Novel Drugs in Myeloma: Harnessing Tumour Biology to Treat Myeloma

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Abstract Steroids and alkylating agents have formed the backbone of myeloma therapy for decades with the result that patient outcomes improved very little over this period. The situation has changed recently with the advent of immunomodulatory agents and bortezomib, and patient outcomes are now improving. The introduction of bortezomib can be viewed as particularly successful as it was designed in the laboratory to fit a target that had been identified through biological research. As such, it has formed the template for new drug discovery in myeloma, with an increased understanding of the biology of the myeloma cell leading to the definition of upregulated pathways which are then targeted with a specific agent. This chapter will examine novel agents currently in development in the context of the abnormal biology of the myeloma cell and its microenvironment.

8.1 Introduction

Conventional chemotherapy, including high dose chemotherapy with autologous stem cell rescue, is successful in producing responses in the majority of patients with newly presenting multiple myeloma. However, relapse is almost universal

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T. Moehler and H. Goldschmidt (eds.), *Multiple Myeloma*,
Recent Results in Cancer Research 183, DOI: 10.1007/978-3-540-85772-3_8,

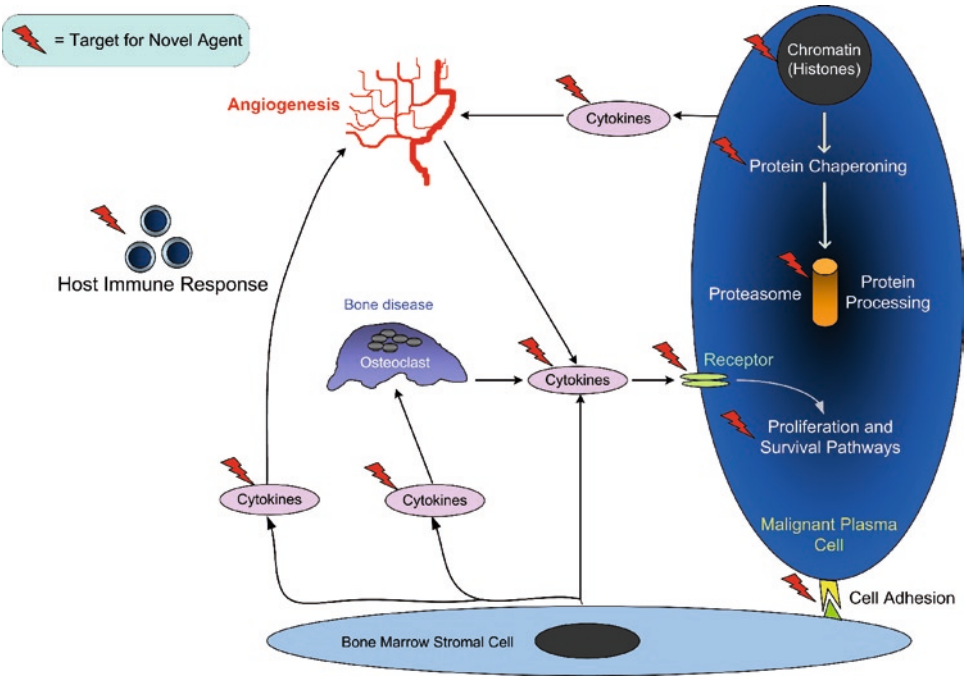

Fig. 8.1 Potential targets for novel agents within the myeloma cell and in the bone marrow microenvironment. Within the cell, drugs can target chromatin, protein chaperoning, protein processing, and intracellularsignalling pathways. Cell surface receptors and cell adhesion to stromal cells are also valid targets. In the bone marrow microenvironment, cytokines that stimulate the myeloma cell, and promote angiogenesis and bone disease can be targeted, whilst immunomodulatory drugs up-regulate the host immune system

and relapsed disease is more difficult to treat due to intrinsic or acquired drug resistance. Myeloma remains an incurable disease with a median survival of 4–5 years, and there is clearly a role for new drugs in its treatment. The development of novel agents has followed on from the huge expansion of knowledge of the biology of the myeloma cell and its interaction with the bone marrow milieu. Within the malignant plasma cell, constitutively activated signalling pathways resulting in cell growth, proliferation and avoidance of apoptosis have been defined. Within the bone marrow milieu, cytokines that stimulate angiogenesis and lytic bone disease and act as growth factors for the malignant clone have also been described. All of these pathways are potential targets for new drugs, and there are currently a raft of phase I and II trials of agents that target the myeloma cell and the cytokines and cells that make up its environment. This chapter will examine novel agents in the context of the biology of the tumour, first examining targets within the tumour cell, and then looking at targets within the bone marrow microenvironment (Fig. 8.1).

8.2 Intracellular Drug Targets

8.2.1 Targeting Signalling Pathways Within Myeloma Cells

External influences such as cytokine stimulation and physical interaction with bone marrow stromal cells trigger intracellular signalling

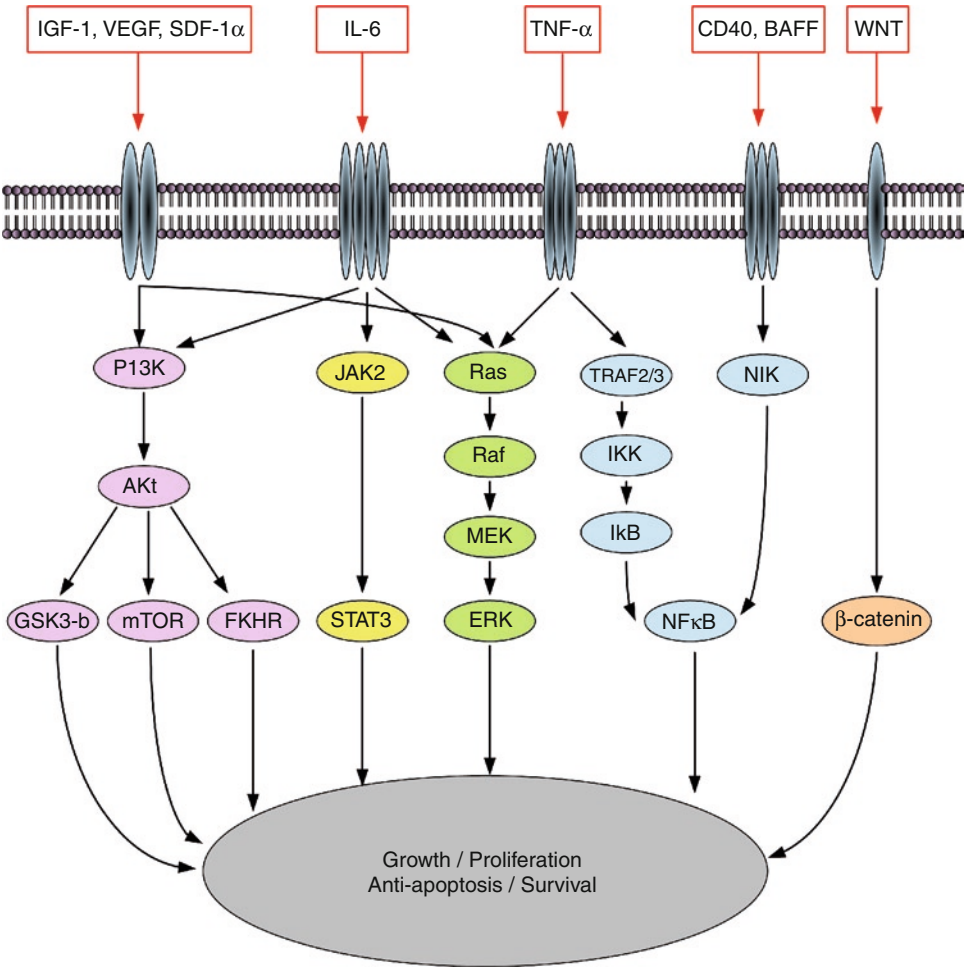

Fig. 8.2 Intracellular signalling pathways

cascades that contribute to the malignant phenotype of the myeloma cell, namely, avoidance of apoptosis, survival, growth and proliferation. Signalling pathways thought to be important in myeloma are the Ras/Raf/MEK/MAPK pathway, the JAK/STAT3 pathway, the P13K/Akt/mTOR pathway, the canonical and non-canonical NF-κB pathway and the Wnt/β-catenin pathway. In addition, the Wnt pathway has been identified as being important in osteolytic bone disease. Inhibition of any of these pathways has the potential to decrease the survival advantage of the myeloma cell and they all therefore constitute valid drug targets (Fig. 8.2).

8.2.1.1 The Ras/Raf/MEK/MAPK Pathway

The Mitogen-activated protein kinase (MAPK) cascade is a key signalling pathway involved in the regulation of cell differentiation, proliferation and survival. The pathway can be stimulated by many cytokines, including interleukin-6 (IL-6), insulin-like growth factor-1 (IGF-1), vascular

endothelial growth factor (VEGF), tumour necrosis factor-α (TNFα), interleukin−21 (IL-21) and stromal cell derived factor-1 (SDF-1), which activate the Ras/Raf serine/threonine kinases. Raf activates the MAPK/ERK kinase (MEK) which in turn activates the extracellular signal-related kinase (ERK) (Roberts and Der 2007). ERK causes autocrine upregulation of the pathway, essential for the malignant phenotype. Mutations of the oncoprotein Ras were found in 23% of patients with myeloma in one case series, which makes it the most commonly mutated gene in myeloma (Chng et al. 2008b). Mutated cases had features associated with greater tumour burden such as higher plasma cell percentage on bone marrow biopsy, higher beta-2-microglobulin (β2m) and more advanced International Staging System (ISS) stage. Moreover, although mutated cases had similar response rates to non-mutated cases, responses were short-lived with shorter progression-free survival (PFS) and overall survival (OS) times.

One of the first steps of signalling in the pathway involves the transfer of farnesyl groups from farnesyl diphosphate to Ras, which allows Ras to be attached to the intracellular membrane. This step is targeted by *farnesyl transferase inhibitors* (FTIs), three of which have been demonstrated to have anti-myeloma activity. *Perillic acid* and *FTI-277* have induced apoptosis and inhibited the growth of myeloma cell lines (Beaupre et al. 2003; Bolick et al. 2003). *Tipifarnib (R115777)* has undergone a phase II trial involving 43 myeloma patients that showed it to be well tolerated and associated with disease stabilisation in 64% of patients (Alsina et al. 2004). This trial showed treatment with Tipifarnib to be associated with decreased levels of phosphorylated Akt and Signal Transducer and Activator of Transcription 3 (STAT3) but not ERK, suggesting that it may act through Ras-independent pathways. This has been borne out in research showing that Tipifarnib's in vitro efficacy does not correlate with Ras mutation status or inhibition of farnesyl transferase (Beaupre et al. 2004; Buzzeo et al. 2005; Armand et al. 2007). FTIs can potentially inhibit multiple farnesylated protein substrates involved in tumour proliferation, so although the initial rationale for their use was based on the knowledge of the importance of Ras in myeloma, they may work primarily through pathways other than the MAPK cascade. The pathway can, however, be targeted at other points. *Sorafenib (BAY 43-9006)* is a multikinase inhibitor that targets Raf and is being used in trials including patients with myeloma.

MEK has been targeted by the drug *AZD6244*. In vitro studies of this drug show it to trigger apoptosis in myeloma cell lines, sensitise cells to other chemotherapy agents, and inhibit cytokine-induced activation of osteoclasts which have an important role in the development of myeloma associated bone disease (Tai et al. 2007). Activation of MEK/ERK is one of the mechanisms of acquired steroid resistance, so combination regimens that involve blockade of this pathway may show synergism or sensitise previously drug-resistant tumours (Tsitoura and Rothman 2004).

8.2.1.2 The Janus Kinase (JAK)/STAT Pathway

The Janus Kinase (JAK)/STAT3 pathway is stimulated by cytokines such as IL-6 and IL-21 and has been shown to be constitutively activated in ~50% of primary myeloma samples, whilst suppression of STAT3 in these samples with curcumin leads to apoptosis (Bharti et al. 2004). Several other agents have been used to block STAT3 activity in myeloma cells, including the JAK 2 inhibitor *AG490* (De Vos et al. 2000), the pan-JAK inhibitor *pyridine 6* (Pedranzini et al. 2006) and *atiprimod* (Amit-Vazina et al. 2005), with similar results of decreased tumour cell viability and increased apoptosis. However, if myeloma cells are in the

presence of bone marrow stromal cells, simultaneous inhibition of the MEK/ERK pathway and the JAK/STAT pathway is required in order to induce apoptosis (Chatterjee et al. 2004). This may be a consideration in the design of future drug trials. Currently atiprimod is the only agent targeting this pathway that has been moved forward into phase I/II patient trials. Preclinical studies have shown this anti-inflammatory agent to inhibit STAT3 activation, reducing the proliferative cytokine IL-6 and down-regulating the anti-apoptotic proteins bcl-2, bcl-X(L) and mcl-1 leading to cell death (Amit-Vazina et al. 2005). Mouse models have confirmed the potential utility of this drug (Neri et al. 2007).

8.2.1.3 The Phosphatidylinositol-3 Kinase (PI3-K)/Akt Pathway

IL-6 and IGF-1 have been shown to mediate proliferation and drug resistance through this pathway (Tu et al. 2000). Activation of Akt has been identified in ~50% of primary myeloma samples (Zollinger et al. 2008). IL-6 induces Akt phosphorylation, which in turn phosphorylates several downstream targets including mammalian target of rapamycin (mTOR), GSK-3B and forkhead transcription factor (FKHR). Upregulation of this pathway has been shown to inactivate FKHR, leading to G1/S phase transition. mTOR, a serine/threonine protein kinase, also regulates the G1/S phase gateway, up-regulating expression of downstream targets such as the D-Cyclins resulting in cellular proliferation. Akt activation has also been linked to resistance to dexamethasone-mediated apoptosis, mediated through inactivation of capsase-9(Hideshima et al. 2001). Blockade of this pathway should therefore lead to G1 growth arrest, and sensitization to steroid-induced apoptosis, and when Akt is down-regulated by siRNA constructs in activated samples, apoptosis is induced.

The pathway has been targeted at several points. P13-K has four class I isoforms (p110α, p110β, p110δ, p110γ), all of which can be inhibited in the laboratory with agents such as LY294002 which have poor solubility. *SF1126* is a soluble conjugate of this agent and retains its efficacy against all class I P13-K isoenzymes. It has demonstrated synergy with bortezomib, vincristine, steroids and alkylating agents and is currently in phase I trials (David et al. 2008). Pichromene is another drug capable of inhibiting all four P13-K enzymatic activities, leading to reduced expression of cyclins D1, D2 and D3 (Mao et al. 2008) Inhibitors of the individual enzymatic isoforms are also in development, including *CAL-101*, which has been shown to induce apoptosis in cell lines even in the presence of bone marrow stromal cells (Ikeda et al. 2008b). Downstream from P13-K, Akt has been targeted by *Perifosine*, a synthetic alkylphospholipid that inhibits phosphorylation, and therefore activation, of Akt. It has been shown to be cytotoxic to myeloma cell lines both when they are in isolation and when they are in the presence of bone marrow stromal cells, to show synergism to MEK inhibitors, dexamethasone and doxorubicin, and to have anti-myeloma activity in a mouse model (Hideshima et al. 2006a). A clinical trial using perifosine monotherapy in 48 patients induced a minor response in one patient, with stable disease in 22 patients. Perifosine with dexamethasone was used in the same trial by 31 patients with progressive disease, with four showing a partial response, eight a minor response and 15 with stable disease (Richardson et al. 2007c). It has also been used in combination with bortezomib with an overall response rate (ORR) of 40%, and with lenalidomide + dexamethasone with an ORR of 50% (Richardson et al. 2007b; Jakubowiak et al. 2008). These response rates are similar to those obtained with their respective partner drugs without perifosine, so it is difficult from these small trials to assess if any benefit is derived from the addition of perifosine. In the bortezomib trial, a 37% response rate was reported in patients classed as previously

resistant to bortezomib, so its role may lie in sensitising chemotherapy-resistant myeloma. Other Akt inhibitors have been developed such as phosphatidylinositol ether lipid analogues (PIAs) and API-2, but these have not been characterised in myeloma yet.

Rapamycin is an antifungal compound produced naturally by *Streptomyces hygroscopicus* which has been discovered to be a specific inhibitor of mTOR, inducing G0/G1 arrest, and sensitising myeloma cells to dexamethasone-induced apoptosis (Stromberg et al. 2004). In vitro studies suggest that it may be synergistic with both bortezomib and lenalidomide (Raje et al. 2004; O'Sullivan et al. 2006). Two rapamycin analogues, *temsirolimus (CCI-779)* and *RAD-001*, have been developed which work by binding to the FK506 binding protein FKBP-12 which then binds to and inhibits mTOR (LoPiccolo et al. 2008). Temsirolimus has demonstrated induction of apoptosis and inhibition of proliferation in a myeloma mouse model (Frost et al. 2004), and also down-regulation of VEGF thereby reducing angiogenesis (Frost et al. 2007). A phase I trial combining temsirolimus with bortezomib showed a tolerable side-effect profile with an overall response rate (ORR) of 33%, and phase II studies are ongoing (Ghobrial et al. 2008). The use of RAD-001 has yet to be reported in myeloma. It has been suggested that inhibition of mTOR may feedback to produce upregulation of upstream elements of the pathway such as Akt, and that P13-K and Akt may be better targets within this pathway (Yap et al. 2008). This will need to be examined in further experimental work.

8.2.1.4 The Nuclear Factor-Kappa B (NF-κB) Pathway and the Ubiquitin Proteasome System

NF-κB is a transcription factor which has been found to be upregulated in both tumour and stromal cells of patients with myeloma. The constitutive activation of NF-κB in myeloma cells deregulates cell cycle and apoptotic pathways, whilst in the stromal cells it triggers the production of cytokines such as IL-6 and B-Cell Activating Factor (BAFF) which cause paracrine stimulation of the myeloma cells, including upregulation of the NF-κB pathway itself (Chauhan et al. 1996; Tai et al. 2006). Within the tumour cells, there are two NF-κB pathways, the canonical and non-canonical, both of which involve the proteasome. In the canonical pathway, an inhibitor of IκB kinase β (IKKβ) phosphorylates the inhibitory IκB proteins which are then processed by the proteasome leading to their inactivation. In the non-canonical pathway, IKKα phosphorylates p100/NFκB2, leading to the removal of an inhibitory C-terminal by the proteasome. The end result of both pathways is accumulation of NFκB in the nucleus. Constitutive activation of both pathways has been linked to mutations of multiple genes such as *TRAF3* and *CYLD* in myeloma (Annunziata et al. 2007; Keats et al. 2007).

The ubiquitin proteasome system (UPS) is responsible for intracellular protein degradation. Proteins are conjugated with a polypeptide, ubiquitin, and are then processed by the 26S proteasome, which consists of 19S flanking units controlling entrance to the 20S core (Peters et al. 1991). Within the 20S core proteolysis occurs by three activities; chymotrypsin-like (CT-L), trypsin-like (T-L) and caspase-like (C-L) (Chauhan et al. 2008). The proteasome is involved in processing many proteins involved in progression through cell cycle and survival. Pharmacological inhibition of the proteasome causes a build-up of mis-folded and unwanted proteins, including the inhibitory NF-κB proteins such as IκB, resulting in apoptosis.

Bortezomib (PS-341) is a boronic acid dipeptide which inhibits the 26S Proteasome, specifically the CT-L and C-L proteasomal activities. Although inhibition of IκB degradation appears to be the major target of bortezomib, it has been shown to affect other pathways through

inhibition of ubiquitinated proteins other than those of the NF-κB pathway and through other direct mechanisms. For example, it may indirectly downregulate both the JAK/STAT and P13-K/Akt pathways via downregulation of gp130 (Hideshima et al. 2003a). It may also affect DNA repair by cleavage of DNA repair enzymes, and phosphorylate p53 causing its activation (Hideshima et al. 2003b). The APEX study showed bortezomib monotherapy to be superior to dexamethasone in patients with relapsed myeloma (Richardson et al. 2005b). OR rates were 43% in the bortezomib group versus 17% in the dexamethasone group, which resulted in a superior overall survival (OS) of 29.8 months versus 23.7 months, despite 62% of the steroid group crossing over to the bortezomib arm (Richardson et al. 2007). Although it remains the only single-agent to show a survival benefit in relapsed myeloma patients, the fact that the majority of relapsed patients fail to respond to monotherapy has lead to a multitude of clinical trials incorporating bortezomib with other agents in order to improve response rates. The combination of bortezomib and liposomal doxorubicin has been shown to lead to improved time to progression and improved survival compared to bortezomib monotherapy (Orlowski et al. 2007b). Bortezomib has been shown to improve progression free survival (PFS) when combined with melphalan+prednisolone in a large phase III trial in newly presenting patients not suitable for auto-transplantation (San Miguel et al. 2008). Bortezomib has therefore been demonstrated to show synergism with conventional chemotherapy and novel agents, and to improve outcome in newly presenting and relapsed patients. Its current place in the treatment of myeloma, both in terms of optimal partner drugs and sequence of treatment, remains to be defined and is likely to change as newer agents become available.

Based on the success of bortezomib, other proteasome inhibitors have been developed with a view to increasing efficacy, altering the side-effect profile and providing more convenient dosing. *Carfilzomib (PR-171)* is an intravenous preparation that blocks CT-L activity but differs from bortezomib in that it shows minimal cross-reactivity with other catalytic sites within the proteasome or with other proteasome classes and may therefore have a more favourable side-effect profile. It activates apoptosis via caspase 8 and 9 in a similar fashion to bortezomib, but is more potent. Two phase II trials have been reported using carfilzomib as monotherapy in relapsed patients. One demonstrated an ORR of 54% in bortezomib naïve patients, which dropped to 19% in patients previously exposed to bortezomib. Deterioration in renal function was seen in five patients, two of which were related to tumour lysis syndrome which may be evidence of potency of the drug (Orlowski et al. 2007; Vij et al. 2009). A second was in a particularly treatment refractory group of patients which may account for the disappointing response rate of 14%. Increases in creatinine were again seen in this trial, although there was no study discontinuation due renal failure (Jagannath et al. 2009). More recent patient assessments have suggested that concurrently administering intravenous fluids with carfilzomib resolves this issue. Neurotoxicity was reported at low rates in these two trials, and chronic administration of carfilzomib in experimental animals does not result in neurotoxicity, raising the possibility that bortezomib-associated peripheral neuropathy may not be a class effect (Demo et al. 2009). A new approach to improving the side-effect profile of proteasome inhibitors is to target the immunoproteasome, a proteasomal variant that is only found in haemopoietic cells. *NPI0052* differs from bortezomib in several ways, not least of which is that it is orally active in animal models. It blocks all three proteasomal activities (CT-L, T-L and C-L) compared to the CT-L and T-L inhibition of bortezomib, and irreversibly binds to the proteasome whereas bortezomib is a reversible inhibitor. It also appears to induce apoptosis via

different signalling pathways, with NPI0052 apoptosis mediated via caspase 8, whereas bortezomib requires activation of both caspase 8 and caspase 9 (Chauhan et al. 2005). It is a more potent inhibitor of the NF-κB pathway. Whether these biological differences translate into improved efficacy needs to be evaluated in clinical trials. The compound *IPSI-001* has been identified as a potent immunoproteasome inhibitor that is effective at inhibiting the proliferation of patient myeloma samples, and is able to overcome drug resistance including bortezomib resistance (Kuhn et al. 2008). Carfilzomib also has some anti-immunoproteasomal activity.

Although NF-κB pathway inhibition appears to be central to the mechanism of action of proteasome inhibitors, their effects are broader than this and attempts have therefore been made to specifically target the pathway. Kinase inhibitors of IκB have been developed and shown to have anti-myeloma efficacy in vitro and in mouse models (Hideshima et al. 2006b), and other novel inhibitors are in development (Meinel et al. 2008). However, no clinical trials have been reported to date, so it is not clear whether inhibiting the pathway through targets other than the proteosome will provide any advantage.

8.2.1.5 The Wingless/int (Wnt)/β-Catenin Pathway

Wnts are a family of glycoproteins that bind to frizzled transmembrane receptors on the myeloma cell, leading to intracellular accumulation of unphosphorylated β-catenin which is normally degraded by the proteasome. Stimulation of myeloma cell lines with Wnt-3a has been shown to lead to cytoplasmic accumulation and nuclear localization of β-catenin, which then binds to T-cell factor transcriptional factors to activate downstream targets such as c-myc and Cyclin D2 resulting in cellular proliferation (Derksen et al. 2004). It has been suggested that the pathway is constitutively activated in myeloma due to hypermethylation (and therefore suppression) of genes acting as negative regulators of the pathway (Chim et al. 2007). A small molecule inhibitor of the nuclear binding of β-catenin to its transcriptional factor, *PKF115-584*, has been shown to be cytotoxic to myeloma cell lines and patient myeloma cells (Sukhdeo et al. 2007). There are no clinical trials at present, and the effect of any Wnt pathway inhibitor on osteolytic bone disease, which is thought to be largely mediated by the Wnt-signalling antagonist dickkopf1, would need to be carefully monitored, as our current understanding of this pathway suggests that its inhibition may result in decreased myeloma cellular proliferation, but a paradoxical increase in osteolytic activity that may exacerbate destructive bone disease.

8.2.2 Targeting the Unfolded Protein Response

The endoplasmic reticulum is responsible for post-translational modification and folding of proteins. This system is placed under stress in myeloma cells, where there is overproduction of secretary proteins, triggering a complex pathway known as the Unfolded Protein Response (UPR) which aims to prevent the accumulation of misfolded proteins. If this system fails, proteins are eliminated by ubiquitination and proteasomal digestion, or alternatively by the aggresome.

The UPR is complex, initiated by three transmembrane receptors which diverge into several pathways. The protein chaperone heat shock protein 90 (HSP90) is involved in the functioning of all three endoplasmic reticulum bound receptor pathways. HSP90 overexpression is seen in myeloma tumour cells, but not in normal plasma cells (Chatterjee et al. 2007). HSP90 inhibition should be a promising treatment as, although it only targets a single biological function, the number of chaperone client proteins affected is large and includes IGFR1 and FGFR3 as well as key proteins of the NF-κB pathway such as NIK

and IKK (Qing et al. 2006). Upregulation of HSP90, HSP70 and HSP27 is seen in myeloma cells treated with bortezomib, suggesting a protective effect to the stress induced on the cell by proteasome inhibition, and providing the rationale for combining proteasome inhibition with HSP inhibition in clinical trials.

The antibiotic geldanamycin binds to HSP90, interfering with its chaperone function, and treatment of cell lines with analogues of geldanamycin causes myeloma cell death due to the unfolded protein response death pathway (Davenport et al. 2007). The first anti-cancer agents directed against HSP90 were therefore analogues of geldanamycin. *Tanespimycin* (*KOS-953*) showed some activity in phase I trials with two partial responses (PRs), one minimal response (MR) and six stable diseases (SDs) recorded in 22 patients so was moved into a phase II trial combined with bortezomib (Richardson et al. 2005a). Preliminary data from this showed three responses in bortezomib refractory patients, and responses of > PR in 7/19 bortezomib naïve patients (Richardson et al. 2007d), and there is now a phase III trial underway of bortezomib + tanespimycin versus bortezomib. More recently, several HSP90 inhibitors that are not derived from geldanamycin have been developed with some evidence that they may have unique properties and actions relative to geldanamycin (Okawa et al. 2008). *NVP-AUY922* is a diarylisoxazole-based drug that has been shown to effectively induce apoptosis in some myeloma cell lines (Stuhmer et al. 2008). A phase I trial is underway in solid tumours, and this agent warrants further attention in myeloma. A phase I trial of another non-geldanamycin derived HSP 90 inhibitor, *KW2478*, is underway and to date has demonstrated tolerability, but no significant clinical responses at the doses used (Cavenagh et al. 2008). However, the maximum tolerated dose has not been reached in this study and dose escalation is ongoing. There is some evidence that inhibition of HSP90 leads to upregulation of the HSP70 family of heat-shock proteins, which may be a mechanism of resistance to this class of drug. Simultaneous inhibition of HSP90 and HSP72 may therefore be required for maximum anti-tumour effect (Davenport et al. 2008).

Another way of targeting the cell protein handling system is through inhibition of the aminopeptidase enzyme system that catalyses the hydrolysis of amino acids from the N terminus of proteins. The aminopeptidase inhibitor *CHR-2797* has been demonstrated to induce apoptosis in a panel of myeloma cell lines and patient samples, and to show synergy with dexamethasone (Davies et al. 2007a). A Phase I trial of patients with haematological malignancies demonstrated efficacy in acute myeloid leukaemia but only enrolled two patients with myeloma, but based on the encouraging in vitro data, further clinical trials in myeloma are warranted (Davies et al. 2007b).

8.2.3 Targeting Chromatin

Epigenetic changes constitute alterations in the gene expression pattern not attributable to the primary base sequence, and include the way that our DNA is packaged by histones and abnormal DNA methylation. Histones are the protein spools around which DNA is wound, without which it would not be possible to package the genome into the nucleus. For mRNA transcription to occur, the tight histone coils need to relax and open, so the histones act as transcription regulators. Histone deacetylation by histone deacetylase (HDAC) results in a closed chromatin pattern to which transcription factors cannot bind, leading to gene silencing, whereas acetylation by histone acetyltransferase (HAT) opens up the chromatin structure to allow transcription. Haematological malignancies have been shown to mediate transcriptional repression of tumour suppressor genes through recruitment of HDAC (Marks et al. 2001) and

HDAC inhibition of myeloma cells has been shown to result in apoptosis (Catley et al. 2003). HDAC inhibitors also act on a number of non-histone proteins that are associated with oncogenesis such as p53, HSP90 and α-tubulin. α-tubulin is deacetyated by HDAC6 and is part of the aggresome system, a protein disposal system analogous to the proteasome where misfolded proteins are transported to lysosomes by the microtubule organising centre (MTOC) to be degraded by autophagy. Inhibition of α-tubulin by tubacin produces synergy with bortezomib, providing some evidence that inhibiting both of the cell's protein disposal mechanisms is beneficial (Hideshima et al. 2005). Some of these compounds may therefore simultaneously relax the chromatin structure to allow transcription of tumour suppressor genes, inhibit the aggresomal pathway and inhibit HSP90. HDAC inhibitors are divided into six classes based on their structure, and several have been shown to have anti-myeloma efficacy.

8.2.3.1 Histone Deacetylase (HDAC) Inhibitors

Suberoylanilide hydroxamic acid (SAHA) (vorinostat) has been shown to upregulate $p21^{WAF1}$ and p53 expression and dephosphorylate Rb via inhibition of HDAC (Mitsiades et al. 2003). It has been used as a single agent in a phase I trial involving ten patients, when it was well tolerated orally and induced one MR (Richardson et al. 2007e). It has been shown to decrease proteasomic activity, so may act synergistically with bortezomib. Two phase I studies and a case series of the combination of SAHA and bortezomib have been reported. In the first study of 16 relapsed patients, eight achieved a PR or near complete response (nCR) (Badros et al. 2007). In the second two-centre study, a 26% PR rate was reported in 34 patients at one centre, and 9/22 patients showed a response at the second centre (Weber et al. 2009), whilst in the small series of six patients, one VGPR and four MRs were seen (Mazumder et al. 2008).It has also been used in a phase I trial in combination with lenalidomide + dexamethasone in nine patients and demonstrated tolerability (Siegel et al. 2009). A phase III study of SAHA + bortezomib versus bortezomib is underway.

The **depsipeptide** FK228 (Romidepsin, FR901288) was demonstrated to induce apoptosis was demonstrated to induce apoptosis in myeloma cell lines and patient tumour cells (Khan et al. 2004) and has recently been reported to show encouraging activity in a small phase II trial when given in combination with bortezomib and dexamethasone. A 67% ORR + 28% MR were seen in 18 relapsed patients, including a response in two patients who were progressing on a bortezomib maintenance programme. It is impossible to know whether the addition of depsipeptide or dexamethasone overcame the drug resistance in these cases.

LBH589 (Panobinostat) has shown potent anti-myeloma activity in vitro and potentiates the effects of other drugs such as dexamethasone, bortezomib and melphalan (Maiso et al. 2006). As well as upregulating $p21^{WAF1}$ and p53, it has been shown to control cell proliferation and survival through HSP90 and induce apoptosis through the aggresome pathway. A phase II study of panobinostat monotherapy has been reported which showed good oral tolerability, a VGPR on one patient who had previously received five lines of therapy, and a MR in one patient post ten lines of treatment (Wolf et al. 2008). Phase I trials combining panobinostat with bortezomib (Siegel et al. 2008) and lenalidomide (Spencer et al. 2009) have demonstrated the safety of these combinations.

PXD101 has demonstrated antiproliferative activity in cell lines and shows additive/synergistic effects with other agents. Its use has been reported in a phase II trial where it was given as monotherapy for two courses, and then with dexamethasone if progressive disease was reported. Twenty-four patients were enrolled,

with no objective responses reported in patients exposed to monotherapy, although some stable disease was seen. A minimal response was seen with the addition of dexamethasone (Sullivan et al. 2006). *ITF2357* has been given to 15 patients with myeloma, inducing one PR (Galli et al. 2007). *SRT501 (resveratrol)* is a naturally occurring polyphenol found in red wine and is one of the sirtuin family of NAD-dependant histone deacetylases. It has been shown to induce apoptosis in myeloma cell lines by down-regulating anti-apoptotic proteins such as cyclin D1, cIAP, XIAP, survivin and bcl-2 and up-regulating pro-apoptotic gene products such as Bax and apoptosis proteasome activating factor-1 (Apaf-1), resulting in suppression of the NF-κB and STAT3 pathways and activation of apoptosis via caspase 3. A phase II trial is underway in myeloma patients starting with resveratrol monotherapy with bortezomib being added for progressive disease. *NVP-LAQ824* has been shown in vitro to inhibit the growth of tumour cells at a much lower concentration than SAHA, so may be a more potent drug (Atadja et al. 2004). It has been shown to induce apoptosis in myeloma cells and to have efficacy in a myeloma murine model, but no clinical trial data has been reported to date (Catley et al. 2003). Multiple other HDAC inhibitors are in development, including *KD5170* and *tubacin*.

8.2.3.2 Hypomethylating Agents

Hypermethylation of the 5′ gene promoter region of genes is an epigenetic mechanism of tumour suppressor gene silencing that has been shown to be present in myeloma (Takahashi et al. 2004). Inhibition of this process may therefore allow increased expression of tumour suppressor genes so constitutes a valid drug target. The DNA methyltransferase inhibitor *5-azacytidine* has been shown to induce apoptosis in myeloma cells, to overcome the survival advantage conferred by IL-6, IGF-1 and adherence to bone marrow stromal cells, and to enhance the activity of doxorubicin and bortezomib (Kiziltepe et al. 2007). It has been stated that the kinetics of its action suggests that its effect may not be mediated via DNA hypomethylation, but by protein synthesis inhibition (Khong et al. 2008). There is extensive clinical data on the use of these agents in other haematological conditions such as myelodysplasia and acute myeloid leukaemia, but none in myeloma to date.

8.2.3.3 New Alkylators

Bendamustine was synthesised in the former East German Democratic Republic in the 1960s and was used in East Germany for 30 years before German unification for the treatment of lymphoma, myeloma and breast cancer, although there were few validated studies from this time to support its use. It has structural similarities to both alkylating agents and purine analogues, and has been demonstrated to have a substance specific interaction with DNA, resulting in minimal cross resistance with other alkylators (Strumberg et al. 1996). A phase III trial has been conducted comparing melphalan + prednisolone with bendamustine + prednisolone in 131 newly presenting patients (Ponisch et al. 2006). Overall response rates were 75% in the bendamustine group and 70% in the melphalan group, with CR rates of 32% in the bendamustine group and 13% in the melphalan group. It has also demonstrated efficacy when used in combination with novel agents in the relapsed patient setting. A phase I trial of bendamustine, prednisolone and thalidomide showed a response in 24/28 patients (Ponisch et al. 2008), whilst it has been used in two phase I trials in combination with bortezomib and dexamethasone and shown impressive response rates of 84% and 88% respectively (Hrusovsky and Heidtmann 2005; Fenk et al. 2007).

8.2.4 Targeting Intracellular Cell Cycle Regulatory Proteins

8.2.4.1 Cyclin D Kinases

Cyclin D dysregulation is central to myeloma pathogenesis, and a classification system has been proposed which shows dysregulation of cyclin D pathways to be a unifying result of at least seven different disease initiating events (Bergsagel et al. 2005). D Cyclins are involved in progression through the G1/S stage of the cell cycle, and their over-expression therefore allows for uncontrolled cellular proliferation without the normal pause that allows for cells with genetic defects to be detected and undergo apoptosis. As cyclin D dysregulation is seen in virtually all myeloma samples, they make an attractive drug target, and several companies have compounds with preclinical data. *P276-00* inhibits CDK4/cyclin D1 and has been shown to inhibit cell growth and induce apoptosis through regulation of cell cycle progression, as well as overcoming the proliferative stimuli of cytokines such as IL-6 and IGF-1. Its in vitro efficacy was borne out in a myeloma mouse model, and a phase I trial is underway (Raje et al. 2009). Similar preclinical efficacy has been demonstrated for the plant cytokinin *kinetin riboside* which inhibits transactivation of *CCND2*, reducing levels of Cyclin D1 and D2 proteins, resulting in cell cycle arrest in vitro and tumour growth inhibition in xenografted mice (Tiedemann et al. 2008). Some of the novel agents seem to have narrow specificity, such as *Purvalanol* against CDK1 and *NVP-LCQ195/AT9311* against CDK1/2, whilst others are broad antagonists of cyclin D pathways with *AT7519* having activity against CDK1,2,4,5,9 and glycogen synthase (GSK) 3b, *SNS-032* inhibiting CDK2,7 and 9 and *RGB286638* having broad activity (McMillin et al. 2007; Cirstea et al. 2008; Santo et al. 2008; Wierda et al. 2008; Zeng et al. 2008). As the initial classification system suggests that either CDK1, 2 or 3 is dysregulated, it would appear that this is an example of an area where, in the future, it may be possible to tailor the drug to the patient based on genetic abnormalities detected in their myeloma clone. Before technology allows that approach, broad spectrum cyclin inhibitors may be more likely to have efficacy in any individual patient, but they may be found to have broader side effects. Clinical data are available from a Phase I trial of SNS-032 (a CDK2, 3 and 9 inhibitor) where patients with myeloma and Chronic Lymphocytic Leukemia (CLL) were treated with a once weekly infusion. Dose-limiting toxicity and tumour lysis were seen in CLL patients, but not in patients with myeloma. No objective responses were recorded, but this remains an exciting therapeutic area (Wierda et al. 2008).

8.2.4.2 Aurora Kinases

The three aurora kinases (aurora A, B and C) regulate the G2 cell cycle checkpoint and as such are intimately involved in centrosome and spindle formation. Targeting these kinases should allow for the arrest of cells at the G2 checkpoint to allow for the recognition of genomic abnormalities that would normally result in apoptosis. Over-expression of *RHAMM,* a centrosome associated gene, has been demonstrated to correlate with the degree of centrosome amplification, whilst centrosome amplification correlates with poor prognosis (Shi et al. 2007; Chng et al. 2008a). Recent data has shown that the presence of aurora A over-expression is an independent poor prognostic factor (Hose et al. 2009). It may therefore be possible to target these new agents to patients with centrosomal amplification of aurora kinase over-expression and thus improve the outlook for a group of patients who have a poor prognosis with current therapies. Data have been

published on multiple aurora kinase inhibitors, including *VX-680, ZK, ADZ 1152, VE-465, ENMD-2076* and *MLN8237*, showing that they are effective in inducing apoptosis of myeloma cell lines and patient samples (Shi et al. 2007; Evans et al. 2008a, b; Gorgun et al. 2008; Wang et al. 2008b). Some of these have isoenzymatic specificities, whilst others such as *ENMD-981693* are multikinase inhibitors which also have activity against proteins such as FGFR3 (Hembrough et al. 2007). Several of these compounds have been taken forward into phase I trials, but no clinical data is available in a myeloma cohort.

8.2.4.3 Pim Kinases

The three Pim kinases are a recently described family of serine/threonine kinases which are potent inhibitors of apoptosis. They mediate this via phosphorylation of the cyclin-dependant kinase inhibitor p27(Kip1) which overcomes G1 arrest thereby allowing cell cycle progression, promoting cellular proliferation (Morishita et al. 2008). Pim-2 has been found to be transciptionally upregulated in myeloma cell lines, and its over-expression is increased by cytokine such as IL-6, BAFF and TNF-α (Asano et al. 2007). Down-regulation of Pim by inhibitory short inhibitory RNAs (siRNAs) has been shown to decrease the proliferation induced by stimulatory cytokines, and to augment the effect of dexamethasone and mTOR inhibitors. On this basis, Pim inhibitors are being taken into phase I trials.

8.2.4.4 Inhibitor of Apoptosis Proteins

Inhibitors of Apoptosis Proteins (IAPs) are a family of proteins that inhibit caspases 8 and 9 and thereby act as regulators of programmed cell death. IAPs include X-chromosome linked IAP (XIAP or BIRC4), cellular IAP 1 (c-IAP1 or BIRC2), c-IAP2 (BIRC3) and survivin, of which XIAP is the best described and possibly the most potent suppressor of apoptosis (Vucic and Fairbrother 2007). XIAP has been found at high levels in patient samples, and has been demonstrated to fall following successful treatment with both conventional chemotherapy and bortezomib (Gaponova et al. 2008). IAP inhibitors are in development and have demonstrated in vitro efficacy against a number of myeloma cell lines, as well as synergy with a range of conventional and novel agents (Khong and Spencer 2007).

8.3 Extracellular Drug Targets

Conditions outside the myeloma cell are essential to its survival. Physical interaction with bone marrow stromal cells trigger the release of cytokines from the stromal cells that mediate destructive bone disease and angiogenesis as well as directly stimulating growth and survival pathways within the tumour cells via the pathways discussed previously such as JAK/STAT3 and NF-κB. Drug resistance also seems to be mediated by cell adhesion to stromal cells.

8.3.1 Targeting Cytokines or Their Receptors

A possible advantage of abrogating upstream targets such as IL-6 and IGF-1 compared to components of the intracellular signalling pathways that they induce is the ability to affect several pathways simultaneously. Myeloma cells show a remarkable capacity to adapt and escape drug toxicity by upregulation of alternative pathways, so blockade of IL-6, which stimulates the JAK/STAT, Ras/Raf/MEK/MAPK

and P13K/Akt, may be more efficacious than targeting any one of the individual downstream targets.

8.3.1.1 Interleukin-6 (IL-6)

IL-6 is possibly the most important, and certainly the most studied, of the cytokines known to be important in myeloma cell survival. High levels of IL-6 or soluble IL-6 receptor have been shown to be associated with adverse prognosis in myeloma, acting as surrogate markers of disease bulk much in the same way as β2m (Bataille et al. 1989; Pulkki et al. 1996). A proportion of tumour cells show autocrine IL-6 production, and patients with higher autocrine production have more advanced disease, whilst their tumour cells are more resistant to apoptosis (Frassanito et al. 2001). However, the major site of IL-6 production in the myeloma bone marrow appears to be stromal cells, with secretion being triggered by tumour/stromal cell binding or by secretion of cytokines such as TNFα from the tumour. Activation of the IL-6 receptor on myeloma cells triggers three signalling pathways; Ras/Raf/MEK/MAPK which appears to mediate cellular proliferation, JAK/STAT3 which mediates survival and P13K/Akt which has been shown to be important in drug resistance (Ogata et al. 1997; Catlett-Falcone et al. 1999; Hideshima et al. 2001). However, some myeloma cell lines show proliferation and survival independent of the presence of Il-6 in the growing medium, so although IL-6 does appear to be important in myeloma pathogenesis, it is not the *sine qua non* of the myeloma cell. This is reflected in disappointing results reported of clinical trials of IL-6 blockade. The IFM 99-03 trial randomised 166 patients to receive a murine anti-IL-6 monoclonal antibody (*BE-8*) or placebo as part of a tandem transplant trial in patients defined as high risk(Moreau et al. 2006). No difference was seen in OS or EFS, although this may have been due to the efficiency of the IL-6 inhibition with this particular agent. Another monoclonal antibody directed against IL-6 (*CNTO 328*) has been produced which, when used as monotherapy in relapsed patients, produced a PR in 3/13 patients (Kurzrock et al. 2008). As with the majority of novel agents, synergy with existing agents is likely to be where any clinical utility lies, and augmentation of bortezomib's effect has been demonstrated in vitro. Preliminary results of the combination of CNTO 328 and bortezomib demonstrated a 57% response rate in 21 relapsed patients (Rossi et al. 2008), and on this basis, a phase III study randomising to bortezomib +/− CNTO 328 is underway.

8.3.1.2 Insulin-Like Growth Factor-1 (IGF-1)

Binding of IGF-1 to its receptor on the myeloma cell induces activation of the Ras/Raf/MEK/MAPK, P13K/Akt pathways, and indirectly the NF-κB pathway, contributing to proliferative and anti-apoptotic cell signalling (Qiang et al. 2002). It has also been shown to inhibit the anti-myeloma activity of dexamethasone, cytotoxic chemotherapy and bortezomib providing the rationale for antagonism of IGF-1 to augment the response to these agents (Mitsiades et al. 2002, 2004). IGF-1 has been suggested to be a prognostic factor with low levels (<13 nmol/l) being associated with a median prognosis that has not been reached at 80 months (Standal et al. 2002). There is good evidence that it plays a role in myeloma pathogenesis and is therefore a valid drug target. Inhibition of the IGF-1 receptor has been achieved with monoclonal antibodies that block IGF-1 binding, and by tyrosine kinase inhibitors, with both approaches showing in vitro activity (Maiso et al. 2008). Clinical data is available on two monoclonal antibodies; *AVE1642* was administered to 14 patients with relapsed disease and was well tolerated, although no objective responses were recorded (Moreau et al. 2007). *CP-751,871* was given to 47 patients either as monotherapy, or with dexamethasone or

rapamycin. Of the 27 patients that received the combination of the antibody and steroids, 2CRs and 4PRs were recorded. Interestingly, the patients with the CRs had previously been classified as refractory to dexamethasone which may provide some evidence for uncoupling of cell adhesion-mediated drug resistance (CAMDR) pathways through IGF-1 inhibition (M Lacy et al 2007).

8.3.1.3 Fibroblast Growth Factor (FGFR3)

Fifteen percent of primary myeloma samples have the t(4;14) which results in the dysregulation of two genes; fibroblast growth receptor 3 (*FGFR3*) and *MMSET*, and leads to ectopic expression of the FGFR3 tyrosine kinase receptor. This cytogenetic subgroup has a particularly poor prognosis, so finding a treatment that specifically targets this group is attractive. βFGF has been shown to be secreted by myeloma cells and is important in tumour angiogenesis. It upregulates production of IL-6 by bone marrow stromal cells, so will feed back to augment the growth and survival pathways in the tumour. Similarly, IL-6 has been shown to increase βFGF production, so there is a paracrine mutual stimulatory circuit in place (Bisping et al. 2003). Multiple tyrosine kinase inhibitors active against the FGFR3 receptor have been shown to be active against myeloma cells in vitro and in murine myeloma models, including *PRO-001*, *TKI 258 (CHIR-258), PKC-412, ENMD-981693* and *XL999* (Chen et al. 2004; Trudel et al. 2005, 2006, 2007; Hembrough et al. 2007). Clinical data is available on only one molecule, *AB1010*, which produced two responses in 19 patients (Arnulf et al. 2007).

8.3.1.4 Vascular Endothelial Growth Factor (VEGF)

As well as its role in tumour angiogenesis, VEGF has been demonstrated to be involved in cell migration via the P13K/AKT pathway, proliferation via the MEK/ERK pathway and survival through upregulation of mcl-1 (Podar and Anderson 2005). Increased bone marrow angiogenesis has been linked to poor outcome in myeloma, and although VEGF has not been directly linked to prognosis, it makes an attractive therapeutic target. Tyrosine kinase inhibitors that block the VEGF receptor, and anti-VEGF antibodies have both demonstrated efficacy in cell line experiments and mouse models of myeloma (Podar et al. 2004, 2006; Campbell et al. 2006). One of these molecules, *pazopanib (GW786034)*, was shown to be ineffective as monotherapy in relapsed myeloma patients (Prince et al. 2007). A humanised monoclonal antibody targeting VEGF (*Bevacizumab*) has been used in two small phase II trials, combined with lenalidomide and dexamethasone with responses reported in 7 out of 10 patients (Raschko et al. 2007) and in combination with thalidomide (Somlo et al. 2005). Larger trials will be needed to ascertain if inhibition of VEGF adds value to existing immunomodulatory drug regimens.

8.3.1.5 Platelet Derived Growth Factor Receptor β (PDGFRβ)

Dasatinib is a tyrosine kinase inhibitor that has activity against a number of receptor and non-receptor kinases, including bcr-abl, Src family kinases, c-KIT, PDGFRβ and FGFR3. FGFR3 is known to be of relevance in the t(4;14) group of patients, whilst PDGFRβ and c-Src have recently been identified as being constitutively activated in the plasma cells and endothelial cells of myeloma patients, and to mediate the release of pro-angiogenic factors such as VEGF (Coluccia et al. 2008). Dasatinib may target myeloma primarily through these receptor kinases, and it has been shown to inhibit tumour growth and angiogenesis in vitro and in myeloma mouse models. Early results of a phase II trial using dasatinib at the same dose as is used in chronic phase chronic myeloid

leukaemia (70 mg BD) showed that it was reasonably well tolerated. No responses were reported at that dose, although the trial was ongoing with a planned dose increase (Wildes et al. 2007).

8.3.1.6 CD40 Ligand

Binding of CD40 ligand to its receptor triggers myeloma cell proliferation via p53-dependant pathways and migration via P13K/Akt and NF-κB signalling (Tai et al. 2003). CD40 also mediates cell binding to fibronectin, and therefore drug resistance mechanisms, in a similar way to SDF-1. Blockade of CD40 ligand binding with a monoclonal antibody (*SGN40*) has been shown to induce apoptosis in cell line experiments, to inhibit proliferation stimulated by IL-6 but not IGF-1 and to be augmented by lenalidomide (Tai et al. 2004, 2005). A second monoclonal antibody, *XmAb5485*, has been shown to induce potent antibody-dependant cell-mediated cytotoxicity against myeloma both in vitro and in mouse tumour models (Zhukovsky et al. 2008).

8.3.1.7 B-Cell Activating Factor (BAFF) and a Proliferation-Inducing Ligand (APRIL)

These two TNF family members have a similar structure and share receptor targets, BAFF interacting with transmembrane activator and calcium modulating cyclophilin ligand interactor (TACI), B-cell maturation antigen (BCMA) and B-cell activating factor receptor (BAFF-R), whilst APRIL can only bind to TACI and BCMA. Through these receptors, they stimulate Ras/Raf/MEK/MAPK, P13K/Akt and NF-κB signalling and have been shown to mediate steroid resistance in this way (Moreaux et al. 2004). They constitute possible drug targets.

8.3.1.8 TNF-Related Apoptosis-Inducing Ligand (TRAIL)

TRAIL ligands induce apoptosis in myeloma cells. This effect is inhibited by osteoprotegerin produced by osteoblasts, another example of pro-survival pathways mediated by the bone marrow milieu. However, this effect has been overcome by stimulating the TRAIL death receptor with agonists of DR4 or 5, and stimulation of this pathway has potential therapeutic implications (Locklin et al. 2007). Monoclonal TRAIL agonists such as *LBY135* exist and have shown synergy with other agents (Khong and Spencer 2007).

8.3.1.9 Fas

The stimulation of Fas receptor by Fas Ligand promotes caspase-dependant apoptotic signalling. *APO010* is a recombinant form of Fas ligand which has been shown to have preclinical anti-myeloma activity against cell lines, and to inhibit tumour growth in mouse models (Ocio et al. 2007). It has been taken forward into phase I clinical trials.

8.3.1.10 p38 mitogen-activated protein kinase (MAPK)

p38 MAPK mediates the production of multiple cytokines including IL-1, IL-6, TNFalpha, VEGF and MIP-1alpha. p38 MAPK has been targeted by the agent *SCIO-469* which decreases constitutive p38a MAPK phosphorylation, with downstream effects of inhibition of HSP27 and upregulation of p53 (Navas et al. 2006; Vanderkerken et al. 2007). In cell line experiments, it has been shown to augment bortezomib activity, whilst in myeloma mouse models, it reduced tumour size and paraprotein levels whilst reducing angiogenesis and having a beneficial effect on destructive bone disease (Hideshima et al. 2004). A phase II trial has been reported

which started relapsed refractory patients on SCIO-469 monotherapy and then instituted bortezomib for patients with no response (Siegel et al. 2006). Of 62 patients treated, the best responses to monotherapy were stable disease in 24%. Combination therapy produced a PR in 26% of patients, including 4 who had previously been classed as bortezomib refractory. Preliminary work has been done on a second agent, *LY2228820*, which showed similar effects of decreased HSP27 activation, modest cytotoxicity as monotherapy but synergism with bortezomib, and inhibition of tumour growth and osteoclastogenesis in mouse models, suggesting that this class of drug may have a beneficial effect on skeletal disease (Ishitsuka et al. 2008).

8.3.2 Targeting Myeloma Cell Adhesion Molecules

8.3.2.1 Stromal Cell Derived Factor-1 (SDF-1)

SDF-1α is produced by both myeloma and stromal cells, and through binding to its receptor CXCR4 (CD184), it plays a critical role in up-regulating binding of myeloma cells to stromal cells and fibronectin. An inhibitor to CXCR4 has been developed which has been used to enhance the mobilisation of CD34+ cells for harvesting prior to autologous transplant. *AMD3100* has been shown to effectively mobilise stem cells from 71% of myeloma patients who have previously failed peripheral stem cell harvesting with chemotherapy and growth factor stimulation, thus giving the option of the proven benefit of autologous transplantation to a significant number of patients who would previously have been denied this treatment (Calandra et al. 2008). However, of equal interest may be the role of this agent in sensitising myeloma cells to other chemotherapy agents by disrupting their interaction with the protective environment of the bone marrow milieu. AMD3100 does not induce apoptosis of tumour cells by itself. However, cell lines that are resistant to bortezomib, dexamethasone, melphalan and doxorubicin in the presence of stromal cells become sensitised to these agents in the presence of AMD3100. In a murine model, mice treated with the combination of AMD3100 and bortezomib show a higher rate of tumour regression than those treated with bortezomib alone, with circulating apoptotic myeloma cells present in the circulation (Azab et al. 2009). There is one report of AMD3100 stimulating plasma cell proliferation (Kim et al. 2008) so careful evaluation of this agent will be required in clinical trials, but it has promise as an agent to overcome CAMDR.

8.3.2.2 Cell Surface 1 Surface Antigen (CS1)

CS1, a member of the immunoglobulin gene superfamily, is universally and highly expressed on myeloma cells, where it has been shown to function as a cell adhesion molecule. A humanised monoclonal antibody (*Elotuzumab (HuLuc63)*) with CS1 specificity has been produced and has been shown in preclinical trials to decrease bone marrow stromal adherence and to induce antibody-dependant cell cytotoxicty (Tai et al. 2008). This effect was augmented by dexamethasone and other chemotherapies in dexamethasone resistant cell lines, suggesting a possible role in sensitisation of tumours to other agents. In mouse xenograft models, it showed significant antitumour activity. Early results of a phase I trial showed no clinical responses at the first dose level, although pharmacokinetic investigations suggested that higher doses will be needed to maintain the drug concentration at the minimal biological activity level defined in the mouse models (Bensinger et al. 2007). Dose finding studies are also exploring the feasibility of its combination with lenalidomide and

dexamethasone (Singhal et al. 2009) and with bortezomib (Jakubowiak et al. 2009). A concern for this drug, and anti-CD56 treatments, is that the NK cell cytotoxic anti-tumour effect may be inhibited as both of these markers are present in high numbers on NK cells.

8.3.2.3 CD56

CD56 (neural cell adhesion molecule) is present on the surface of the plasma cells of ~70% of patients with myeloma. It has a role in myeloma cell adhesion to the bone marrow stroma, and lack of expression of CD56 has previously been linked to extramedullary disease, plasma cell leukaemia and an aggressive clinical phenotype (Pellat-Deceunynck et al. 1998). However, recent analysis of a large series of myeloma patients showed that the presence or absence of CD56 had no effect on overall survival. (Mateo et al. 2008). There are two approaches to targeting cell surface molecules such as CD56 with monoclonal antibodies. One is to use the antibody to block binding of the surface receptor to its ligand to make the cell more immunogenic and therefore trigger antibody dependant cell cytotoxicity (ADCC). The second is to use the monoclonal antibody as a vehicle for delivery of toxins to the cell, in which case the cell surface marker should be chosen on the basis of being highly expressed in the malignant clone, but present in small numbers in the surface of normal cells in the haemopoietic compartment and in other tissues. This approach is taken by *IMGN901* (*huN901-DM1*) which is a humanised monoclonal antibody conjugated with the cytotoxic agent maytansinoid. Once bound to CD56, the antibody is internalised to release the cytotoxic agent within the cell. Eighteen patients with CD56+ disease have received the antibody in a phase I trial, with three MRs recorded (Chanan-Khan et al. 2008).

8.3.2.4 CD38

CD38 is a transmembrane glycoprotein involved in cell adhesion and calcium mobilisation. It is almost universally present on the surface of myeloma cells and is present in much lower numbers on other haemopoietic cells so is therefore an obvious targets for the delivery of immunotoxins. Indeed, an anti-CD38 monoclonal antibody conjugated to an analogue of ricin was one of the earliest attempts at delivering targeted treatment to the myeloma cell (Goldmacher et al. 1994). Although preclinical data looked promising with effective killing of myeloma cells with minimal cross-reactivity with haemopoietic cells, it did not lead on to clinical applications. More recently newer antibodies have been produced with the aim of instigating ADCC, and although limited preclinical data is available on these agents, one (*SAR650984*) has been shown to have an effect in mouse xenograft models (Stevenson 2006; Park et al. 2008).

8.3.2.5 CD138

CD138 is another cell surface molecule that is almost universally expressed on myeloma cells. There is recent preclinical data on *nBT062*, a monoclonal antibody conjugated to maytansinoid, with promising in vitro results and efficacy in mouse xenograft models (Ikeda et al. 2008).

8.3.2.6 CD66

CD66 has been shown to be co-expressed with CD38 in nearly all patients with myeloma (Richardson et al. 2008). It has been used in a novel way to deliver targeted radiotherapy to patients before stem cell transplantation. A monoclonal

antibody conjugated to yttrium-90 has been infused as part of the conditioning therapy prior to high dose melphalan in a phase I study and was shown to be well tolerated with no significant increase in time to engraftment (Orchard et al. 2008). Focal uptake of the antibody was seen in two patients suggesting that in vivo tumour targeting was occurring. This is a novel method of delivering up to 25 Gy of radiotherapy to the bone marrow with minimal additional toxicity.

8.3.3 Targeting the Host Immune System

8.3.3.1 Immunomodulatory Drugs (IMiDs)

Thalidomide and its analogues lenalidomide and pomalidomide are collectively known as the immunomodulatory agents. They share similar mechanisms of action, although they have differing potencies and slightly different side effect profiles. Firstly, they directly activate apoptotic signalling in the myeloma cell. This is primarily achieved through capsase 8 mediated pathways, but the IMiDs also affect the cell at the mitochondrial level, causing c-jun terminal kinase (JNK) dependant release of cytochrome-c and Smac into the cytoplasm, where they regulate other cell survival pathways to mediate apoptosis (Anderson 2005). Secondly, they increase NK cell number and function to augment the host immune response against the tumour. Thalidomide has been shown not to stimulate T cells alone, but to act as a co-stimulator to trigger proliferation of anti-CD3 stimulated T cells. They cause nuclear factor of activated T-cells 2 and activator protein 1 to translocate to the nucleus via activation of P13K signalling pathways, resulting in increased IL-2 and IF-γ secretion. The end result is increased NK cell numbers, and increased antibody-dependant cell cytotoxicity (Davies et al. 2001; Hayashi et al. 2005). Thirdly, they decrease the secretion of key cytokines such as IL-6, TNF-a, VEGF and IGF-1 from bone marrow stromal cells, and in this way affect multiple downstream signalling pathways including NF-κB. The net result of these mechanisms is decreased angiogenesis, a decrease in the supportive and protective effect of the bone marrow milieu including reduced cell adhesion-mediated drug resistance, a decrease in multiple intracellular growth and proliferation pathways, a direct triggering of apoptosis and augmentation of the host cell-mediated anti-tumour immune response.

Thalidomide

Since it was withdrawn from the market in 1961 following its implication as the causative factor in phocomelia, thalidomide has enjoyed a revival based on the same anti-angiogenic properties that cause its most serious side effect, first as a treatment for erythema nodosum associated with leprosy, and more recently as an effective treatment for myeloma. The seminal study of thalidomide in myeloma treated 84 relapsed/refractory patients with a dose starting at 200 mg/day and increasing to 800 mg/day, a higher dose than that employed in most regimens today. A 32% response rate was observed (Singhal et al. 1999). A recent 10 year update on this study, which was expanded to include 169 patients, showed that 17 are still alive with ten remaining event free (van Rhee et al. 2008). Subsequent trials have demonstrated superior response rates when used with dexamethasone and highlighted some potentially serious side effects such as peripheral motor and sensory neuropathy and deep vein thrombosis (Rajkumar et al. 2006). The addition of thalidomide to the commonly used regimen of melphalan and prednisolone for patients not deemed suitable for autologous transplantation has been demonstrated to prolong progression free and overall survival in a large randomised phase III trial (Palumbo et al.

2006). Based on this data, thalidomide, usually in combination a steroid and an alkylator, has become a commonly used regimen both as induction prior to autotransplantation and as therapy for elderly patients, based on proven improved response rates in the former and proven improved survival in the latter. However, in an attempt to increase potency and to reduce side effects, analogues have been produced which may eventually supersede thalidomide.

Lenalidomide (CC-5013)

Structurally, lenalidomide has the same backbone as thalidomide, but with the addition of an amino (NH_2) group and the removal of a carbonyl (C=O) group from the phthaloyl ring. It has good oral bioavailability when administered as a once daily dose, with renal drug excretion of the unmetabolised drug. Care is therefore needed in administering lenalidomide to patients with severe renal impairment to avoid drug accumulation and toxicity, and a recommended dosing system for patients with severe renal impairment based on pharmacodynamic studies has been proposed (Chen et al. 2007). The main potential side effects of this drug are similar to thalidomide, but it has more of a propensity for causing bone marrow suppression with resulting neutropenia, so is generally administered for 21 days followed by a rest week in order to allow for recovery of blood counts.

The evidence for efficacy of lenalidomide in relapsed myeloma patients comes from two large phase III trials of essentially identical design involving 705 patients in total (MM-009 and MM-010) which were reported in the same issue of the New England Journal of Medicine (Dimopoulos et al. 2007; Weber et al. 2007). Both trials involved the administration of dexamethasone 40 mg once daily, initially for D1–4, 9–12, 17–20 for 4 months and then D1–4 only. To this was added either lenalidomide 25 mg for 21/28 or placebo. Both trials had very similar results with significantly increased overall response rates in the lenalidomide group (MM-009:61% vs 20%, $p<0.001$; MM-010: 60% vs 24%, $p<0.001$), increased time to progression (MM-009 and MM-010: 11 months vs 5 months, $p<0.001$) and improved OS (MM-009: 30 months vs 20 months, $p<0.001$; MM-010: not reached vs 20 months, $p=0.03$). The high rates of thromboembolism in this and subsequent trials containing the combination of lenalidomide and dexamethasone mean that some form of thromboprophylaxis is considered mandatory. Taken together, these trials were proof that lenalidomide improves response and survival rates in relapsed myeloma patients when used with dexamethasone. Several subgroup analyses from these trials have been reported. Given the similar structures of thalidomide and lenalidomide, it was important to establish whether patients who had previously received thalidomide derived benefit from subsequently being treated with lenalidomide. Of the 704 patients in the two pooled trials, 39% had previous exposure to thalidomide. The thalidomide naïve group had experienced less lines of therapy and a shorter duration of living with myeloma. Thalidomide exposed patients treated with lenalidomide showed higher response rates and longer PFS compared to the placebo group, although the PFS was less than in those treated with lenalidomide who were thalidomide naïve. There was no difference in survival based on previous thalidomide exposure (Wang et al. 2008a). Another interesting subgroup analysis suggested that patients who required a steroid reduction had superior response and survival rates compared to those who continued on dexamethasone 40 mg (San-Miguel et al. 2007).

Further trials have since been carried out combining lenalidomide with a variety of other agents in the relapsed setting including alkylating agents, anthracyclines and bortezomib, with various response rates (Baz et al. 2006; Morgan et al. 2007; Richardson et al. 2007a). Its use has also been reported in newly presenting patients with promising results. Used in combination with dexamethasone in an early phase II trial a

91% response rate was reported in 34 patients (Rajkumar et al. 2005). The same group is conducting a larger, randomised trial comparing lenalidomide in combination with high or low dose dexamethasone. The final results of this are not available, but preliminary results were published that showed a significantly higher OS in the low dose steroid group at 18 months follow-up (91% vs. 80% in 445 randomised patients) (Rajkumar et al. 2007). This is the second large trial to show a poorer outcome with high dose steroids in combination with lenalidomide than with low dose steroids, an effect probably attributable to increased infection rates in the high dose group. A large trial combining lenalidomide with melphalan and prednisolone in elderly patients is also underway, the preliminary results of which were encouraging (Palumbo et al. 2007). Like all novel agents, lenalidomide's role in new and relapsed patients requires further elucidation by well-conducted randomised trials, buts its potential efficacy in both these setting is proven.

Pomalidomide (CC4047)

Although there are several other IMiDs in development, pomalidomide is the only one with published clinical trial data at present. It has been shown to have efficacy in phase I trials, with a 50% response rate being reported in 20 relapsed patients treated with oral pomalidomide monotherapy (Streetly et al. 2008). A phase II trial using pomalidomide in combination with high-dose dexamethasone showed a 62% response rate in 37 relapsed patients. Neutropenia was the most common serious adverse event. Of interest, four patients who had previously been classed as refractory to lenalidomide showed responses (Lacy et al. 2008). Also reported are responses to thalidomide in patients who have progressed on pomalidomide therapy, supporting the notion that cross-resistance between the IMiDs is not absolute (Mughal et al. 2009).

8.3.4 Targeting Bone Disease

Myeloma bone disease is due to an imbalance between bone resorption by osteoclasts and new bone formation by osteoblasts. Myeloma cells produce osteoclast activating factors such as receptor activator of nuclear factor-kappa B ligand (RANKL), macrophage inflammatory protein-1α (MIP-1α) and IL-6. Conversely, osteoblast activity is suppressed by cytokines such as dikkopf-1 (DKK1), frizzled-related protein 2, IL-7 and IL-3 (Roodman 2008). This tips the balance towards bone resorption, resulting in the osteoporosis and lytic lesions that characterise destructive myeloma bone disease, but it also provides several targets whose manipulation may alter this balance. There is some evidence that novel agents such as proteasome inhibitors and IMiDs have a direct effect on bone disease that is supplementary to the beneficial effect of tumour bulk reduction. Bortezomib has been shown to induce the differentiation of mesenchymal stem cells into osteoblasts (Mukherjee et al. 2008), and responding patients in the APEX trial were shown to have increases in their serum alkaline phosphatase levels as a marker of increased osteoblastic activation (Zangari et al. 2007). The new IMiDs such as lenalidomide and CC-4047 have been shown to alter the balance of bone resorption by inhibiting osteoclast formation (Anderson et al. 2006; Breitkreutz et al. 2008). The effects of these new drugs on bone disease are welcome side effects of drugs whose primary role is to reduce tumour burden, whilst bisphosphonate therapy is currently the standard of care for prevention of bone lesions. However, although bisphosphonates have been proven to reduce skeletal events, primarily vertebral crush fractures, these events still occur at a higher rate than in an age-matched population, which will become more relevant as myeloma patients live for longer with improved therapies. This fact, and concern over bisphosphonate side effects such as osteonecrosis of the jaw, means that there

remains a role for novel agents to specifically target myelomatous bone disease. Agents that disrupt the abnormal osteoclast/osteoblast balance in myeloma have the potential to make the bone marrow niche a less conducive place for myeloma cells to thrive, and there is hope that these agents could inhibit myeloma cell growth and there is hope that these agents could inhibit myeloma cell growth as well as improving rates of skeletal related events. Evidence of this effect comes from the MRC Myeloma IX trial, where patients were randomised to an oral bisphosphonate, clodronic acid, or an intravenous bisphosphonate, zoledronic acid. Zoledronic acid decreased skeletal events, but also reduced mortality by 16%, resulting in an extension of median OS by 5.5 months (p=0.04) Morgan GJ et al. 2007. It is likely that in the future the treatment of myelomatous skeletal disease may involve combination therapy, incorporating a bisphosphonate with one of the agents mentioned below.

8.3.4.1 Receptor Activator of NF-κB Ligand (RANKL)

RANKL is a potent stimulator of osteoclastogenesis, but in the normal bone marrow milieu, its effects are largely blocked by its decoy receptor osteoprotegrin (OPG) which is present in higher numbers than RANKL. This balance is upset in myeloma as OPG is decreased which tips the balance in favour of osteoclast mediated bone resorption. *Denosumab* (*AMG162*) is a humanised monoclonal antibody that binds to RANKL and neutralises it in a similar way to endogenous OPG, tipping the balance back in favour of osteoblastic bone formation. It has been shown to increase bone mineral density in osteopenic post-menopausal women in a large phase III trial, where it was given either 3 monthly or 6 monthly (McClung et al. 2006). There is some thought that bone destruction leads to the release of factors that promote myeloma cell growth, and animals treated with denosumab have shown decreased paraprotein levels and prolonged survival. However, preliminary data from a phase II study of denosumab in plateau phase or relapsed myeloma showed no impact on disease burden, (Vij et al. 2007). A phase III trial in myeloma patients is underway, the results of which are likely to be available within a year.

8.3.4.2 Dickkopf-1 (DKK1) and Wingless/int (Wnt)

Wnt/b-catenin signalling plays a central role in bone homeostasis through promotion of osteoblast differentiation. It may also regulate OPG expression and therefore impact on RANKL mediated osteoclastogenesis. DKK1 inhibits Wnt by binding to its co-receptor lipoprotein-related protein 5 (LRP5). Plasma cells from patients without myeloma, and from patients with MGUS do not express DKK1, whereas it is found in high levels in myeloma bone marrow samples, making it likely to be a key player in the development of myeloma bone lesions (Yaccoby et al. 2007). Treating mice with an anti-DKK1 antibody (*BHQ880*) was shown to prevent the normal inhibition of osteoblasts seen in myeloma, although no change in osteoclast numbers was seen. Treatment resulted in decreased numbers of osteolytic bone lesions and a 25% increase in new bone formation (Yaccoby et al. 2007; Heath et al. 2009). This agent is in trials in osteoporosis, and a trial in myeloma is being planned.

8.3.4.3 Macrophage Inflammatory Protein 1-α (MIP-1α)

MIP-1α (also known as chemokine-chemokine ligand 3 (CCL3)) is another inflammatory cytokine released by myeloma cells that upregulates the number and function of osteoclasts. It

is present in high levels in patients with significant myeloma bone disease, and its production has been shown to be upregulated by interactions between myeloma cells and stromal cells via VCAM-1 (Hashimoto et al. 2004; Abe et al. 2009). Binding of MIP-1a to its receptor CCR1 has been shown to stimulate osteoclast formation independently of RANKL, and to induce myeloma cell migration and proliferation via the Akt pathway. MLN3897 is a specific antagonist of CCR1 which has demonstrated in preclinical data a 40% decrease in osteoclast number and a 70% decrease in osteoclast function, as well as affecting myeloma cell migration and adhesion (Vallet et al. 2007). It is currently in phase II trials in rheumatoid arthritis and multiple sclerosis, and warrants further examination in myeloma bone disease.

8.3.4.4 Activin A

Activin A is a member of the TNF-α superfamily. It is produced by bone marrow stromal cells and its expression has been found to be increased fourfold in myeloma patients with multiple bone lesions compared to those with one or less lesions (Vallet et al. 2008). *ACE-011* is a clinical grade Activin A inhibitor that has been shown to stimulate osteoblast differentiation and inhibit osteoclastogenesis in vitro, and to inhibit myeloma cell growth in vivo. A single dose reduced markers of bone resorption in postmenopausal women (Ruckle et al. 2008).

8.4 Conclusion

Steroids and alkylating agents have formed the backbone of myeloma therapy for decades, with other conventional agents such as anthracyclines, platinum drugs and vincristine adding minimal additional benefit. As a result, patient outcomes showed little real improvement until recently, with the most important breakthrough being proof of dose escalation as opposed to drug discovery. This has changed in the last decade with the advent of the IMiDs and bortezomib. These drugs came to be used in myeloma through very different routes, thalidomide having been in existence for over 50 years and utilised for myeloma because of its known anti-angiogenic properties, whilst bortezomib was designed in the laboratory specifically to target myeloma through inhibition of the proteasome. Thalidomide, lenalidomide and bortezomib have widened the treatment options for both the newly presenting and the relapsed patient. All these drugs have been proven to improve responses in both newly presenting and relapsed patients. Optimum combinations of these agents within regimens, and optimum sequencing of regimens are points for debate and will be covered in other chapters.

Bortezomib could be viewed as especially successful as it arose directly from laboratory research into myeloma cell biology, being designed to fit a specific target. As such it has formed a template for the design of other novel agents, with upregulated pathways being defined within the plasma cell and then targeted with a specific agent. There has been a huge expansion in research in myeloma molecular biology in the last decade which has led to a long list of potential drug targets within the cell. Increasing understanding of the role that the bone marrow microenvironment plays in promoting myeloma cell survival and drug resistance has also led to the definition of targets outside the myeloma cell. As a result of this expansion in knowledge of cell biology, there are now a huge number of novel agents in phase I and phase II trials. The current challenge in myeloma therapy is to build on the success of IMiDs and proteasome inhibitors and fit some of these promising new agents into current treatment paradigms.

The best results in these early phase trials have been seen with new analogues of existing drugs, i.e. new proteasome inhibitors and new IMiDs. Other truly new agents have so far been relatively disappointing when used as monotherapy. This is maybe not surprising given the specific nature of some of these drugs. Myeloma is a biologically heterogeneous disease and is the end product of dysregulation of multiple different pathways in individual patients. It is currently drugs that have quite a broad spectrum of action that are the most effective, such as proteasome inhibitors which affect not only the NF-κB pathway but also all other proteins that are degraded by the proteasome. Until we have better technology to define dysregulated pathways within the individual patient, drugs which have multiple targets such as HSP90 inhibitors are most likely be clinically effective in a group of patients. Novel agents that do find their way into clinical practise are likely to do so because they demonstrate synergism with existing agents or uncouple drug resistance mechanisms to existing agents. Because these new drugs are not conventionally cytotoxic, they are likely to have non-overlapping side effects so may be suited to being used in combination regimens. To put things into context, one has to remember that both thalidomide and bortezomib showed response rates of 30–40% when used as monotherapy in relapsed patients, which is the context that most new drugs are introduced. However, when combined with steroids and alkylating agents the response rates double. There is often in vitro evidence of synergy for these new agents and existing agents that provide rationale for certain combinations. Treatment of a myeloma cell with bortezomib, for example, is known to result in activation of the unfolded protein response; blocking this escape mechanism with heat shock protein inhibitors may therefore augment response to bortezomib. This, and other combinations of novel agents, will need careful evaluation in well-designed randomised trials with the addition of novel agent or placebo to existing gold standard treatments.

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