MMRF Research Roundtable: Novel Targeted Therapeutics for the Treatment of Multiple Myeloma

20-21 April 2004
Torino, Italy

Supported by unrestricted educational grants from

Accelerating the Search for a Cure
## NOVEL TARGETED THERAPEUTICS FOR THE TREATMENT OF MULTIPLE MYELOMA

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21 April 2004

Dear Colleague:

Welcome to Torino, Italy, and to the MMRF Research Roundtable: Novel Targeted Therapeutics for the Treatment of Multiple Myeloma.

We are pleased and privileged to bring together key thought leaders, both research scientists and clinicians, in this evolving and exciting field of cancer research. With this roundtable, we hope to expand our horizons by examining and discussing the advancement of innovative, targeted therapies for the treatment of multiple myeloma. We look forward to an open and stimulating discussion that will challenge our current thinking and increase our collective knowledge.

This experts roundtable is made possible by the Multiple Myeloma Research Foundation (MMRF) through an educational grant from Celgene Corporation, McCarty Cancer Foundation, Millennium Pharmaceuticals, Inc., Novartis Oncology, and Ortho Biotech International.

Once again, thank you for joining us. We look forward to working together today and in future endeavors.

Kenneth C. Anderson, MD
Chief, Division of Hematologic Neoplasia
Dana-Farber Cancer Institute
Kraft Family Professor of Medicine
Harvard Medical School

Mario Boccadoro, MD
Professor of Medicine
Director, Section of Hematology
University of Torino
Tuesday, 20 April 2004

6:00 PM  Welcome Dinner
Keynote Speaker: Giovanni Apolone, MD

Wednesday, 21 April 2004

7:30 AM  Continental Breakfast

8:30 AM  Welcome & Introductions

Session I – Novel Therapies Targeting the Tumor Site
Session Chair: Kenneth C. Anderson, MD

8:40 AM  PTK787
Kenneth C. Anderson, MD and Dorianno Fabbro, PhD

9:00 AM  FGFR3
Keith Stewart, MBCHB, FRCPC

9:20 AM  Histone Deacetylase Inhibitor
Constantine S. Mitsiades, MD, PhD and Dorianno Fabbro, PhD

9:40 AM  Group Discussion

10:10 AM  Break

10:30 AM  MAP2 Kinase
Ralf C. Bargou, MD, PhD

10:50 AM  Mcl-1
Martine Amiot, PhD

11:10 AM  IGF-1 Receptor Inhibitors
Constantine S. Mitsiades, MD, PhD and Francesco Hofmann, PhD

11:30 AM  Inhibition of Rank Ligand (OPG/Rank Fc)
Peter Croucher, PhD

11:50 AM  Group Discussion

12:20 AM  Lunch
Session II – Novel Therapies Targeting the Tumor and its Microenvironment

Session Chair: Mario Boccadoro, MD

1:20 PM  Arsenic Trioxide
Pieter Sonneveld, MD and Jack Singer, MD

1:40 PM  Bortezomib Upfront Therapy
Sundar Jagannath, MD and David P. Schenkein, MD

2:00 PM  Revlimid™ (CC-5013)
Kenneth C. Anderson, MD and Robert Knight, MD

2:20 PM  Thalidomide Plus Melphalan and Prednisone
Antonio Palumbo, MD and H. Grant Prentice, MD

2:40 PM  Aplidin® (Aplidium albicans)
Faustino Mollinedo, PhD and Jose A. Lopez-Martin, MD

3:00 PM  Group Discussion

3:30 PM  Publication Discussion and Conclusion

4:00 PM  Adjournment
Co-Chairmen

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Session I: Novel Therapies Targeting the Tumor Site

Session Chair:
Kenneth C. Anderson, MD
The Vascular Endothelial Growth Factor Receptor Tyrosine Kinase Inhibitor PTK787/ZK222584 Inhibits Growth and Migration of Multiple Myeloma Cells in the Bone Marrow Microenvironment: Clinical Application

K. Anderson

Our prior studies show that vascular endothelial growth factor (VEGF) is produced in the bone marrow (BM) microenvironment and is induced by multiple myeloma (MM) cell adhesion to bone marrow stromal cells (BMSCs). We have also shown that CD40 induces p53-dependent VEGF secretion in MM cells. Our studies further reveal that both MM cell lines and patient cells express high affinity for VEGF receptor (VEGFR) Flt-1, but not for Flk-1/KDR. Moreover, these studies showing that VEGF induces proliferation and migration of MM cells have begun to delineate the signaling cascades mediating those sequelae. In an attempt to target VEGF therapeutically, we have studied PTK787/ZK 222584 (PTK787), a molecule designed to bind specifically to the tyrosine kinase domain of VEGFR and inhibit angiogenesis. We show that PTK787 acts directly both on MM cells and in the BM microenvironment. Specifically, PTK787 (1 to 5 µM) inhibits proliferation of MM cells by 50%, as assayed by [3H]-thymidine ([3H]-dT) uptake. This effect of PTK787 is dose-dependent in both MM cell lines and patient MM cells that are both sensitive and resistant to conventional therapy. PTK787 enhances the inhibitory effect of dexamethasone (dex) on growth of MM cells, and can overcome the protective effect of interleukin 6 (IL-6) against dex-induced apoptosis. PTK787 (1µM) also blocks VEGF-induced migration of MM cells across an extra-cellular matrix. Importantly, PTK787 also abrogates the increased MM cell proliferation and increased IL-6 and VEGF secretion in cultures of MM cells adherent to BMSCs. These findings therefore demonstrate that PTK787 acts directly on MM cells and also inhibits paracrine IL-6-mediated MM cell growth in the BM milieu. The demonstrated preclinical anti-MM activity of PTK787, coupled with its anti-angiogenic effects, provides the framework for an ongoing clinical trial of this agent to overcome drug resistance and improve outcome in patients with relapsed MM. Finally, our most recent studies show that GW654652, a novel pan-inhibitor of VEGF receptors, also blocks growth and migration of MM cells in the BM microenvironment, setting the stage for clinical evaluation of this novel targeted therapeutic agent, as well.

Submitted by Kenneth C. Anderson, MD, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA

References
Tumor growth is dependent on increased angiogenesis, the development of new blood vessels from existing vasculature. Vascular endothelial growth factor (VEGF) is a key factor driving tumor angiogenesis. It is secreted by tumor cells, tumor stromal cells, and infiltrating inflammatory cells. VEGF binds to its receptors on endothelial cells and promotes angiogenesis by increasing vascular permeability and stimulating endothelial cells to divide, migrate, and form a new vasculature. Myeloma cells are also reported to express VEGF receptors, suggesting a paracrine role for VEGF pathways in myeloma. In addition, serum levels of VEGF are reported to correlate with disease severity, and increased angiogenesis and microvessel density have been correlated with decreased survival in multiple myeloma. PTK787/ZK 222584 (PTK/ZK) is a novel oral angiogenesis inhibitor that potently inhibits all VEGF receptor tyrosine kinases, and to a lesser extent, the PDGF-b receptor and c-kit. In preclinical models, PTK/ZK has been shown to inhibit growth of multiple myeloma cells in the bone marrow microenvironment, and has also been shown to reduce tumor growth and microvasculature in experimental tumors in rodent tumor models. In clinical trials, PTK/ZK induced statistically significant reductions in tumor blood supply measured by dynamic-contrast-enhanced magnetic resonance imaging, a biomarker for tumor blood flow, vascularity, and vascular permeability, and this biologic effect has been positively correlated with improved patient outcomes. Responses (partial and minor) have been observed in colorectal cancer, renal cell cancer, and glioblastoma patients in phase I/II trials with PTK/ZK monotherapy. Oral, once-daily administration of PTK/ZK alone or in combination with standard chemotherapy has been generally well tolerated in over 1000 patients, enabling the drug to be given chronically, a potentially important consideration in angiogenesis inhibition. The most frequently reported adverse events (all grades) with PTK/ZK monotherapy have been nausea, fatigue, vomiting, and dizziness. These events were mild-to-moderate in severity and easily managed with supportive care. PTK/ZK (1,250 mg/day) is currently in phase III trials for metastatic colorectal cancer, and a trial for multiple myeloma is planned.

*PTK787/ZK 222584 is developed as a collaboration between Novartis Pharmaceuticals, East Hanover, New Jersey, USA and Schering AG, Berlin.

Submitted by Doriano Fabbro, PhD, Novartis Institute for BioMedical Research, Basel, Switzerland
Fibroblast Growth Factor Receptor 3 as a Molecular Target in Multiple Myeloma

K. Stewart

Dysregulation of fibroblast growth factor receptor 3 (FGFR3) by the translocation t(4;14)(p16;q32) occurs in 15% of multiple myeloma (MM) patients and confers a growth and survival advantage to malignant plasma cells. We have previously demonstrated that FGFR3 functions as an oncogene in murine transplant models and confers a growth advantage to B cells which proliferate more rapidly in the face of interleukin 6 (IL6) and may even become independent of IL6 in the presence of activating mutations. In that light, activating mutations of FGFR3 are relatively common in MM cell lines but not in newly diagnosed myeloma in which less than 5% of t(4;14) positive patients also have an FGFR mutation. Recent studies demonstrate that FGFR3 translocation can also be detected by gene expression profiling in 16% of patients. The clinical impact of t(4;14) translocation has subsequently been demonstrated in 3 large studies each reporting a marked reduction in overall survival. In our own series, we investigated the frequency and prognostic relevance of the t(11;14) and t(4;14) in 120 patients with multiple myeloma who received intensive chemotherapy (melphalan 200mg/m²) followed by autologous stem cell transplant at our institution. Myeloma cells were evaluated by immunofluorescence detection of cytoplasmic light chain combined with fluorescence in situ hybridization (cIg-FISH) for the t(11;14) and t(4;14). Overall, the t(11;14) was detected in 16/118 (13.5%) and the t(4;14) in 14/108 (13%) of the patients. Patients with a t(4;14) had a significantly higher relapse rate (79%) and shorter event-free survival (median 9.5 months) than patients without the t(4;14) (49%, 25.8 months) (P=0.0001). Patients with a t(4;14) also had a significantly shorter overall survival compared with patients without the translocation (median 18 months vs 46.3 months; p=0.0053). As in other studies, an association was found between the presence of the t(4;14) and IgA immunoglobulin isotype. Our results indicate that the detection of the t(4;14) by cIg-FISH is associated with a poor prognosis in MM patients receiving intensive chemotherapy and autotransplant.

As FGFR3 is therefore a poor prognostic indicator and a molecular target, we assessed the therapeutic potential of two FGFR-specific tyrosine kinase inhibitors, SU5402 and CHIR258, in MM. Both compounds inhibited FGFR3 phosphorylation in vitro and in murine MM tumor models. Murine B cells dependent on FGFR3 for survival were specifically sensitive to the drugs. A panel of eleven human myeloma cell lines was studied, five bearing the t(4;14) translocation. The KMS11 human myeloma cell line, which expresses constitutively active mutant FGFR3, displayed an 85% decrease in S-phase cells, a 95% increase in G0/G1 cells, and a 4.5-fold increase in apoptotic cells after 72 hours of treatment with 10 M SU5402. CHIR258 also induced delayed, dose-responsive apoptosis of these cells. Activated ERK1/2 and STAT3 were rapidly downregulated after treatment. In human myeloma cell lines expressing wild type FGFR3, the stimulating effect of aFGF ligand was abrogated by treatment. Myeloma cells lacking the t(4;14) or with the t(4;14) and a secondary ras mutation did not respond to therapy. Studies assessing in vivo activity of CHIR258 against FGFR3-expressing tumors in xenograft mice have demonstrated in vivo activity against myeloma xenografts. Primary patient samples with known t(4;14) also respond. These data indicate that the small molecule inhibitors SU5402 and CHIR258 potently inhibit FGFR3 and have activity against t(4;14) human MM cells. These findings support the development of clinical trials of early intervention with FGFR3 inhibitors in t(4;14) myeloma.

Submitted by Keith Stewart, MBCHB, FRCPC, Princess Margaret Hospital, Toronto, Ontario, Canada
Molecular Profile of the Anti-Myeloma Activity of Histone Deacetylase (HDAC) Inhibitors: Biological Insight and Implications for the Therapeutic Management of Myeloma

C. Mitsiades, N. Mitsiades, K. Anderson

Histone deacetylases (HDACs) affect cell differentiation and survival at the transcriptional level by regulating the acetylation status of nucleosomal histones and the function of transcription factor complexes. HDAC inhibition induces differentiation and/or apoptosis in transformed cells. We recently showed (Blood, 2003;101(10):4055-4062) that HDAC inhibitors, such as the prototypic hydroxamic acid-based HDAC inhibitor suberoylanilide hydroxamic acid (SAHA), potently induce cell death (through caspase-independent/calpain-dependent mechanism) of human multiple myeloma (MM) cells, including cell lines and MM patient-derived tumor cells, either sensitive or resistant to conventional or novel anti-tumor agents. SAHA also sensitized MM cells to death receptor (e.g. Fas or TRAIL receptor)-mediated apoptosis and inhibited IL-6 secretion in co-cultures of bone marrow stromal cells (BMSCs) with MM cells. These comprehensive effects of SAHA, both on MM cells directly and on their microenvironmental interactions, prompted further investigation of the molecular sequelae of this class of agents, with particular focus on the transcriptional profile of SAHA treatment, since HDAC inhibition exerts its anti-tumor activity by targeting predominantly the regulation of gene expression. HDAC inhibition was originally pursued with intent to induce differentiation of malignant (e.g. leukemic) cells, by de-repressing transcriptional programs of cellular differentiation. Interestingly, however, our gene expression profiling (using U133A Affymetrix oligonucleotide microarrays) and subsequent confirmatory mechanistic and functional assays (Proc Natl Acad Sci USA, 2004;101:540-5) indicate that HDAC inhibition in MM triggers a distinct transcriptional signature hallmarked by suppression of pathways critical for tumor cell proliferation, survival and drug resistance, including downregulation of insulin-like growth factor (IGF) / IGF-1 receptor (IGF-1R) and interleukin-6 receptor (IL-6R) signaling cascades; suppression of anti-apoptotic molecules (e.g. caspase inhibitors); oncogenes (e.g. myb, maf, pim-1, Axl, Polo and Aurora kinases, abl, vav, PAK-1, ASK); DNA synthesis or repair enzymes; transcription factors (e.g. XBP-1, E2F-1); nucleocytoplasmic transport regulators; and adhesion molecules (e.g. RHAMM, integrins) implicated in MM pathophysiology. SAHA treatment upregulates p53 transcriptional activity, represses the activity of HIF-1α and NF-κB, and suppresses 26S proteasome subunits and proteasome activity, but does not trigger major heat shock protein upregulation, in contrast to pronounced stress responses generated by treatment of MM cells with other anti-tumor agents, e.g. proteasome inhibitors. Importantly, SAHA enhances MM cell sensitivity to other anti-MM agents, including dexamethasone and cytotoxic chemotherapy, as well as thalidomide analogs, proteasome inhibitors or hsp90 inhibitors. SAHA treatment does not indiscriminately suppress or activate gene transcription: it modulates expression of a wide constellation of molecular targets, which correspond, however, to highly specific functional clusters, with known direct or indirect involvement in tumorigenesis and/or proliferation, survival, and drug-resistance of MM cells, specifically, or malignant cells, in general. Our studies indicate that HDAC function is critical for MM cells by actively maintaining a transcriptional program indispensable for their uncontrolled proliferation and/or inappropriate resistance to pro-apoptotic stimuli. The pleiotropic anti-tumor effects of SAHA, its ability to enhance the anti-MM activity of multiple conventional or novel agents and, importantly, the fact that it was bioavailable, well-tolerated, and achieved objective responses after oral administration in phase I clinical trials, have provided the preclinical framework for clinical applications of SAHA in MM. A phase I trial of oral SAHA in relapsed or refractory MM patients is currently underway in our Center. The results of these studies and ongoing further preclinical translational efforts will further clarify the potential future role of HDAC inhibitors as a therapeutic strategy against MM.

Submitted by Constantine S. Mitsiades, MD, PhD, Jerome Lipper Multiple Myeloma Center, Department of Medical Oncology, Boston, Massachusetts, USA
Histone deacetylases (HDACs) play key roles in maintaining chromatin structure and regulation of gene expression. Recent studies indicate that abnormal histone deacetylase activity may lead to aberrant expression of oncogenes and/or tumor suppressor genes resulting in cancer. We have developed HDAC inhibitors NVP-LAQ824 and NVP-LBH589, which inhibited partially purified histone deacetylase activity purified from H1299 lung carcinoma cells, transcriptionally activated the p21 promoter, and inhibited tumor cell line growth at sub-micromolar concentrations. In cell proliferation assays, low concentrations of the compounds caused tumor cells to die by apoptosis; however, even at higher concentrations, they only produced reversible cell cycle arrest in normal human dermal fibroblasts. Cell cycle analysis revealed that both tumor and normal cells accumulate in the G2/M phase of the cell cycle after NVP-LAQ824 treatment, but a sub-G1 phase subsequently develops only in tumor cell lines. In vivo, NVP-LAQ824 at tolerable doses inhibited the growth or induced stasis of established human lung, colon, or breast tumor xenografts in nude mice independent of p53 tumor suppressor gene status of the tumor. Analysis of histone-H3 and H4, in NVP-LAQ824-treated cells and tumor xenografts showed increased histone acetylation, suggesting that inhibition of histone deacetylases might be the mechanism of its anti-tumor activity. We have also observed that treatment of tumor cell lines with LAQ824 affected protein stability through hsp90 protein acetylation, resulting in decreased levels of oncogenic and tumor cell survival proteins such as Her2/neu, BCR-abl, and phospho-AKT, as well as depletion of anti-angiogenic factors such as HIF-1α, VEGF, and Tie-2. These studies demonstrate that our histone deacetylase inhibitors have high potential for anti-tumor efficacy through multiple anti-cancer mechanisms.

Submitted by Doriano Fabbro, PhD, Novartis Institute for BioMedical Research, Basel, Switzerland
Combined Disruption of Both the MEK/MAPK and the IL-6/STAT3 Pathway is Required to Induce Apoptosis of Myeloma Cells in the Presence of Bone Marrow Stromal Cells

R. Bargou

The IL-6/STAT3 pathway has been reported to play an important role in the pathogenesis of multiple myeloma (MM) and in the survival of MM cells. Recently we have shown, however, that human MM cells become independent of the IL-6/gp130/STAT3 pathway if cells were cocultured with bone marrow stromal cells (BMSCs), suggesting that the bone marrow microenvironment stimulates additional IL-6-independent pathways that protect MM cells from apoptosis. Therefore, it was the aim of this study to determine the contribution of IL-6-independent signaling mechanisms to the survival of MM cells. Here we show that selective targeting of MAPK (Erk 1, 2) with either a small compound inhibitor of MEK or MAPK-directed siRNA constructs is not sufficient to induce apoptosis of MM cells, either in the presence or in the absence of BMSCs. Thus, targeting of IL-6/STAT3 or MAPK alone is not sufficient to induce apoptosis in MM cells cocultured with BMSCs. In contrast, combined targeting of both IL-6/STAT3 and MAPK led to strong induction of apoptosis even in the presence of BMSCs. This effect could be observed with MM cell lines as well as primary myeloma cells freshly isolated from bone marrow aspirates of patients. Detailed pathway analysis revealed that BMSCs stimulate STAT3 via IL-6 and MAPK in part via IL-6-independent mechanisms. Thus, we provide experimental evidence that combined targeting of different independently activated pathways is required to efficiently induce apoptosis of MM cells, in particular if they grow in the presence of cells from the bone marrow microenvironment. This might have direct implications for the development of future therapeutic strategies in MM.

Submitted by Ralf C. Bargou, MD, PhD, Dept. of Hematology, Oncology, and Tumorimmunology, Robert-Rössle Cancer Center at the Max-Delbrück-Center for Molecular Medicine, Charité, Humboldt University of Berlin, Berlin, Germany
The Disruption of the Mcl-1/Bim Interaction is an Interesting Target in Multiple Myeloma.

P. Gomez and M. Amiot

Multiple myeloma (MM) is a plasma cell malignancy that accumulates within the bone marrow. IL-6 is the essential factor for both survival and proliferation of myeloma cells. Other studies and ours studies have demonstrated that Mcl-1 is tightly regulated by IL-6 in myeloma cells. Mcl-1 belongs to the pro-survival Bcl-2 subfamily and can associate with proapoptotic counterparts. Mcl-1 differs from Bcl-2 and Bcl-x\textsubscript{L} in its structure, short half-life, and ability to protect cells from a variety of cytotoxic stimuli. Mcl-1 antisense triggers an important decrease of viability associated with an induction of apoptosis in all myeloma cell lines tested, whereas neither Bcl-2 nor Bcl-x\textsubscript{L} antisense affects the viability of myeloma cells. These results suggest that Mcl-1 is one of the key proteins in regulating myeloma cell survival and that Mcl-1 antisense strategy could be of considerable importance in the treatment of MM.

Besides antisense strategy, we investigated the capacity of different signaling pathway inhibitors to inhibit Mcl-1 protein expression. Indeed, we show that for one third of myeloma cell lines and MM patients, AG490, an inhibitor of JAK2, dramatically downregulated Mcl-1 expression. The downregulation of Mcl-1 by AG490 in these cell lines is well correlated to its capacity to induce apoptosis. In the same way, in some other myeloma cell lines, we demonstrated that an inhibitor of IGF-1 receptor downregulates Mcl-1 in relationship with the induced apoptosis.

Recently, it became clear that the proapoptotic proteins that have only a single Bcl-2 homology domain, BH-3-only proteins, are the essential initiators of apoptosis and regulators of antiapoptotic Bcl-2 family proteins. Bim, a BH-3-only protein, has a pivotal role in cytokine regulation of hematopoietic cell survival. The three major Bim isoforms are expressed in myeloma cells and are negatively regulated by IL-6. Blockade of IL-6 signaling induces an upregulation of Bim concomitant to the downregulation of Mcl-1. We demonstrated that Bim is strongly associated with both Mcl-1 and Bcl-2 in myeloma cells. Of major interest, we found that upon apoptosis induction, the endogenous complex Mcl-1/Bim falls to barely detectable levels, whereas under the same conditions, Bcl-2 is still strongly associated with Bim. These observations suggest that the decrease of Mcl-1/Bim interaction may be prompted by a loss of Mcl-1 protein level. In myeloma cells undergoing apoptosis, Bim protein level was found to be upregulated, while Mcl-1/Bim complex was nearly abolished; these findings suggest that Bim is released and becomes available to exert its proapoptotic function.

A recent study provides evidence that Bim, like Bid, activates Bax or Bak by inducing their oligomerization. We can assume that the control of the balance between Mcl-1 and Bim regulates cell fate through either IL-6 regulation or changes in the local environment of myeloma cells. Thus, an interesting clinical approach may be focused on drugs that can affect the balance of Mcl-1/Bim. Until now, it has been shown that different compounds are able to modulate Mcl-1 levels. Our study provides evidence that another therapeutic target could be the disruption of the Mcl-1/Bim interaction by BH-3-peptides or BH-3-peptidomimetics. The feasibility of such an approach has been recently evaluated in other cellular systems.

Submitted by Martine Amiot, PhD, INSERM U601, Nantes Cedex, France
We and others have previously shown that insulin-like growth factors (IGFs) and their growth-signaling receptor IGF-1R (CD221) not only stimulate the proliferation of multiple myeloma (MM) cells, but also protect them from pro-apoptotic anti-MM agents, such as dexamethasone (Dex), Apo2L/TRAIL (Oncogene, 2002;21(37):5673-83), or the proteasome inhibitor bortezomib (Proc Natl Acad Sci USA, 2002;99(22):14374-9). However, until very recently, this pathway had not attracted major interest as a potential therapeutic target for MM (or other tumors). This was primarily related to concerns that the IGF-1R expression in a broad range of normal tissues would not allow for its inhibition to be associated with a clinically useful therapeutic window; that the very high degree of homology between the IGF-1R and the insulin receptor (InsR) would preclude the development of sufficiently selective inhibitors against IGF-1R, but not InsR; and finally, that no studies had previously addressed (in MM or other disease settings) the relative importance of proliferative/anti-apoptotic signaling via IGF-1R vs. other receptors traditionally considered as major therapeutic targets for MM. As part of our expanded research program focusing on the IGFs/IGF-1R pathway, we recently characterized in detail the expression of IGF-1R and its functional role in MM vs. other tumor cells to assess its potential role as a therapeutic target (Mitsiades et al. Cancer Cell, 2004;3:221-230). We observed that IGF-1R is expressed on all MM cell lines and primary tumor samples that we tested (including cells resistant to Dex, melphalan, anthracyclins, Apo2L/TRAIL, thalidomide/IMiDs, or even bortezomib). Inhibition of IGF-1R (with neutralizing anti-IGF-1R-specific monoclonal, antagonistic peptides or small-molecule selective IGF-1R kinase inhibitors) had pronounced anti-proliferative/pro-apoptotic effect against malignant cells from a broad range of hematologic malignancies and solid tumors, while MM cells (including both drug-sensitive and drug-resistant ones) were among the tumor types most responsive to IGF-1R inhibition. In contrast to the aforementioned IGF-1R inhibitory strategies (which profoundly suppressed serum-induced proliferation/survival of all MM cells), specific anti-IL-6R neutralizing MAbS had minimal, if any, effect on serum-cultured MM cells, suggesting high serum levels of IGFs (but not IL-6). Furthermore, we found that IGFs are not only present in circulation, but are also produced in the bone marrow microenvironment by autocrine (MM cells) and paracrine (bone marrow stromal cells [BMSCs], osteoblasts) sources and that IGF-1R inhibitors overcome the protection conferred to MM cells by their co-culture with BMSCs. Through gene expression and proteomic profiling of IGF-1R-inhibitor-treated MM cells, we found that IGF-1R inhibition blocks key growth/survival pathways (e.g. PI-3K/Akt, Ras/Raf/MAPK, IKK-α/NF-κB); blocks expression of several inhibitors of apoptosis (e.g. FLIP, XIAP, cIAP-2, survivin); neutralizes pro-apoptotic Forkhead transcription factors; suppresses both constitutive and serum- or IGF-1-induced upregulation of proteasome activity and telomerase activity; and increases sensitivity of MM cells to Dex, chemotherapy, and PS-341. Based on these encouraging in vitro data and because of the potential of small molecule IGF-1R inhibitors for translation to clinical applications, we placed significant emphasis on the in vivo anti-MM activity of the selective IGF-1R kinase inhibitors NVP-ADW742 and NVP-AEW541, synthesized by Carlos-Garcia Echeverria and Francesco Hofmann (Novartis Institute for BioMedical Research, Basel, Switzerland). Importantly, in our SCID/NOD mouse model of diffuse MM bone lesions, NVP-ADW742, both as a single agent, as well as in combination with cytotoxic chemotherapy, such as melphalan, significantly suppressed MM tumor growth and improved overall survival of mice. Importantly, the IGF-1R administration, either alone or in combination with chemotherapy, was well-tolerated and no major treatment-related toxicity was seen. Most notably, these IGF-1R kinase inhibitors can be administered orally with sufficient bioavailability, without adverse effects on oral glucose tolerance and intraperitoneal insulin tolerance.
IGF-1 Receptor Inhibition: A Novel Therapeutic Strategy for Multiple Myeloma (con’t)

Our studies indicate that IGFs (present in serum or from autocrine/paracrine sources) and IGF-1R signaling in MM cells play critical roles in proliferation, survival and drug-resistance of MM. Most importantly, our studies provide comprehensive first proof-of-principle that inhibition of IGF/IGF-1R pathway can be achieved with a favorable therapeutic window and that small molecule IGF-1R kinase inhibitors constitute promising therapeutic agents for MM. Because our in vitro and in vivo data show that MM is one of the most sensitive neoplasias to IGF-1R inhibitors, MM constitutes a most appropriate testing ground for their clinical development.

Submitted by Constantine S. Mitsiades, MD, PhD, Jerome Lipper Multiple Myeloma Center, Department of Medical Oncology, Boston, Massachusetts, USA
NVP-AEW541 - A Novel, Potent and Selective Inhibitor of the IGF-1R Kinase


IGF-1R-mediated signaling promotes survival, anchorage-independent growth, and oncogenic transformation, as well as tumorgrowth and metastasis formation in vivo. Targeting IGF-1R function by different approaches, including antisense oligonucleotides, antibodies, and dominant negative mutants, was shown to reverse the transformed phenotype of cancer cells in vitro, to inhibit tumor formation and to reduce the metastatic potential in vivo.

In our drug discovery approach, we have targeted the intracellular kinase domain of the IGF-1R by interfering with ATP binding. Following High-Throughput-Screening, we identified pyrrolo[2,3-d]pyrimidine derivatives as inhibitors of the IGF-1R kinase. Upon optimization, we have characterized NVP-AEW541 as a representative small molecular weight kinase inhibitor of the IGF-1R, capable of distinguishing between the IGF-1R (IC$_{50}$ = 0.086 µM) and the closely related InsR (IC$_{50}$ = 2.3 µM) in cells. As expected for a specific IGF-1R kinase inhibitor, NVP-AEW541 abrogates IGF-1R mediated survival and colony formation in soft-agar at concentrations that are consistent with inhibition of IGF-1R autophosphorylation. In vivo, this orally bioavailable compound inhibits IGF-1R signaling in tumor xenografts and significantly reduces the growth of IGF-1R-driven fibrosarcomas. Thus, NVP-AEW541 represents the first class of selective, small molecule IGF-1R kinase inhibitors with proven in vivo anti-tumor activity and potential therapeutic application.

Submitted by Francesco Hofmann, PhD, Novartis Institute for BioMedical Research, Novartis Pharma AG, Basel, Switzerland
Session II: Novel Therapies Targeting the Tumor and its Microenvironment

Session Chair: Mario Boccadoro, MD
Arsenic Trioxide in Multiple Myeloma

P. Sonneveld, E. Kamst

Arsenic trioxide (AsO₃) has anti-tumor activity in hematological malignancies, such as acute promyelocytic leukemia (APL), chronic myeloid leukemia (CML), and relapse or refractory multiple myeloma (MM). AsO₃ induces a loss of mitochondrial potential and cytochrome c release. It induces apoptosis in association with downregulation of Bcl-2. In addition, multiple other effects have been observed, including inhibition of NF-κB and VEGF. The in vitro effects in MM include inhibition of STAT3 activation and JAK-STAT signaling. AsO₃ decreases MM cell adhesion to the bone marrow microenvironment and sensitizes chemo-resistant cells to chemotherapy. The anti-myeloma effects seem to be inversely related to intracellular glutathione (GSH) levels, possibly because of the rescue of mitochondrial damage.

Glutathione-S-transferases (GSTs) are phase II detoxification enzymes that form a large family, consisting of several isoforms. They catalyze the conjugation of glutathione (GSH) to various compounds, including many chemotherapeutics. The isoform GSTP1 has been identified as a direct inhibitor of the c-Jun N-terminal kinase (JNK). GSTP1 knockout mice show increased constitutive activity of the JNK and JAK/STAT pathways. JNK mediates apoptosis through the mitochondrial pathway, which is, at least in part, due to phosphorylation of Bim-related members of the Bcl-2 family. Stimulation of cell survival involves activation of JunD by JNK, but downstream genes involved in survival such as IL6 may also be induced via their AP-1 responsive elements.

A few years ago, GSTP1 was shown to also directly interact with the C-terminus of JNK in resting cells. Upon stress induction by exposure to hydrogen peroxide or UV radiation, the GST dissociates from JNK, leading to an increased JNK kinase activity. More recently, specific GSTP1 inhibitors have been synthesized. One of them, TLK199 has been shown to mimic the phenotype of GSTP-deficient mice in vivo. Its administration leads to increased constitutive JNK activity, a higher leukocyte count in blood, and an increased proliferative potential of bone marrow cells ex vivo. Several reports have shown that overexpression of GSTP or altered GSH levels frequently occur in cancer cell lines. In addition, experimental overexpression of GSTP1 or increased availability of GSH in tumor cell lines of various origins consistently leads to increased drug resistance. Hypermethylation of the GSTP promoter region is the most common somatic alteration in prostate cancers and results in a loss of GSTP expression.

Genetic polymorphism in the GSTP1 gene was recently shown to correlate with the response to treatment with a combination of ABCM in MM patients. Individuals carrying the 105Val allele instead of 105Ile showed improvement in primary response, and progression free survival. The GSTP1 variant containing 105Val has been shown less active in conjugating GSH to a cyclophosphamide metabolite. It was speculated that the improved therapy response was due to reduced detoxification in tumor cells. The combination of recent insights outlined above suggests that in addition to possible effects on drug detoxification, altered GSTP1 expression or function will also lead to altered drug-induced modulation of JNK activity, which is expected to influence the activity of many anti-cancer drugs including AsO₃.

At Erasmus MC in Rotterdam, we are working on a project to identify the role of GSTP1 interaction with the JNK and STAT3 pathways in multiple myeloma and how this affects the activity of AsO₃ and of inhibitors of NF-κB and histone deacetylation. In this program, the downstream effectors of these pathways and how they are expressed following therapeutic intervention are investigated. These pathways will also be studied in myeloma plasma cells from a recently initiated phase II study of AsO₃ with dexamethasone in relapse MM.

Submitted by Pieter Sonneveld, MD, Erasmus MC, Rotterdam, Netherlands, Rotterdam, The Netherlands
Trisenox® (Arsenic Trioxide) In Multiple Myeloma (MM): Current Status and Future Development

J. Singer

Trisenox® is marketed in the US and EU for the treatment of relapsed or refractory acute promyelocytic leukemia (APL) on the basis of a high proportion of patients achieving durable molecular remissions. Trisenox blocks the transcriptional repressor function of PML-RARα resulting in terminal differentiation of neoplastic promyelocytes. However, it also affects signaling pathways of interest in other neoplastic disorders: it suppresses NF-κB function, presumably through inhibition of IKK; activates oxidative stress pathways; suppresses Bcl-2; and induces apoptosis by activating caspases. Trisenox increases levels of reactive oxygen species, such as hydrogen peroxide, and inhibits the activity of scavenging enzymes, such as glutathione peroxidase and catalase. Its effects are most pronounced in cells with low levels of reduced glutathione (GSH) and can be enhanced by agents that reduce levels of GSH, such as BSO and ascorbic acid (AA). These findings provide a mechanism for the observation by many investigators in a variety of models that Trisenox has additive or synergistic effects when combined with drugs such as alkylating agents. Decreasing levels of GSH enhance activity of these drugs, and high levels are associated with resistance.

On the basis of in vitro studies and clinical activity observed during phase I trials, clinical studies were initiated to explore the activity of Trisenox in patients with advanced MM. It was used initially as a single agent; subsequent studies used it in combination with dexamethasone (DEX), AA, AA+DEX, and AA + low dose melphalan (MEL). Although it was initially used on a daily schedule or a two-week-on and two-week-off schedule at 0.25 mg/kg, current protocols use Trisenox on a twice-weekly schedule (0.25 mg/kg) for up to 12-week cycles following a 4-5 day loading period. Responding patients have been continued on therapy for > one year. Trisenox has been generally well tolerated and associated with few severe drug-associated nonhematopoietic toxicities. Rapidly reversible neutropenia and thrombocytopenia were seen in some heavily pretreated patients.

Using the BLADÉ criteria for response, single agent therapy with Trisenox in 38 predominantly refractory patients in two trials produced a response rate (RR) of 29%. Addition of AA in a similar group of patients appeared to substantially increase the response rate to Trisenox. Partial remissions by SWOG criteria (> 50% reduction of paraprotein) were observed only when Trisenox was used in combination with AA, DEX and AA, or MEL and AA. In each of these studies, the overall RR was > 50% and the SWOG criteria response rate was > 30%. CRs and near CRs were observed. Many responses proved remarkably durable. A number of patients had sustained reversal of severe renal impairment. A preliminary comparative analysis of the trials suggests that the time to maximal response is decreased and the response rate is enhanced when AA is administered in conjunction with Trisenox. Additional phase II trials are currently evaluating the efficacy of Trisenox + AA in combination with bortezomib (Velcade®), thalidomide, and MEL. Taken together, these studies indicate that Trisenox is an active agent in patients with MM, even in those refractory to MEL, DEX, thalidomide, and Velcade®, and that it has a favorable safety profile. The overall development plan for Trisenox will be reviewed.

Submitted by Jack Singer, MD, Cell Therapeutics Inc., Seattle WA, USA
First-Line Therapy With Bortezomib (Velcade®, Formerly PS-341) in Patients With Multiple Myeloma (MM)

S. Jagannath, B. Durie, J. Wolf, E. Camacho, D. Irwin, J. Lutzky, M. McKinley, E. Gabayan, J. Crowley, D. Schenkein

Introduction: Preclinical evidence has suggested that the proteasome is an effective therapeutic target in MM. The proteasome inhibitor bortezomib demonstrated sustained efficacy and acceptable toxicities among patients (pts) who had failed two lines of prior therapy and refractory disease (SUMMIT trial), as well as in pts failing front line therapy (CREST trial). Since bortezomib induced CR and durable responses in pts with advanced disease, the current study examines the response rate and safety of bortezomib in pts with newly diagnosed MM.

Methods: Pts received bortezomib 1.3 mg/m² 2x/wk x2 q3wk for a maximum of 6 cycles. Dexamethasone 40 mg the day of and after each bortezomib dose was allowed after 2 cycles for pts with < PR, and after 4 cycles for pts with < CR. Response criteria was based on modified EBMT criteria, with the addition of a near CR category (disappearance of all M protein by electrophoresis, but positive immunofixation, with normal bone marrow). Stem cells could be harvested at the discretion of the physician. Neurologic tests are being performed before and after bortezomib.

Results: Nineteen pts (47% males, median age 63 yrs) have been accrued. Pts presented with IgG (58%), IgA (32%), or light chain (10%) disease, with a median KPS of 90 (50 - 100). The majority of pts were Durie-Salmon Stage III (55%). As of November 2003, 12 pts have completed 6 cycles and were evaluable for response: there were 4 (33%) near CR, 5 (42%) PR, 1 (8%) MR, and 2 (17%) PD. One pt has undergone stem cell transplant with complete hematologic recovery. The most common adverse events (grades 1 – 3) were fatigue (67%), diarrhea (58%), constipation (42%), nausea (42%), peripheral neuropathy (33%), and vomiting (33%). One episode of each of the following grade 3 events occurred: abdominal pain, diarrhea, dizziness, dyspnea, fever, neuropathic pain, neutropenia, syncope, and vomiting. One pt required dose modification. No grade 4 toxicity was observed.

Conclusion: In this study, bortezomib appeared to be promising as initial therapy in patients with newly diagnosed MM and had manageable toxicities. Major responses (near CR and PR) were seen in 75% of the pts by 6 cycles. Study accrual is ongoing, and the full complement of 42 pts is expected to be recruited quickly.

Keywords: bortezomib/multiple myeloma/first line therapy.

Submitted by Sundar Jagannath, MD, St. Vincent’s Comprehensive Cancer Institute and New York Medical College, New York, New York, USA
Bortezomib as Front-line Therapy in the Treatment of Multiple Myeloma

D. Schenkein

The proteasome is a key regulator of multiple cellular processes in myeloma cells, and its inhibition is the focus of a novel therapeutic strategy. Bortezomib (VELCADE®, formerly PS-341), the only proteasome inhibitor used clinically, was approved in 2003 for the treatment of patients with multiple myeloma who have received at least 2 prior therapies and are progressing on their last therapy. This approval was based, in part, on the phase 2 SUMMIT and CREST trials. In SUMMIT, patients with relapsed and refractory multiple myeloma received bortezomib 1.3 mg/m² 2x/wk x2 q3wk. The overall response rate (complete response [CR] + partial response [PR] + minimal response [MR]) in this heavily pretreated population (median of 6 prior therapies) was 35%. In CREST, patients with relapsed or refractory disease following front-line therapy received bortezomib 1.0 or 1.3 mg/m² on the same schedule as in SUMMIT. Preliminary results of CREST suggest that efficacy and toxicity are potentially dose-related. APEX, a phase 3 trial, was designed to show clinical benefit of bortezomib over high-dose dexamethasone in patients with relapsed or refractory multiple myeloma. APEX was recently stopped early due to a significant positive benefit in the bortezomib arm noted at interim analysis. Data are currently under review.

Results of these trials suggest that bortezomib may be effective in newly diagnosed multiple myeloma. In a preliminary report of an ongoing trial focusing on newly diagnosed symptomatic disease, patients were treated with bortezomib 1.3 mg/m² 2x/wk x2 q3wk. Dexamethasone 40 mg 4x/wk was given with every dose of bortezomib for all < PR patients after 2 cycles and all <CR patients after 4 cycles. At the end of 6 cycles, all patients (N = 12) had received dexamethasone in addition to bortezomib. Major responses were achieved by 75% of the patients (near CR [100% disappearance of M-protein by electrophoresis but not immunofixation, and normal bone marrow] = 33%, PR = 42%), and the overall response rate was 83%. One patient received a stem cell transplant and experienced complete hematologic recovery. The most common adverse events in this trial were constipation, diarrhea, fatigue/malaise, nausea, sensory neuropathy, and vomiting. Additional ongoing trials are examining the use of the PAD (bortezomib, doxorubicin, dexamethasone) regimen prior to stem cell harvest and high-dose therapy/stem cell transplantation (HDT/SCT), and the use of bortezomib before stem cell mobilization and as maintenance following HDT/SCT.

Conclusions: Efficacy with manageable toxicities associated with bortezomib treatment has been established in heavily pretreated patients. In addition, several studies are examining bortezomib in less heavily treated patients (e.g., CREST, APEX), and newly diagnosed patients. Early data suggest efficacy with manageable toxicities in treatment-naive patients, without an effect on stem cell collection and transplantation. Studies in this patient population are ongoing.

Submitted by David P. Schenkein, MD, Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts, USA

References:
Revlimid™ (CC-5013) in Myeloma

K. Anderson

Thalidomide and Revlimid represent novel agents, which target the tumor and microenvironment.\textsuperscript{1} Specifically, Revlimid induces apoptosis of drug resistant multiple myeloma (MM) cells, downregulates tumor cell adhesion to bone marrow stromal cells (BMSCs) and induction of cytokines, inhibits angiogenesis, and upregulates T and NK cell responses.\textsuperscript{2-4} We have shown that it directly co-stimulates T cells via phosphorylation of CD 28, thereby triggering IL-2 transcription and secretion as a mechanism of its immunomodulation.\textsuperscript{5-6} We have also shown inhibition of human MM cell growth and associated angiogenesis as well as prolongation of host survival, in a SCID mouse model.\textsuperscript{7} A phase I trial of Revlimid demonstrated 79% stabilization of disease or response, without somnolence, constipation, or neuropathy attendant to thalidomide use. A phase II trial in 102 patients with relapsed and relapsed/refractory MM demonstrated 37% response, including 10% complete response, and a second phase II trial of 200 patients is now completed. Addition of dexamethasone augmented response in 41% of patients. A phase III trial comparing Revlimid/dexamethasone versus dexamethasone alone is now fully enrolled. We have studied the effects of Revlimid both at the level of mitochondria and cell signaling. For example, it activates JNK and triggers ROS, with release of Smac and cytochrome c from mitochondria.\textsuperscript{8-10} It induces activation of caspase 8 and augments apoptosis induced via TRAIL (caspase 8) and Velcade® (caspase 9), both delineating its mechanism of action and providing the framework for clinical protocols combining novel agents.\textsuperscript{11} Activity of Revlimid in advanced MM has set the stage for ongoing protocols testing its efficacy for patients earlier in their disease course, and as maintenance therapy to prolong response to stem cell transplantation.

Submitted by Kenneth C. Anderson, MD, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA

References


Revlimid™ (CC-5013) in Myeloma (con’t)


Novel Immunomodulatory Drug Therapy for Multiple Myeloma

R. Knight

CC-5013 (Revlimid™) is the lead compound in a new class of oral immunomodulatory drugs (IMiDs), which inhibit the secretion of pro-inflammatory cytokines (TNF-2, 1L-1B and 1L-6), increase the secretion of the anti-inflammatory cytokine 1L-10, induce 1L-2 and IFN-8 production and T-cell proliferation, and inhibit neo-angiogenesis. Preclinical studies have shown CC-5013 to be approximately 50 to 2000 times more potent than the parent IMiD compound, thalidomide, as an activator of immunomodulatory effects and an inhibitor of angiogenesis. Animal studies have not found CC-5013 to have teratogenic effects. CC-5013 has also been demonstrated to induce apoptosis or growth arrest in resistant multiple myeloma cells in vitro. A phase I study in healthy volunteers showed that oral CC-5013 was well tolerated, rapidly absorbed, and largely excreted unmetabolized in urine.

A subsequent phase I dose-finding study of CC-5013 (5-50 mg/day) in patients with relapsed or refractory multiple myeloma was conducted. The MTD was 25 mg/day and DLT was identified as myelosuppression. No cases of sedation, constipation or neuropathy (the dose-limiting toxicities of thalidomide) were reported. Importantly, >25% reductions in paraprotein occurred in 63% of patients and another 16% of patients had stable disease. The greatest paraprotein reductions were seen in patients treated at doses of 25 and 50 mg/day.

A randomized phase II study comparing CC-5013, 15 mg bid vs. 30 mg qd given for 3 weeks followed by 1 week of rest enrolled 101 patients with relapsed or refractory multiple myeloma. High-dose dexamethasone was added to CC-5013 therapy for patients with disease progression and to patients with stable disease after > 8 weeks of single-agent treatment. An analysis showed an increased incidence of dose reduction for myelosuppression in the 15 mg bid patient cohort, although response rates were similar. The overall response rate in this heavily pretreated population was 37% (10% CR, 12% PR, 15% MR), and another 43% of patients achieved stable disease. Forty-nine patients received high-dose dexamethasone in addition to CC-5013, and 20 (41%) patients attained a response. These findings demonstrated that CC-5013, either as a single agent or in combination with high-dose dexamethasone, shows promising clinical activity in patients with advanced multiple myeloma.

International phase III studies of CC-5013 therapy in patients with resistant multiple myeloma are ongoing.

Submitted by Robert Knight, MD, Celgene Corporation, Warren, New Jersey, USA
Oral Melphalan, Prednisone, and Thalidomide for Multiple Myeloma Patients

A. Palumbo, A. Bertola, P. Musto, M. Nunzi, V. De Stefano, V. Callea, B. Rotoli, M. Petti, T. Caravita, V. Lauta, C. Patti, M. Ambrosini, S. Bringhen, F. Cavallo, P. Falco, A. Carella, A. Liberati, M. Boccadoro

Thalidomide is a new drug for treatment of myeloma. In newly diagnosed patients, thalidomide induced a 35% response rate. The addition of dexamethasone increased the response rate to 70%. The combination of thalidomide with chemotherapy induced a 70%-80% partial response rate, and 10%-25% complete remission rate. No data are present on the association of thalidomide with standard oral melphalan and prednisone (MP). To address this issue, we evaluated the potential additive and synergistic effect of the combination melphalan, prednisone, and thalidomide (MPT).

Between June 2002 and June 2003, 49 patients with newly diagnosed symptomatic multiple myeloma received 6 monthly courses of MP (melphalan 4 mg/sqm and prednisone 40 mg/sqm for 7 days every month) plus thalidomide that was delivered at 100 mg/day continuously until any sign of disease progression or relapse. The dose of thalidomide was reduced to 50% when grade II WHO toxicity occurred, and suspended for any grade III.

Patient characteristics were as follows: median age 71 (range: 61-82), female sex (44.8%), stage IIA (36.8%), IIIA (46.9%), B (16.3%); myeloma protein IgG isotype (63.3%), IgA (20.4%), Bence Jones proteinuria (16.3%); median bone marrow plasmocytosis 40% (range 5-70), WHO performance status ≥3 (10.4%), median hemoglobin 10.9 g/dL (range: 7.4-14.7), median plasma creatinine 0.8 mg/dL (range: 0.7-3.6), serum albumin 2.83 g/dL (range: 1.8-4.3), median serum calcemia 2.25 mmol/L (range: 2.1-2.58), median serum β2 microglobulin 3.7 mg/L (range: 0.36-14.8), median plasma C-reactive protein 3 mg/L (range: 0.005-157).

Forty-one patients completed the entire program. At the end of the study, the frequency of immunofixation negative (complete response) was 24.5%, immunofixation positive (near complete remission) 14.3%, myeloma protein reduction 90%-99% (very good partial response) 6.1%, myeloma protein reduction 50%-89% (partial response) 34.7%, myeloma protein reduction 0%-49% (no response) 8.2%, and (progressive disease) 2%.

After a median follow-up of 13 months (range 6.1 to 23.6), 89.6% of patients were alive in remission and 5 patients had relapsed at 3.87, 5.83, 7, 9.33, and 13.1 months from the start of treatment. One patient died of acute pulmonary thromboembolism and one of pneumonia.

Hematologic toxicity occurred in 55.2% of patients, neurologic toxicities in 39%, constipation in 32.6%. Infections and thrombosis were the most prominent adverse events. Infections were observed in 28.7% of patients, most related to pneumonia (12.2%). Without of any kind of prophylaxis, symptomatic and objective diagnoses of thromboembolism were 20.4%: 1 arterial peripheral occlusion, 3 pulmonary thromboembolism and 6 distal deep-vein-thrombosis.

MPT is a feasible and promising approach for newly diagnosed patients. Response rate is significantly higher than those previously reported with any other conventional poly-chemotherapy regimen. The complete remission rate was quite similar to those observed after transplant. The occurrence of a significant incidence of sepsis and deep-vein thrombosis suggests the need for antibiotic and anticoagulant prophylaxis.

Submitted by Antonio Palumbo, MD, University of Torino, Department of Hematology, Torino, Italy
Thalidomide Plus Melphalan and Prednisone

G. Prentice

Melphalan plus prednisone, remains the gold standard conventional chemotherapy in the treatment of MM. This is so despite numerous studies of other combinations, including the infusional regimen VAD (and variants) and other promising combinations such as the MRC ABCM protocol. High dose therapy (HDTx) with mobilized stem cell support is increasingly recognized as a significant advance, giving further tumor cytoreduction. Despite documented improvements in response rates to melphalan over non-alkylator containing treatments and HDTx over conventional treatment, these strategies rarely, if ever, result in cure or a substantial advantage. HDTx is considered by many to be inappropriate for a significant proportion of the older patients but is the preferred treatment for younger patients lacking a suitable BM donor. Curative treatment in many malignancies, like infectious diseases, is most likely to be successful with the application of immunotherapy or “treatment with memory.” Allogeneic BMT is currently the only such strategy but is hampered by considerable toxicity due to nonselective targeting resulting in substantial morbidity and mortality. Until more selective and effective treatments are developed, there remains a need for agents capable of blocking the various functions required for the tumor cell survival. Treatment likely to extend disease control with a minimum impact on quality of life is desirable for those ineligible for curative treatment such as allogeneic BMT. The genetic and epigenetic pathophysiology of myeloma is increasingly understood due to the work of many, in particular the Boston group of Anderson. The autocrine and paracrine networks supporting proliferation and survival of the tumor cell are, at least in early stage disease, highly dependent upon adherence to the bone marrow microenvironment. Thalidomide, a drug with a dark past, has remarkable properties that result in the downregulation of several cytokines involved in myeloma cell survival and proliferation (including TNFα, IL-1, IL-6), while it also has a similar effect on adhesive molecule expression (ICAM1/VECAM). By blocking VEGF and bFGF production, new blood vessel formation is inhibited. Indeed it was this anti-angiogenic property of the drug that first led to its use in myeloma by the UARK team.

Thalidomide also increases Th1 lymphocyte numbers and causes activation of NK cells. Thus there exists the possibility that an immune response directed against a myeloma-specific antigen induced either by immunization or allogeneic BMT might be further enhanced by the application of thalidomide. While much is now understood of the targets of thalidomide in the treatment of myeloma, we remain ignorant of, and in dispute on, the rank order of these effects. There are data to suggest that thalidomide might also possess direct cytotoxic properties against myeloma cells. The application of thalidomide as a single agent in relapsed or refractory disease results in an approximate 30% PR rate. The original findings of the UARK studies are supported by a meta-analysis of greater than 1000 patients (Glasmacher, personal communication). A number of studies including combinations with dexamethasone, alkylating agents, or anthracyclines show either additive or synergistic activity.

Thus the potential benefit of combining thalidomide with other agents requires exploration in patients unsuitable for HDTx. MP + thalidomide is currently favored for the older patients, and several studies are well advanced in Europe. Those near completion include the IFM 99-06 and already reported in abstract form the Italian Multiple Myeloma Study Group programme (Palumbo ASH ’04 No. 509). This study employed melphalan 4mg/m² 7d/m for 6 cycles, prednisone 40mg/m²/m for 6 cycles, and thalidomide 100mg/d until relapse. The HOVON 49 study is at a somewhat earlier stage. IFM 99-06 also tests HDTx using a reduced dose of HD melphalan (x2) likely to further our understanding of the best strategies in older patients.
Thalidomide Plus Melphalan and Prednisone (con’t)

The Italian Group has shown, in a preliminary analysis (Palumbo, personal communication update) of this 1st line study, quite remarkable activity; Of 42 evaluable patients (61 – 80 years of age, median 72) 93% show PR (50% + reduction in M protein) or better, including 45% with either CR or nCR. This was mirrored by clinical benefit. The median time to maximum response was only 2 months. These results approach those seen after HDTx. Further follow up is required to determine the long-term outcome. The cumulative discontinuation rate for thalidomide at 16 months was 35%. The major toxicity was thromboembolic with 19% DVTs. This issue is now being addressed with low molecular weight heparin. Neurotoxicity of grade 3 – 4 was seen in 7%.

Alternatives include the combination of cyclophosphamide with prednisone or dexamethasone) and thalidomide. Pulsed combinations have shown promise, and this combination may also be administered orally. This combination is presently favored in the younger (<65 years) population that might proceed to HDTx, given the known lack of toxicity of cyclophosphamide to the marrow repopulating “stem” cell.

Outstanding issues include the dose of thalidomide that is probably best restricted, mainly to reduce the risk of neurotoxicity. The other toxicities are substantially soluble. The scheduling of thalidomide and its role in maintenance therapy also requires exploration in this setting.

Studies of other combinations are warranted, including some of the newer agents to explore synergies while reducing the toxicity. Meanwhile, the Italian study illustrates the potential of the MP + thalidomide combination in the older population.

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Induction of Apoptosis in Multiple Myeloma Cells by the Novel Antitumor Agent Aplidin® (Aplidium albicans): Pre-clinical Studies and Mechanism of Action.

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Aplidin (dehydrodidemnin B) is a naturally occurring cyclic depsipeptide isolated from the Mediterranean tunicate Aplidium albicans that shows a promising in vitro and in vivo antitumor activity. We have found that Aplidin at nM concentrations (10-100 nM) induced apoptosis in human leukemic cell lines and primary leukemic cell cultures from leukemic patients. Aplidin behaved as an extremely potent and rapid apoptotic inducer on leukemic cells that triggers Fas/CD95-, c-Jun NH2-terminal kinase (JNK)-, and mitochondrial-mediated apoptotic signaling routes. We have also found that Aplidin is very active against a panel of six human multiple myeloma (MM) cell lines, as well as against primary MM tumor cells freshly isolated from patients, as assessed by cell proliferation assays, apoptosis measurements, and activation of caspase-3 and apoptotic-signaling pathways. The efficiency of the cytotoxic action of Aplidin on MM cells is in the same range (nM) as the proteasome inhibitor PS-341, even though the corresponding mechanisms of action differ from each other. Aplidin treatment resulted in a rapid and persistent activation of JNK activation, whereas PS-341 induced a late stimulation of this signaling pathway. Aplidin treatment also induced a rapid activation of p38, but not ERK. Aplidin and PS-341 also differed in their respective actions on cell cycles in MM cells. Our findings suggest that Aplidin is a potent activator of the apoptotic-signaling pathway in MM cells. In addition, we found that normal cells were more resistant to Aplidin than tumor cells. Thus, primary cultures of normal human resting peripheral blood lymphocytes were spared or weakly affected following Aplidin treatment. Nevertheless, mitogen (phytohemagglutinin/interleukin-2)-activated T-lymphocytes resulted that were sensitive to the drug apoptotic action. Overall, these findings provide the framework for putative future clinical trials of Aplidin in MM.

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Aplidin® (Aplidium albicans): Non-Clinical and Clinical Bases For a Clinical Evaluation in Patients With Multiple Myeloma

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Aplidin (APLD) is a cyclic depsipeptide isolated from the marine tunicate, Aplidium albicans. APLD induces cell cycle arrest at G2 and G1; triggers both the death receptor and mitochondrial apoptotic pathways to cause cell death; inhibits the secretion of VEGF and downregulates flt-1; and inhibits endothelial cell proliferation and angiogenesis (chick embryo chorioallantoic membrane assay).

In vitro studies demonstrated antiproliferative activity against a broad spectrum of tumor types, namely bladder, breast, gastric, lung carcinoid, melanoma, neuroblastoma, prostate, thyroid (with IC50 values ranging from 10-7 to 10-9 M), and leukemia and lymphoma (with IC50 values ranging from 10-8 to 10-9 M). Of note, APLD exhibited in vitro antitumor activity (i.e., IC50 ≤ 10-8 M) against a broad panel of human multiple myeloma (MM) cells including those resistant to conventional and novel therapies. Consistent with these results, APLD induced cell death of primary tumor cells isolated from patients. This effect appeared to be independent of well-known tumor survival signals such as Bcl-2, Akt, and certain bone marrow-derived cytokines, as well as cell adhesion to stromal elements in the bone marrow microenvironment. In addition, using a series of bioinformatics algorithms to analyze the in vitro drug-sensitivity data, the pattern of sensitivity of myeloma cells to APLD was clearly distinct from that of other conventional or investigational antineoplastic drugs (Mitsiades CS et al. Blood 2003;102:11s, abstract #250). Using the hollow fiber in vivo model, bladder, gastric, and prostate tumors were shown to be susceptible to APLD. With xenograft models, activity was noted against Burkitt’s, gastric, pancreas, and renal tumors. Even though APLD appeared to display substantial antineoplastic effects in certain human solid tumors, the most salient antitumor activity of APLD was anti-leukemic.

To the cut-off date of 15 March 2004, a total of 327 patients have been recruited into clinical trials with APLD: 215 into phase 1 and 112 into phase 2 clinical studies. At the proposed recommended dose for phase 2 (RD) most of the dosing schedules tested within the phase 1 programme intended to deliver a similar dose intensity of around 2.5 mg/m2/week. The selected schedules for phase 2 are a 3-h infusion every two weeks and a 1-h weekly infusion three out of four weeks. Out of the patients included in the phase 1 program, 77 were treated at the various RDs. These patients received a median of 2 cycles of APLD. The following is a description of the main adverse events observed in these phase 1 cohorts.

The major dose limiting toxicity is musculoskeletal adverse events. At the RDs, reversible grade 3-4 increases in CPK appeared in 9% of the patients; grade 3-4 myalgia and muscular weakness were respectively reported in 3% and 4% of the patients. Resolution of myalgia or muscle weakness typically was observed 1-3 weeks after onset. The other main dose limiting toxicity is abnormalities in liver function tests. At the RDs, grade 3-4 increases in AST/ALT were the most common (13%), followed by elevations in alkaline phosphatase (8%). Hematological laboratory abnormalities were not DLTs in any of the schedules used in phase 1. Severe emesis despite maximum antiemetic prophylaxis was noted as a DLT. However, grade 3-4 emesis was reported in only 2 patients (3%) at the RD. Grade 3 diarrhea was DLT, but appeared in less than 5% of patients treated at the RD. Grade 3-4 constipation was described in the same proportion of patients. No cases with grade 3-4 mucositis was reported. Acute renal failure was a DLT in one trial. It appeared in the context of acute liver failure and muscular adverse events (myalgia and a grade 4 increase in creatin kinase), and had a fatal outcome. No Grade 3-4 creatinine abnormalities were reported at any RD, apart from a single case of transient grade 3...
increase in creatinine in a patient with renal adenocarcinoma. Fatigue was one of the most frequent adverse events experienced by the patients treated at the RDs. Its significance may be confounded, since underlying progressive disease is usually an early event in these phase 1 patients. Grade 3 hypersensitivity reactions were described in 2 patients in this pooled cohort. No routine prophylaxis with H₁-receptor blocking agents was given in any of the phase 1 trials.

When central venous access was not used, local problems related to drug administration were frequent, including erythema, pain during infusion, and phlebitis. Signs or symptoms of injection-site reactions were reported in about 20% of the patients treated at the RD. Pulmonary embolism and deep venous thrombosis were observed in patients with advanced, progressive disease. In 2 patients treated at the RD, the investigator’s assessment was unable to exclude a relationship with APLD. Similar assessments were also made for 4 cases of grade 3-4 dyspnea, and 3 cases of supraventricular tachycardia were observed. APLD at the doses proposed for further development does not manifest alopecia.

During the phase 1 trials, tumor shrinkages and/or long-lasting disease stabilizations were reported in patients with colorectal, renal, neuroendocrine (carcinoid, medullary thyroid carcinoma, and pheochromocytoma), head and neck, and lung carcinomas, melanoma, sarcoma, and non-Hodgkin lymphoma, warranting its further clinical development. Efficacy of APLD is currently being assessed in several phase 2 studies in solid tumors and hematological malignancies. Based on its preclinical activity and on its safety profile, a phase 2 trial in multiple myeloma is currently under implementation in US and Europe (Spain).

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