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Multiple myeloma: Monoclonal antibodies-based immunotherapeutic strategies and targeted radiotherapy

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ABSTRACT

Multiple myeloma (MM) is an incurable B-cell malignancy of terminally differentiated plasma cells. Besides conventional treatments, several targeted therapies are emerging for MM. We review recent developments in monoclonal antibodies (MoAbs) and (radio)immunoconjugates-based targeted immunotherapeutic (serotherapies) strategies, as well as skeletal targeted radiotherapy (STR™) in MM. MoAbs-based strategies include the targeting of cytokines and their receptors as well as toxins, drugs or radionuclide delivery to MM cells. Both targeted radioimmunotherapy (RIT) and STR™ have proved efficient in the treatment of radiosensitive tumours. We conclude that there is a need for more mechanistic investigations of drug action to identify novel therapeutic targets in myeloma cells, as well as in the bone marrow microenvironment.

1. Introduction

Multiple myeloma (MM) is a haematologic malignancy associated with a variety of clinical manifestations, including osteolytic lesions due to uncoupled bone metabolism, anaemia and immunosuppression due to loss of normal haematopoietic stem cell function, and end-organ damage due to monoclonal immunoglobulin secretion.[1] In the United States, the diagnosis of MM involves 15 000 new patients per year with a median expectation of life of 3–4 years. Despite this MM remains incurable, while recent advances in basic biology have led to important insights into its pathophysiology, which in turn are rapidly changing the management and therapeutic strategy of this malignancy. Novel agents, such as thalidomide, lenalidomide and bortezomib, have recently demonstrated an increasing therapeutic role in the treatment of MM patients.[2] However, MM can relapse even after complete remission and therefore new drugs and therapeutic strategies are urgently needed. Here we will review the potential of immunotherapeutic and targeted radiotherapy strategies for treating MM.

2. Therapeutic strategies in MM

2.1. *Immunotherapy*

Immunotherapy is an experimental treatment strategy for MM.[3–7] Strategies to harness the powerful immune system are mainly at the pre-clinical stage of development for MM, but they are moving towards clinical testing. There is wide variety in the techniques used and the outcomes achieved so far. MM patients do not mount a strong immune response

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against their disease. The goal of using immunotherapy is to either help the body elicit an "active" immune response to attack myeloma cells (active immunotherapy) or to substitute an alternative "passive" response elicited outside the body and administered to the patient (passive immunotherapy).[3,6,7] Immunotherapy is likely to be most effective when the burden of disease has been minimized, such as after high-dose chemotherapy and stem cell transplantation (SCT). It seeks to destroy the remaining cells, which are thought to be responsible for relapse after therapy. However, immunotherapy may also have other indirect anti-MM effects,[7] such as: 1) slowing MM cell growth; 2) making MM cells more vulnerable to destruction by other therapies; or 3) affecting the bone marrow (BM) microenvironment to make it less hospitable to MM cells.

Immunotherapy is an active area of research in MM. In addition to monoclonal antibodies (MoAbs), other types of immunotherapy being investigated in MM include vaccines, cytokines as well as manipulation of immune cells. Researchers are investigating new molecular targets and strategies for immunotherapy, as well as working toward a better understanding of the immune defects present in patients with MM.[6–9] (Table 1) The following section deals with various types of MoAb-based strategies under investigation in MM.

2.1.1. *Monoclonal antibodies*

MoAbs target specific molecules and are used as passive immunotherapy to treat various diseases, including certain types of cancer.[3,7] These MoAbs selectively target tumour tissues and have been safely administered in cancer patients.[7] The recent approvals of MoAbs for clinical use[7,9–12] have renewed interest in MoAb-based targeted serotherapies in MM.

Several MoAbs directed against marker proteins expressed on MM cells are being investigated as potential therapeutic tools for MM. MRA (humanized anti-interleukin-6 (IL-6) antibody, atlizumab) is being evaluated in an open-label phase I and II trials to assess the safety and efficacy in MM. Blockade of IL-6R may prove effective in limiting MM cell growth. It is given as monotherapy to patients with MM who are not candidates for, or who have relapsed after SCT.[7,9] The TRM-1 (Fully Human TRAIL-R1 MoAb) is directed against a specific receptor on MM cells known as TRAIL (TNF-related apoptosis-inducing ligand). Binding of the MoAb to TRAIL contributes to MM cell death. TRM-1 is being evaluated in a phase I, open-label, dose-escalation trial to assess the safety, tolerability, immunogenicity, and pharmacokinetics in subjects with MM.[7] The AHM (anti-HM1.24 MoAb) is presently in phase I clinical trials. HM1.24 is a marker expressed on MM cells. MoAbs to HM1.24 have been shown to induce killing of MM cells *in vitro* and *in vivo*.[7] The mKap is a murine MoAb that is specific for free human kappa light chains (the small arms of antibody molecules) and a marker on the surface of plasma cells (PCs). This antibody induces apoptosis in MM cell lines that express the surface antigen KMA *in vitro* and exhibited antitumour activity in a mouse model of disease. Complete data of the preclinical animal trials are expected by late 2005–06.[7,9] The SGN-40 (anti-huCD40 MoAb) is a humanized anti-CD40 MoAb directed against the CD40 receptor on MM cells. Currently, it is being investigated in a phase I, multi-dose trial in patients with refractory or recurrent MM.[7,9] Anderson and co-workers[13] examined the potential therapeutic utility of SGN-40 for treating human MM using MM cell lines and patient MM cells ($CD138^{++}$, $CD40^{+}$). Results showed that, SGN-40 induced modest cytotoxicity, and in the presence of *de novo* protein synthesis inhibitor cycloheximide, it significantly induced apoptosis in dexamethasone (Dex)-sensitive and Dex-resistant MM cells and in patient MM cells. Pretreatment of MM cells with SGN-40 blocked sCD40L-mediated phosphatidylinositol 3′-kinase/akt and nuclear factor-$\kappa\beta$ activation. Moreover, SGN-40 suppressed IL-6R expression at mRNA and protein levels, and importantly, pretreatment of MM cells with SGN-40 inhibited proliferation triggered by IL-6 but not by insulin-like growth factor-1 (IGF-1). SGN-40 has direct anti-MM effects that are unrelated to antibody-dependent cell cytotoxicity (ADCC), highlighting the preclinical rationale for the evaluation of SGN-40 as a potential new therapy. A recent similar study focused on the efficacy of a fully human anti-CD40 MoAb, CHIR-12.12 against human MM cells.[14] CHIR-12.12 triggered lysis of MM cells via ADCC, but did not induce ADCC against CD40-negative MM cells, confirming specificity against CD40-expressing MM cells. The study thus provides the preclinical rationale for clinical trials of CHIR-12.12 in MM.

2.1.1.1. Targeted Anti-IL-6 MoAb therapy. Experimental and clinical findings support the role of IL-6 in cancer and provide a rationale for targeted therapeutic investigations. Various therapeutic agents affect IL-6-mediated effects. Known inhibitors of IL-6 include corticosteroids, non-steroidal anti-inflammatory agents, estrogens, and cytokines (e.g., IL-4).[15] Dex has also been shown to inhibit both IL-6 and IL-6R gene expression in MM cell lines. Targeted biological therapies include IL-6-conjugated MoAbs directed against IL-6 and IL-6R.[15,16] Initial investigations were conducted with mouse MoAb to IL-6 (murine MoAbs BE-4 and BE-8).[17,18] A patient with primary plasma cell leukaemia (PCL) that was resistant to chemotherapy was the first reported recipient of anti-IL-6.[19] There was inhibition of MM proliferation in the BM, along with decreased serum levels of calcium and monoclonal IgG. Levels of C-reactive protein (CRP) became undetectable and no serious side-effects were noted. This study demonstrated the potential of MoAb therapy against IL-6, resulting in a transient tumour cytostasis and reduction in toxicities from IL-6.[16] A subsequent study by Bataille[18] reported the results of MoAb targeting to IL-6 in MM patients with advanced and progressive MM. More recently, a chimeric mouse MoAb to IL-6 (human-mouse cMoAb to IL-6) with the investigational name CNTO 328 was used in a phase I clinical trial in patients with MM.[20,21] van Zaanen[20,21] conducted a phase I/II study based on a dose-escalation analysis of intravenous (i.v.) infusions of murine-human chimeric (cMoAb) CNTO 328 (CCLB8; now called CNTO 328) in patients with end-stage, progressive MM[22] resistant to second-line chemotherapy. Data from the study enabled the development of a method for calculating endogenous IL-6 production and the finding that CNTO 328 therapy normalized endogenous IL-6 production but did not affect the IL-6 production associated with infection. These investigations suggested that CNTO 328 had a low immunogenicity and was able to block IL-6-dependent processes *in vivo*.[20] In a second report,[21] three additional patients were enrolled in the dose-escalation study and received CNTO 328 as before. The dosing regimen was 40 mg/day in these patients. Disease stabilized in 11 of 12 patients;

Table 1 – Various agents and immunotherapies currently in clinical or preclinical trials for multiple myeloma (MM) and myeloma bone disease

Phase	Brand name (other name)	Company (sponsor)	Indication
Phase III	Revlimid® (CC-5013, lenalidomide)	Celgene Corporation	A thalidomide analogue, this is a type of IMiD™ (immunomodulatory drug) with multiple effects in Phase II and III studies.Two randomized multi-center studies approved, not yet active at this time; also in combination with VELCADE for relapse after Total Therapy II.
	Thalomid®* (thalidomide)	Celgene Corporation and the NCI	Randomized study of thalidomide + prednisone as maintenance therapy after auto-transplant; randomized study of combination chemotherapy with or without thalidomide.
	Actimid™ (CC-4047)	Celgene Corporation	Type of IMiD™ (immunomodulatory drug) with multiple effects under study in Europe.
Phase II	Avastin™ (bevacizumab, rhuMAb-VEGF)	Genentech Inc.	For relapsed or refractory myeloma, with and without thalidomide.
	Bexxar (Iodine I-131 tositumomab)	GlaxoSmithKline, U of Michigan	Radioactive antibody to CD20-expressing cells for patients with newly diagnosed or relapsed MM.
	Campath-1H (alemtuzumab, monoclonal antibody CD52)	Berlex, NCI and Fred Hutchinson Cancer Research Institute	Tested in conjunction with allogeneic stem cell transplants to prevent GVHD and in patients with relapsed and refractory myeloma.
	Dendritic Cell Vaccine	Arkansas Cancer Research Center	Autologous dendritic cells for high-risk MM.
	Proleukin® (IL-2, Interleukin-2)	National Cancer Institute and Chiron	For advanced myeloma as part of auto or syngeneic transplant.
	Quadramet® (EDTMP, samarium-153 ethylene diamine tetramethylene phosphonate Samarium)	Mayo Clinic, Rochester, MN	Skeletal-targeting radiopharmaceutical for use in combination with standard or high-dose chemotherapy for patients undergoing stem cell transplant.
	Revlimid® (CC-5013, lenalidomide)	Celgene	In combination with Biaxin and dexamethasone for newly-diagnosed myeloma.
	Rituxan® (rituximab)	NCI	High-dose cyclophosphamide in combination with rituximab in patients with primary refractory, high-risk, or relapsed myeloma.
	Trisenox®* (arsenic trioxide for injection)	Cell Therapeutics, Inc.	For recurrent or refractory myeloma.
	CNTO 328	Centocor	Monoclonal antibody to IL-6
Phase I	anti-IGF-1R MoAb (CP-751,871); monoclonal antibody to insulin-like growth factor-1 receptor)	Pfizer Global Research and Development, Inc.	For relapsed and refractory MM.
	Campath-1H (alemtuzumab, monoclonal antibody CD52)	National Cancer Institute and Fred Hutchinson Cancer Research Institute	Used before mismatched allo transplant to prevent GVHD.
	CNTO 328	Centocor	Chimeric antibody to Interleukin-6 for patients with MM
	CHIR-12.12	Chiron and Xoma Ltd.	Monoclonal antibody to CD40
	CHIR-258	Chiron	Fibroblast growth factor receptor 3 inhibitor for potential treatment of t(4;14) MM
	huN901-DM1 (huN901-N2′-Deacetyl-N2″-(3- Mercapto-1-oxopropyl)- Maytasine)	ImmunoGen, Inc.	Targets and kills cancer cells that express the CD-56 antigen. A humanized monoclonal antibody coupled with a cancer-killing agent.
	MRA (humanized anti-human IL-6 receptor MoAb; Atlizumab)	Chugai Pharma USA	Safety and efficacy study of monoclonal antibody to IL-6 in patients with myeloma. Phase I studies in the US and Phase II in France.
	SGN-40	Seattle Genetics	Monoclonal antibody to CD40 protein on myeloma cell surface.
Preclinical	1D09C3	GPC Biotech, AG	Human monoclonal antibody; clinical trials second half of 2004.
	Antigen-loaded dendritic cell vaccine	National Cancer Institute and Aastrom Biosciences	Idiotype vaccination following allogeneic transplant.
	Anti-CD 38 Saporin (OKT10 Saporin, OKT-SAP)	Cancer Research UK	Saporin toxin linked to anti-CD38 monoclonal antibody; in PH I dose escalation studies for relapsed myeloma in the UK
	BLyS/rGel	Targa Therapeutics	Fusion protein of an antibody tethered to a toxin for the potential treatment of MM

Table 1 – *continued*

Phase	Brand name (other name)	Company (sponsor)	Indication
	CD74-DOX conjugate (anti-CD74 monoclonal antibody conjugated with doxorubicin)	Immunomedics, Inc.	Therapy targeted to CD74-expressing MM cells lines
	cKap	PacMab Ltd.	Chimeric version of the murine antibody, mKap, that induces apoptosis in all myeloma cell lines shown to express the surface antigen KMA. cKap is highly effective in inducing antigen-dependent cellular cytotoxicity.
	HuMax-CD38 (human IgG1,k antibody)	Genmab A/S	Antibody to the CD-38, which is highly expressed on the surface of MM cells.
	Skeletally targeted proteasome inhibitors (bone-targeting nanocapsules)	US Department of Defense. Southwest Research Institute and UT Health Science Center	Targeting proteasome inhibitors to myeloma lesions in bone via nanocapsules.
	StemEx	Gamida Cell	Haematopoietic support in patients with relapsed or refractory haematologic malignancies who are receiving high-dose therapy.
	mKap	PacMab Ltd.	A murine antibody which induces apoptosis in myeloma cell lines that express the surface antigen KMA. Preclinical animal trials expected by late 2005-06.

Source: Multiple Myeloma Research Foundation (MMRF), USA and The International Myeloma Foundation (IMF), USA, September 2005.
n.b.: For Website addresses, vide Refs. [7] and [9].

the twelfth patient with progressive disease responded to the second course of treatment. Despite stabilization of disease, no patient had a clinically significant response (e.g., a reduction in the level of M protein greater than 50%). Moreover, no immune response to chimeric anti-IL-6 MoAb occurred, and CRP levels became undetectable in 11 of 12 patients. The authors concluded that no life-threatening side-effects were associated with CNTO 328 therapy, and pharmacokinetic measurements indicated that the circulating cMoAb had a long half-life (17.8 days). Although no patient achieved remission, the authors hypothesized that the level of M protein did not decrease more than 50% in treated patients who exhibited decreased CRP levels due to the presence of immature and mature MM cells. Immature MM cells are highly proliferative, but mature cells are not. Mature cells are also responsible for synthesizing M protein, implying that some IL-6-independent myeloma cells in end-stage MM[21] cannot be targeted by a MoAb to IL-6. The study indicated that, in MM patients the endogenous IL6 production is significantly increased when compared to healthy individuals. Treatment with cMoAb to IL-6 normalizes endogenous IL-6 production, probably by blocking a positive feed-back loop in MM associated IL-6 production. Recently, Myeloma Institute for Research and Therapy at the University of Arkansas for Medical Sciences (UAMS) and M.D. Anderson Cancer Center (MDACC) at the University of Texas have initiated phase I trials [study summary Nos. UARK 2004-23 (UAMS) and 2004-0492 (MDACC)] involving multiple i.v. infusions of CNTO 328 in subjects with B-cell non-Hodgkin's lymphoma (NHL), MM, or Castleman's disease for evaluating five different dosing regimens of CNTO 328 to see which dose/schedule can best be given safely.[23,24]

Moreau[25] investigated the potential of combination therapy, including a murine MoAb to IL-6 (BE-8; 250 mg), Dex (49 mg/day), and high-dose melphalan [220 mg/m^2 (HDM220)], followed by autologous SCT in the treatment of 16 patients with advanced MM. IL-6 activity was strongly inhibited, as indicated by reduced CRP levels. Overall, 13 of 16 patients (81.3%) exhibited a response, with a complete response (CR) seen in 6 patients (37.5%) without any toxic or allergic reactions. The results of a recent study by Rossi[26] showed that: i) BE-8 was able to fully neutralize IL-6 activity *in vivo* before and after melphalan (HDM: 140 mg/m^2) as shown by inhibition of CRP production; ii) there was no occurrence of haematological toxicity; iii) there was a significant reduction of mucositis and fever; along with iv) a median event-free survival (EFS) of 35 months and an overall survival (OS) of 68.2% at 5 years with a median follow-up of 72 months; and v) the overall daily IL-6 production progressively increased on and after 7 days post-HDM, with the increased serum CRP levels. In the 5/24 patients with uncontrolled CRP production, a large IL-6 production was detected (320 μg/day) that could not possibly be neutralized by BE-8. These data showed the feasibility to neutralize IL-6 *in vivo* with BE-8 in the context of HDM. Another study by Lu[27] demonstrated that BE-8 could not efficiently block daily production of IL-6 at a level greater than 18 μg/day. This particular study observed an inverse correlation between clinical response and daily production of IL-6 during treatment if production exceeded 18 μg/day. This confirmed the importance of calculating this particular parameter for optimizing an anti-IL-6 dosing strategy. It was therefore suggested that the cMoAb CNTO 328 with an 18-day half-life might be beneficial for chronic administration. Furthermore, in a recent report, Moreau[28] showed that in high-risk MM patients, the dose intensity of melphalan at 420 mg/m^2 led to encouraging results, but the addition of BE-8 to the second conditioning regimen, however, did not improve progression-free survival (PFS)/EFS or OS.

Alternative approaches have been developed by using humanized anti-IL-6R MoAb (rhPM-1, IgG1 class). PM1 is currently tested in phase I/II trials in patients with MM.[29] Other approaches include the combination of anti-IL-6 or anti-IL-6R MoAbs that shorten the half-life of the IL-6/IL-6R

complexes (from 4 days to less than 20 minutes) *in vivo*, in addition to the formation of polymeric complexes instead of monomeric complexes, a situation compatible with increased clearance of these IL-6/IL-6R complexes.[16,30] Analysis of data from six structured clinical trials of various MoAbs to IL-6 in the treatment of human malignancies has shown a number of interesting observations. MoAbs were well tolerated and exhibited a decrease in cancer-related symptoms (i.e., fever, cachexia, and pain). Administration of anti-IL-6 MoAbs resulted in inhibition of CRP production below detection limits as well as neutralization of IL-6 production *in vivo*, transiently inhibiting MM cell proliferation. However, many patients had severe disease and produced huge amounts of IL-6, making anti-IL-6 MoAb unable to completely and/or very efficiently neutralize them. Considering the risk of immunization against the murine anti-IL-6 MoAb, humanized anti-IL-6 MoAb might prove useful, especially for treatment of patients with earlier stage disease.[31] Because IL-6 is mainly a survival factor rather than a proliferation factor for human MM cells and blocks Dex-induced apoptosis, a main issue in MM will be to use anti-IL-6 MoAb therapy to potentiate tumour killing by various drugs, including Dex or high-dose chemotherapy.[31] It appears trivial that in a complex disease like MM, growth cannot sufficiently be reduced by blocking a single cytokine signal, which suggests that multidrug regimens may be useful as alternative therapies to improve the poor outcome of MM patients. Honemann, Chatterjee and co-workers[32] analyzed the effect of the IL-6R antagonist SANT-7 on growth and survival of the IL-6-dependent MM cell lines as well as primary MM cells from 7 patients. In particular, the study was aimed to find out whether SANT-7 enhances the growth-inhibitory effects of Dex and all-trans retinoic acid (ATRA). None of the drugs when tested as a single substance, including SANT-7, induced major growth-inhibition if MM cells were co-cultured with primary human BM stromal cells (BMSCs). However, when Dex and ATRA were given in combination with SANT-7, strong growth-inhibition was achieved in cell lines and primary MM cells. This effect was due to cell-cycle arrest and induction of apoptosis. Thus, combining SANT-7 with additional drugs could be a useful approach to the treatment of MM. Tassone and co-workers have shown that, SANT-7 significantly enhanced growth inhibition and apoptosis in both MM cell lines and primary MM cells. The study indicated that, overcoming IL-6 mediated cell resistance by SANT-7 potentiates the effect of glucocorticoides and bisphosphonates on MM cell growth and survival, providing a rationale for therapies including IL-6 antagonists in MM.[33,34] A recent study by the same group demonstrated that, in a novel murine model[35] of human MM in which IL-6-dependent INA-6 MM cells were directly injected into human BM implants in severe combined immunodeficient (SCID) mice (SCID-hu), inhibition of IL-6 signalling by SANT-7 significantly potentiated the therapeutic action of Dex against MM cells.[36] This provides the preclinical rationale for clinical trials of SANT-7 in combination with Dex to improve patient outcome in MM.

2.1.1.2. *Strategies for Targeting IGF-1.* Besides IL-6, the IGF-1 signalling pathway is also implicated in cellular mitogenesis, angiogenesis, tumour cell survival, and tumourigenesis. Inhibition of this pathway results in decreased cell growth, inhibition of tumour formation in animal models, and increased apoptosis in cells treated with cytotoxic chemotherapy.[37] Strategies targeting IGF-1 and IGF-1 receptor (IGF-1R) may therefore be important to develop efficient anti-MM agents. Studies from Anderson's group have shown the functional association of IGF-IR and beta1 integrin in mediating MM cell homing, providing the preclinical rationale for novel treatment strategies targeting IGF-1/IGF-1R in MM.[38] In another study, synergistic combination of rapamycin (Rapamune), a specific mTOR inhibitor with CC-5013 (Revlimid), an immunomodulatory analog (IMiD) of thalidomide was able to overcome drug resistance as well as the growth advantage conferred on MM cells by IL-6, IGF-1, or adherence to BMSCs and induced apoptosis of MM cells.[39] Anderson's group has further demonstrated the potential cytotoxicity and apoptosis-inducing effects of the novel immunomodulator FTY720 on MM cells and MM patient samples.[40] Neither IL-6 nor IGF-1, which both induce MM cell growth and abrogate dex-induced apoptosis, protected against FTY720-induced growth inhibition. FTY720 further down-regulated IL-6-induced phosphorylation of Akt, signal transducers and activators of transcription 3, and p42/44 mitogen-activated protein kinase; IGF-1-triggered Akt phosphorylation; and tumour necrosis factor (TNF) -alpha-induced IkappaBalpha and NF-kappaB p65 phosphorylation. These studies thus provide the rationale for clinical evaluations of mTOR inhibitors combined with IMiDs as well as FTY720 in MM. Recent studies of Menu[41] showed that, IGF-1R tyrosine kinase (IGF-1RTK) inhibitor picropodophyllin (PPP) possesses a marked antitumour activity and strongly points to the possibility of using IGF-1R inhibitors in the treatment of MM. Furthermore, CP-751,871, a fully human anti-type 1 IGF-R IgG2 Ab, both as a single agent and in combination with adriamycin, 5-fluorouracil, or tamoxifen showed significant antitumour activity *in vivo* and blocked the binding of IGF-1 to its receptor, IGF-1-induced receptor autophosphorylation and induced down-regulation of IGF-1R *in vitro* and in tumour xenografts.[37] It is currently being investigated in phase I trials in patients with relapsed and refractory MM.[9]

2.1.2. *Immunotoxins and immunoconjugates*

The direct linking of MoAbs with drugs, toxins, or radionuclides to specifically target cancer cells has been widely studied.[3,7,9,10] MoAbs linked to protein toxins, such as ricin or *Pseudomonas* toxin are highly active *in vitro* and are selective, unlike corresponding unconjugated toxins.[7,42,43] However, *in vivo*, these immunotoxins have significant side-effects and retain their high immunogenicity.[42,44] Immunotoxins are bi-functional protein molecules consisting of a MoAb chemically conjugated or genetically fused to a protein toxin.[42,45] MoAbs are selected for immunotoxins based on their reactivity towards cell surface receptors or antigens that are preferentially expressed on malignant cells. After MoAb-mediated internalization, the toxin portion of an immunotoxin traffics into the cytosol, where the enzymatic activity innate to the toxin catalytically inhibits protein synthesis, resulting in cell death. G28-5 sFv-PE40 is a single-chain immunotoxin targeted to CD40, which is highly expressed in human haematologic malignancies, including NHL, B-lineage leukaemias, MM as well as certain carcinomas. *In vitro* analysis showed

that this monovalent immunotoxin had a binding affinity of 3 nmol/L, within 15-fold of the bivalent parental MoAb.[46] This toxin was effective in treating human Burkitt's lymphoma xenografted SCID mice with complete antitumour responses, defined by an asymptomatic phenotype for greater than 120 days, obtained at doses of 0.13 to 0.26 mg/kg. Francisco's group[47] has further reported the efficacy of BD1-G28-5 single-chain Fv (sFv), another single-chain immunotoxin targeted to human CD40 consists of bryodin 1 (BD1), a plant ribosome-inactivating protein that is 20-30-fold less toxic in animals than commonly used toxins, fused to the sFv region of the anti-CD40 MoAb G28-5. BD1-G28-5 sFv retained the full protein synthesis inhibition activity of recombinant BD1. BD1-G28-5 sFv was potently cytotoxic against CD40-expressing B lineage NHL and MM cell lines, but not against a CD40-negative T cell line. Interestingly, BD1-G28-5 sFv was not cytotoxic against CD40-expressing carcinoma cell lines that were sensitive to a BD1-based immunotoxin conjugate targeted to the Ley carbohydrate antigen. These data represented the first report indicating that BD1 can be used in the construction of potent single-chain immunotoxins against CD40-expressing haematologic malignancies. Goldmacher[48] have reported the *in vitro* cytotoxic properties of a conjugate of the anti-CD38 MoAb HB7 with ricin that has been chemically modified so that its galactose-binding sites of the B chain are blocked by covalently attached affinity ligands (blocked ricin). Conjugation of blocked ricin to the HB7 has minimal effect on the apparent affinity of the antibody. HB7-blocked ricin was tested for its ability to inhibit protein synthesis in fresh patients' MM cells and in normal BM mononuclear cells (BMMCs) isolated from two healthy volunteers; tumour cells from four of five patients were 100-fold to 500-fold more sensitive to the inhibitory effect of HB7-blocked ricin than the normal BM cells. The potent specific cytotoxicity of this immunotoxin for tumour cells combined with its low cytotoxicity for normal cells suggests that it may be efficacious for either the *in vivo* or *in vitro* killing of MM cells. The strong expression of CD38 on MM cells compared with its pattern of expression on normal cells suggests that this antigen may be a useful target for the *in vivo* or *in vitro* depletion of tumour cells while sparing normal cells. Indeed, a chimeric heteroconjugate using the Fab portion of an anti-CD38 MoAb and a human IgG F fragment has been shown to mediate ADCC and is undergoing clinical testing. Previous studies using varying doses and schedules of *in vivo* administration of another blocked ricin immunotoxin, anti-B4-blocked ricin[49] showed that it was well tolerated by patients and that transient hypoalbuminemia, thrombocytopenia and fever were the main side-effects of the immunotoxin serotherapy for B-cell malignancies. The use of these chimeric or humanized MoAb-based immunoconjugates that are relatively non-immunogenic with high affinity for tumour-associated antigens and that are efficiently internalized into cells once they bind to the target antigen, have the potential to both improve antitumour efficacy and reduce the systemic toxicity of therapy.[42] Several immunoconjugates, particularly those that incorporate internalizing Abs and tumour-selective linkers have demonstrated impressive activity in preclinical models. Immunoconjugates that deliver doxorubicin, maytansine and calicheamicin are currently being evaluated in clinical trials for targeted treatment of cancer.[50] An important point for a successful MoAb-delivery of drug cytotoxicity is the antitumour activity of the linked agent. Maytansine derivatives represent a new class of highly cytotoxic agents suitable for conjugation with MoAbs.[51] Maytansine is a natural product, originally derived from the Ethiopian shrub *Maytenus serrata*, which inhibits tubulin polymerization, thereby resulting in mitotic block and cell death. The activity of maytansine is approximately 200-1000-fold greater than that of the Vinca alkaloids, which exert their cytotoxic potential by a similar mechanism. Because of their extremely high potency, maytansinoid derivatives are presently of great interest. Several other related conjugates progressing toward (or undergoing) clinical trials are bivatuzumab mertansine (DM1-Anti CD44V6 MoAb), huN901-DM1,[52,53] BB-10901,[54–56] trastuzumab-DM1,[57] MLN591-DM1,[58] MLN2704-DM1,[59] and My9-6-DM1 targeted against the CD33 antigen on myeloid cells.[60] HuN901 is a humanized MoAb that binds with high affinity to CD56, the neuronal cell adhesion molecule. HuN901 conjugated with the maytansinoid $N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine (DM1), a potent antimicrotubular cytotoxic agent may provide targeted delivery of the drug to CD56 expressing tumours. Based on gene expression profiles of primary MM cells showing expression of CD56 in 10 out of 15 patients (66.6%) and flow cytometric profiles of MM ($CD38^{bright}CD45^{low}$) cells showing CD56 expression in 22 out of 28 patients (79%), Tassone[61] assessed the efficacy of huN901-DM1 for the treatment of MM. They first examined the *in vitro* cytotoxicity and specificity of huN901-DM1 on a panel of $CD56^+$ and $CD56^-$ MM cell lines, as well as a $CD56^-$ Waldenstrom's macroglobulinemia (WM) cell line. HuN901-DM1 treatment selectively decreased survival of $CD56^+$ MM cell lines and depleted $CD56^+$ MM cells from mixed cultures with a $CD56^-$ cell line or adherent BMSCs. *In vivo* antitumour activity of huN901-DM1 was then studied in a tumour xenograft model using a $CD56^+$ OPM2 human MM cell line in SCID mice. They observed the inhibition of serum paraprotein secretion, inhibition of tumour growth, and increase in survival of mice treated with huN901-DM1. These data demonstrated that huN901-DM1 has significant *in vitro* and *in vivo* anti-MM activity at doses that are well tolerated in a murine model. Recently, in a further report, Tassone and co-workers have demonstrated[62] the *in vitro* and *in vivo* antitumour activity of the maytansinoid DM1 covalently linked to the murine MoAb B-B4 targeting syndecan-1 (CD138). They evaluated the *in vitro* activity of B-B4-DM1 against a panel of CD138(+) and CD138(–) cell lines, as well as CD138(+) patient MM cells. Treatment with B-B4-DM1 selectively decreased growth and survival of MM cell lines, patient MM cells and MM cells adherent to BMSCs. Tumour regression and inhibition of tumour growth, improvement in OS, and reduction in levels of circulating human paraprotein were observed in mice treated with B-B4-DM1. Although immunohistochemical analysis demonstrated restricted CD138 expression in human tissues, the lack of B-B4 reactivity with mouse tissues precludes evaluation of its toxicity in these models. The authors concluded that B-B4-DM1 is a potent anti-MM agent that kills cells in an antigen-dependent manner *in vitro* and mediates *in vivo* antitumour activity at doses that are well tolerated, providing the rationale for clinical trials of this immunoconjugate in MM.

2.1.3. *Radioimmunoconjugates*

Targeted radioimmunotherapy (RIT) has proved efficient in the treatment of radiosensitive tumours, such as NHL and MM, but it is less suitable for more radioresistant solid tumours.[42,63] Several methodologic approaches intended to improve RIT efficacy are under evaluation. One involves optimizing the use of radionuclides - for instance, by replacing iodine 131 ((131)I), which has been widely used until 2002, with a more suitable radioisotope, such as yttrium 90. This isotope is particularly useful for lymphomas, which are often treated only when the tumour mass has become large. In case of MM, cells are found either isolated in BM or in small clusters. Among the different beta-emitters available, it would be logical to choose (131)I for the treatment of MM, because the energy of beta particles emitted is distributed within a range of about a millimeter, whereas more energetic beta emitters (yttrium 90, rhenium 186 and 188) deliver their energy over a greater range. However, the notion of energy distribution is not the only parameter to consider; high gamma emission, physical half-life, or the coupling technique used can make the choice of a suitable radionuclide more complicated. For instance in MM, alpha particles have a theoretical advantage over beta particles because of their high linear energy transfer and shorter range of action. Presumably, cell destruction would be more selective and irradiation less harmful to adjacent tissues. RIT in MM causes intense, prolonged blockage of the cell-cycle phase arrest that maintains the cells in a more radiosensitive state, which accounts for greater cell destruction. Using a specific MoAb, B-B4 coupled to bismuth 213 ((213)Bi) by a chelating agent (CITC-DTPA), the feasibility of alpha-RIT for MM has been demonstrated previously.[64–66] In another study,[67] the two MoAbs tested, MA5 and B-B4, targeted the epithelial antigens Muc-1 and syndecan-1, respectively, which are both expressed by MM cell lines. Radiobiologic effects were evaluated for (213)Bi- and (131)I-labelled MoAbs. Supiot[67] assessed *in vitro* that MA5 stained all MM cells in only 50% of patients, whereas B-B4 recognized all MM cells in all patients. B-B4 principally showed hepatic, pulmonary, and duodenal staining, whereas MA5 marked renal and pulmonary tissues. RIT with (213)Bi-B-B4 induced specific mortality and G(2)/M phase cell cycle arrest, which depended on the concentrations and specific activity. For (213)Bi-MA5, this arrest appeared at concentrations above 10 nM, an amount 5-fold higher than that required with B-B4. This difference was also found in thymidine incorporation assays. Furthermore, with (213)Bi-B-B4, the arrest at the G(2)/M phase appeared quickly, within 24 hours after irradiation, and affected up to 60% of the cells (for 20 nM of (213)Bi-B-B4 at 1200 MBq/mg). Conversely, (131)I-B-B4 had a very limited effect on cell mortality and did not induce any cell cycle arrest. The study showed that B-B4 might be the more effective therapeutic tool and further suggests that alpha-RIT might be more suitable than beta-RIT for treating single-cell tumour models. Thus, these findings set the stage for the beginning of phase I/II clinical trials using alpha-emitter-radiolabelled B-B4, with special attention paid to hepatic, pulmonary and intestinal side-effects. Furthermore, the continued over expression of syndecan-1, the target of B-B4 in progressive MM suggests that B-B4-alpha-RIT would be effective even in the treatment of refractory forms of the disease.

2.2. *Targeted radiotherapy*

In contrast to conventional radiotherapy, an outside-in approach - targeted radiotherapy-systemic administration of radioactive agents that home in on a particular tissue, antigen, or receptor type proceeds from the inside out.[68] Several radiolabelled compounds have been shown to be effective in early phase clinical trials as well as in preclinical studies. To be effective by themselves, therapeutic agents have to be potent and penetrating, and they need to kill only those cells that express large amounts of the target molecule. As a component of targeted radiotherapy, MoAb is merely a delivery vehicle. The attached radioisotopes do the killing, and their emissions penetrate tumours reaching diseased cells that may or may not express the target antigen. But antibodies are big; whereas, smaller molecules travel better, penetrate tumour tissue better and are excreted much more swiftly when released into circulation. Research efforts are now shifting to these smaller molecules to better exploit the effects of radioisotopes. Some of these newer radiopharmaceuticals often conjugated with newly tamed radioisotopes are doing a better job than their older counterparts of targeting cancer cells and providing pain relief, and some are even showing promise as therapeutic agents alone or in combination with other chemotherapeutic agents.[68]

2.2.1. *Bone Seekers*

Representatives of the bone-seeking radiopharmaceuticals readily bind to sites where new bone is being deposited - a frequent reaction to the presence of a metastatic lesion.[68] Phosphonate chelates are taken up in the skeleton and could be combined with radioactive isotopes to deliver high levels of radiation to bone and BM while sparing normal tissues. In adults, pronounced bone formation occurs almost exclusively at sites of metastases. In the 1980s, strontium-89 (MetaStron; Alberstham), a radioactive calcium analog was approved for pain palliation.[68] For many cancer indications, chemotherapy has become the standard treatment to control progression of disease in soft-tissue areas. But some chemotherapeutic agents are radiosensitizers. Indeed, like chemotherapy, strontium-89 is itself myelosuppressive - it suppresses BM proliferation. In the late 1990s, the FDA approved Quadramet (153Sm-EDTMP; lexidronam), a short-range, beta-emitting, targeted radioisotopic pharmaceutical with avid skeletal uptake consisting of 1:1 radioactive samarium-153 chelated by a bone-seeking ethylenediaminetetramethylene phosphonate (EDTMP), with the aim of delivering a potentially ablative radiation dose to BM for bone pain palliation in cancer.[68] It concentrates by chemi-absorption in areas of enhanced metabolic activity where it associates with the hydroxyapatite crystal. Samarium-153 has a half-life of just less than 2 days. The rapid depletion in residual radioactivity or very rapid clearance from non-osseous tissues means that patients can move quickly into chemotherapy. It also means that those with diminished blood cell counts can, in short order, be given haematopoietic agents, such as recombinant erythropoietin or granulocyte-macrophage colony-stimulating factor (GM-CSF) without

risking high mutation rates in newly burgeoning blood cells. With Quadramet, the wait time shrinks to about 2 weeks. Total-body irradiation (TBI), followed by haematopoietic system rescue by BMT has been found to improve the response of patients with MM to treatment with melphalan. Moreover, MM is known to be very susceptible to radiation treatment.[68] TBI was once a standard therapy, but as chemotherapy became increasingly a routine in this indication, fell out of favour because of the two modalities' additive toxicity.

Over the last 3 years, Wiseman and Dispenzieri at the Mayo Clinic conducted uncontrolled phase I[69,70] and phase II trials[71] combining Quadramet (at a dose about 30 times that approved for palliation) with a standard/fixed high dose of the cytotoxic drug melphalan (200 mg/m^2) in MM patients, followed by autologous PBSCT conditioning regimen. Patients were further treated at a skeletal-targeted absorbed radiation dose of 40 Gy based on a trace labelled infusion 1 week prior to therapy. Despite rapid elimination of unbound radiopharmaceutical, via kidneys and bladder, no episodes of nephrotoxicity, thrombotic thrombocytopenic purpura (TTP), haemorrhagic cystitis, or delayed radiation nephritis were observed with follow-ups. Overall response rate was 94% in the phase I trial and about 50% of the patients exhibited complete or near-complete responses in the phase II trial. Addition of high dose of 153Sm-EDTMP to melphalan conditioning thus appears to be safe and well tolerated. Its effect on PFS and OS is not known but the very good and CR rates are promising.

Osteolytic bone destruction is the hallmark of MM. The bisposphonate zoledronic acid is one of the most potent anti-resorptive agents used in MM. Iuliano and co-workers[72] investigated the toxicity, clinical impact and quality of life of the synergistic combined effects of a sequential dose of 153Sm-EDTMP and zoledronic acid in 8 elderly symptomatic refractory MM patients. Two GBq (~54 mCi) of 153Sm-EDTMP was scheduled to be administered every 12 weeks and 4 mg of zoledronic acid every 28 days. Two courses of 153Sm-EDTMP plus zoledronic acid were sufficient to produce long-term improvement in MM-related symptoms devoid of any side-effects with a decrease in the M-component more than 25%. The study thus provides a novel palliative approach to the treatment of symptomatic elderly MM patients not eligible for further chemotherapy. The absence of additive metabolic or renal toxicity may warrant the inclusion of such an approach in the clinical practice and the extension of this approach in younger patients in association with chemotherapy. Pharmacokinetics and radiation dosimetry for a commercial preparation of 153Sm-EDTMP were evaluated in a controlled clinical trial in 43 tracer (average dose 740 MBq) studies of 42 patients with haematological malignancies.[73] Activity taken up by the skeletal tissue was firmly bound, and the percentage activity retained in the skeleton at 24 hour with tracer doses was high (62%+/−13%), although this decreased to approximately 30% with therapy infusions. Because of this decrease in retention, the maximum feasible therapy activity for this formulation of Quadramet is 35 GBq. After therapy infusions of up to 50 GBq in 37 patients, non-haematopoietic toxicity was not seen in any patient. In addition, myelosuppression was achieved without evidence of myelofibrosis. A similar trial by Macfarlane and Morton[74] examined the safety of adding an escalating regimen of 153Sm lexidronam (19–45 GBq) 12–14 days prior to the standard SCT regimens for marrow-based haematological malignancies in 10 patients, 7 with MM, in whom TBI as part of conditioning was desirable but not feasible. No adverse events were attributable to 153Sm lexidronam. Of the 7 patients with MM, 4 achieved CR, 2 partial responses, and another had stable monoclonal band at 3 months post-transplant. Dose-limiting toxicity was not attained. At the activities used, 153Sm lexidronam was not associated with additional toxicity in this population.

Turner, Glancy and co-workers have reported through a series of studies on the potential therapeutic efficacy of 153Sm-EDTMP in MM, both in experimental animal models as well as in clinical trials. Chemoradiotherapy with melphalan and 153Sm-EDTMP was used in an experimental rat model to ablate BM, which was subsequently rescued by BMT.[75] In comparison to the singly administered agents, the combination of 9.5 mg kg^{-1} melphalan and 555 MBq kg^{-1} 153Sm-EDTMP caused marrow ablation with high animal mortality. However, the mortality was reduced to 7% by the sequential chemoradiotherapeutic regimen of internal irradiation with 153Sm-EDTMP followed by chemotherapy with melphalan to ablate BM effectively whilst preserving the capacity for recovery following marrow transplantation. To the tune of the prior study, a mouse model system for MM simulating human MM was used for sequential therapy with 22.5 MBq 153Sm-EDTMP, 18.5 mg/kg melphalan, and syngeneic BMT.[76] Results showed that, the sequential treatment regimen was significantly more effective than the single-agent treatment in terms of improved survival without any evidence of radiotoxicity. The survival advantage conferred by these sequential therapeutic protocols suggests its potential clinical usefulness in the treatment of MM and other haematologic malignancies in humans.

A number of studies by Turner have reported the use of samarium in extensive bone lesions, pain exacerbation and disseminated skeletal metastases; the standard recommended administered activity of 38 MBqkg^{-1} may lead to significant myelotoxicity. In one study,[77] the total administered activity of 153Sm-EDTMP predicted on a 2 Gy BM dose varied between 35 and 63% of the standard recommended regimen of 37 MBqkg^{-1} and pain relief was experienced by 8 of the 10 patients. Administration of 153Sm-EDTMP according to the supplier's recommendations would have delivered BM doses of 3.27–5.90 Gy in these patients, doses at which myelotoxicity would have been anticipated. In a clinical trial, the 103 keV gamma emission of 153Sm was utilized for prospective individual estimation of β radiation absorbed dose to red marrow to minimize myelotoxicity and provide optimum internal radiotherapy to skeletal metastases in each of the 28 patients unresponsive to all conventional treatment.[78] Pain relief occurred within 14 days of administration of 153Sm-EDTMP in almost 80% of patients. Bio-distribution studies demonstrated rapid skeletal uptake and long term retention of 153Sm-EDTMP in bone with less than 1.0% of administered activity was retained in non-osseous tissue. Microdensitometry confirmed surface uptake of 153Sm-EDTMP in cortical bone and demonstrated relatively high trabecular bone activity which is the major component of radiation absorbed dose

to BM. Haematological studies in animals showed 153Sm-EDTMP-induced myelotoxicity to be transient and no histopathological abnormalities were demonstrable with doses 10 times greater than those administered to patients. In a different study, 35 patients with disseminated skeletal metastases from a variety of tumour types underwent phase I clinical trial of 153Sm-EDTMP.[79] Pain was relieved in 22 of 34 evaluable patients (65%) following a single administration of 153Sm-EDTMP. Myelosuppression was transient and platelet counts had recovered to pretreatment levels within 10 weeks of treatment. The study indicated that 153Sm-EDTMP is effective for the amelioration of pain due to disseminated skeletal metastases, and in 15 of the 34 evaluable patients, there was evidence of stabilization or regression of skeletal metastases on radiographs and follow-up 99mTc-MDP bone scans. Turner and Claringbold conducted a phase II study of 153Sm-EDTMP palliative treatment on 23 patients with painful disseminated multifocal skeletal metastases.[80] The radiation-absorbed dose to BM was fixed at 2 Gy. Symptomatic relief of bone pain was experienced by 61% of the evaluable patients with a median duration of 8 weeks. Re-treatment with 153Sm-EDTMP was studied in 15 patients, including in 4 of the 23 patients treated with a single dose. Good control of pain was obtained in 13 of these patients (87%). Both the median duration of pain control (24 weeks) and survival (9 months) in the re-treated group were substantially greater than for patients treated with a single dose, thereby suggesting the need for multiple shots of 153Sm-EDTMP for a better therapeutic benefit.

2.2.2. *Skeletal Targeted Radiotherapy (STR™)*

Skeletal Targeted Radiotherapy (STR™) is a novel experimental approach[85,86] based on the use of Holmium-166 1, 4, 7, 10-tetraazcyclododecane-1, 4, 7, 10-etramethylenephosphonate (^{166}Ho-DOTMP) which is a radioactive bone-seeking drug. Because most MM cells are found in the BM and are sensitive to radiation, STR may be effective in the treatment of MM. ^{166}Ho-DOTMP consists of two components that are bound together.[81,82] The first component is DOTMP, a phosphate group that attaches to bone, particularly in areas of rapid bone turnover that occur in MM and various bone cancers. The other component of ^{166}Ho-DOTMP is the radioactive holmium (^{166}Ho). The high-energy and long path-length of radionuclide ^{166}Ho beta particles provide deep penetration and uniform irradiation of disease sites in the bone and its relatively short half-life of 26.8 hours permits reinfusion of stem cells within 6 to 8 days. It also has a minor gamma component suitable for imaging and dosimetry. These properties make ^{166}Ho-DOTMP a potentially useful drug for delivering a bone-based dose of targeted radiation for the treatment of cancers in the bone and BM. By selectively targeting the radiation to the site of disease, the exposure to normal organs can be reduced compared to conventional TBI.[81,82]

Before receiving STR, patient's peripheral blood SCs (PBSCs) are collected and stored for later reinfusion. Then a small dose of ^{166}Ho-DOTMP (a "tracer") is injected intravenously over 1 to 17 minutes, and tests are done to determine if enough ^{166}Ho-DOTMP goes to the bone.[81,82] If so, the patient is then treated with a larger dose of STR, along with high-dose chemotherapy (melphalan). Patients are treated in shielded rooms and remained shielded until their activity decreases to institutional radiation safety standards. The time in a shielded room generally lasts from 6 to 24 hours depending upon the amount of radioactivity delivered and the safety requirements of the institution. The combination of ^{166}Ho-DOTMP and high-dose chemotherapy along with ASCT is designed to produce both a direct therapeutic effect on tumour sites in the bone, plus a general BM-ablative effect to help destroy tumour cells in the marrow. For this reason, patients who receive STR then receive a SCT to replace their BM cells and to restore BM function. Next, the patient is carefully monitored for side-effects and to see how their myeloma responds.

Two phase I/II dosimetry studies[81–83] were conducted to evaluate the maximum tolerated dose (escalating doses) of ^{166}Ho-DOTMP in combination with various melphalan transplant-conditioning regimens (as part of a myeloablative preparative regimen) for ASCT in MM. A total of 83 MM patients were treated with STR in combination with high-dose chemotherapy (melphalan) in two phase I/II clinical trials. Positive clinical activity of STR was noted. Many (64%) of these patients had substantial response rates after the treatment, and 35% of them had a CR as well as 91% achieved disease stabilization or better. The median EFS was 22 months. The CR and EFS rates compared favourably to other published transplantation regimens. Analysis of the data showed that a STR dose of 750 millicuries of radiation per square meter of body surface area (mCi/m^2) provided optimal patient safety and tumour response. Of the 83 evaluable patients in the study, 10 had received this dose. Among these 10 patients, disease control (i.e., disease stabilization or better) was achieved in 8 patients, including 4 who achieved a CR, and the 3-year survival rate was 90%. At this dose, there were no reported cases of TTP/haemolytic uremic syndrome (TTP/HUS) or Grade 4 renal toxicity. In 2004, the 4-year survival rate for these 10 patients was reported to be 70%. These trials demonstrated the feasibility of targeted skeletal radiotherapy with ^{166}Ho-DOTMP as part of a standard preparative regimen with either 200 mg/m^2 melphalan or 140 mg/m^2 melphalan and 800 cGy of TBI.[83] As TBI has been associated with higher rates of regimen-related toxicity, future trials are being designed to determine the safety and efficacy of ^{166}Ho-DOTMP without TBI. The data from this study suggested that ^{166}Ho-DOTMP in addition to a standard preparative regimen can result in a high rate of complete remissions in MM patients undergoing autografts.

Although no engraftment delays were seen even at the highest doses in phase I/II studies, higher absolute exposure to critical non-target organs, such as the kidney and the bladder, was observed and likely contributed to the late, non-haematologic toxicities that developed. Follow-up of these patients revealed 3 main late toxicities: grades 2 to 3 haemorrhagic cystitis (defined by the National Cancer Institute [NCI] Common Toxicity Criteria as greater than grade 2 haematuria with or without associated symptoms) with onset from 1 to 45 months after ^{166}Ho-DOTMP therapy, thrombotic microangiopathy (TMA) of the kidney and late renal dysfunction, and myelodysplastic syndrome.[81–83] Some patients experienced serious bladder problems, that include grade 1 or higher haematuria, haemorrhagic cystitis, diffuse erythaema (particularly around the ureteral outlets), bladder neck obstruction,

radiation-nephritis, and relapse in the bladder after receiving the drug. Almost all of the patients who experienced bladder toxicities did not have a procedure called "continuous bladder irrigation," that helps flush extra radioactivity from the body. All patients who have been treated with STR will now receive continuous bladder irrigation as part of their treatment. A few patients who experienced bladder problems also experienced renal dysfunctions including renal toxicity of grade 3 or higher at a dose of more than 40 Gy targeting the marrow. Moreover, a severe form of sustained renal impairment associated with microangiopathic haemolytic anaemia, thrombocytopenia, uncontrolled hypertension, elevated lactate dehydrogenase, elevated creatinine levels and TMA consistent with a radiation nephropathy were also observed. Most of these problems occurred in patients who received the highest doses of STR, which are no longer used. TTP/HUS occurred in patients receiving doses of STR that are higher than the dose being evaluated in the phase III trial and was possibly caused by a high rate of radiation exposure to the kidney immediately after ^{166}Ho-DOTMP infusion. No patients who received this dose in the earlier trials experienced TTP/HUS. Other possible side-effects of this regimen include nausea, vomiting, diarrhoea, fatigue, fever, increased risk of infection, and organ damage.

In 2002, a small pivotal study[81,82] evaluated the method to be used for calculating radiation dose in the planned phase III study. This dosimetry study included 12 patients at 5 transplant centers in the US. In the study, tracer doses of STR were first administered to determine whether there was sufficient uptake by the bone and to determine the appropriate treatment dose. Eligible patients then received a treatment dose of STR, followed by high-dose chemotherapy and PBSCT to restore BM function. The safety data were encouraging, with no reported cases of TTP/HUS or bladder toxicities. One grade 4 adverse event (severe weight loss) was reported. Seven serious adverse events (fever) were reported but were considered to be related to the high-dose chemotherapy rather than to STR. Two episodes of grade 3 mucositis (mouth sores) were reported.

A multi-center, randomized, phase III study,[68,81,82] which expects to enroll approximately 240 patients with primary refractory MM (patients with newly diagnosed disease who do not respond to standard chemotherapy) is presently ongoing. This trial is designed to evaluate the safety and efficacy of STR in patients with primary refractory MM, defined as patients who have failed to respond to any therapy since the initiation of their first treatment and have been undergoing treatment for less than 18 months. It is expected to complete patient enrollment by the end of 2006 and to file a New Drug Application (NDA) for STR in MM in mid 2007. STR might provide the potential for a novel approach in MM.

MM and other haematological malignancies have been treated by myeloablative radiotherapy/chemotherapy and subsequent SCT. (166)Dy]Dy/(166)Ho-ethylenediaminetetramethylene phosphonate (EDTMP)[84] forms a stable *in vivo* generator system with selective skeletal uptake in mice; therefore, it could work as a potential and improved agent for marrow ablation. Induced BM cytotoxicity and genotoxicity were determined by the reduction of reticulocytes (RET) and elevation of micronucleated reticulocyte (MN-RET) in peripheral blood and ablation by BM histological studies. Enriched (166)Dy(2)O(3) was irradiated and (166)Dy]DyCl(3) was added to EDTMP in a molar ratio of 1:1.75. A group of BALB/c mice was intraperitoneally injected with the radiopharmaceutical and two groups of control animals were injected with the cold complex and with 0.9% sodium chloride, respectively. The RET and MN-RET frequency were statistically different in the treatment at the end of the 12-day period demonstrating cytotoxicity and genotoxicity induced by the *in vivo* generator system. The histological findings showed that there was complete or almost complete acellularity, which means significant suppression of the BM activity. The study indicated that [(166)Dy]Dy/(166)Ho-EDTMP] induces cytotoxicity, genotoxicity and severe myelosuppression in mice. Potentially, it may be a good agent for use in humans.

3. Conclusion

Before more effective immunotherapy strategies can be established, a better understanding of the immune defects that prevent MM patients from mounting a strong response against their tumour cells is required. Researchers are beginning to identify these defects, which include functional deficiencies in T cells and dendritic cells, excessive production of inhibitory cytokines, and inadequate production of stimulatory cytokines.[85] A number of antitumour agents with demonstrated activity against other forms of cancer are showing promise against MM as well. Novel MoAbs that target various receptors are in preclinical trials. Immunomodulatory therapies that induce cell apoptosis and inhibit angiogenesis are now being investigated in newly diagnosed patients as well as in those who have failed previous therapies.[86] We expect that, the use of potentially targeted therapies and host-directed novel treatments (e.g, glucocorticoids, bisphosphonates, MoAbs against IL-6 and IGF-1, novel IMiDs, proteasome inhibitors) would be a useful strategy for the improvement of patient outcome in MM in the near future.[3–10,87,88]

Conflict of interest statement

None declared.

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