumour in the maintenance or adjuvant setting.

Myeloma has for many years been a 'Cinderella' disease which has proved to be resistant to all previous attempts at cure. The development of new scientific techniques has facilitated our efforts to learn how myeloma cell growth is controlled and this has led to the development of a paradigm shift in the way in which we manage and treat this disease. For the first time in a generation, there is real hope of a new way forward in this disease and the future for those practising in this field is likely to be an exciting one.

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