Friday 1 October 08:00–09:45

PLENARY SESSION 8

Optimising targets for angiogeneic inhibition

458 INVITED

What has 30 years of angiogenesis research and drug development taught us about cancer treatment?

J.M. Wood. *Novartis Institutes for Biomedical Research, Oncology, Basel, Switzerland*

The term angiogenesis, referring to the growth of new bloods, was first described in 1787 by a British Surgeon, John Hunter. Two centuries later, it was recognized to play an important role in embryonic development and physiological processes such as placenta formation. In 1971 a Boston surgeon, Judah Folkman hypothesized that tumor growth was dependent upon angiogenesis and that tumor angiogenesis could be a target for cancer therapy. He speculated that targeting genetically stable endothelial cells could inhibit tumor growth without the development of drug resistance which occurs in the genetically unstable tumor cells. Shortly after, Folkman and colleagues discovered that cartilage contains factors that inhibit tumor angiogenesis. In the 1980s, numerous endogenous angiogenic factors and anti-angiogenic factors were discovered and characterized; the most significant factor for the tumor field being vascular endothelial factor (VEGF) first discovered by Harold Dvorak in 1983 as a vascular permeability factor and later by Napoleon Ferrara in 1989 as a critical angiogenic factor in embryonic development and tumor growth. In the 1990s several different molecules with anti-angiogenic properties went into clinical trials in cancer patients. These first trials did not meet the high expectations generated by the experimental data in animal models. Moreover, animal data (Robert Kerbel, 2001) showed that tumors can become resistant to anti-angiogenic therapy. Potential mechanisms of resistance include secretion of alternative angiogenic factors by the genetically unstable tumor cells or selection of tumor cells that either can survive hypoxic conditions or co-opt existing blood vessels in surrounding tissues. This indicated that combination therapies with agents targeting tumor cells or alternative anti-angiogenic pathways may be more effective than single agent therapy, particularly in advanced disease. This has been supported by animal studies and has led to clinical trails of anti-angiogenic compounds in combination with conventional chemotherapy as well as the design of small molecule kinase inhibitors that target multiple signaling pathways in vascular cells as well as in tumor cells. In 2003, the first large scale clinical trial showing a prolonged survival in patients with cancer after treatment with a very selective anti-angiogenic drug (Avastin, an inhibitory antibody against VEGF) in combination with a standard chemotherapy regimen was reported along with promising data from phase I trials with small molecule inhibitors targeting VEGF signaling pathways. This has validated the concept of anti-angiogenic therapy for cancer and generated enormous interest in the area. Attractions of this approach include an improved safety profile compared to conventional cytotoxic therapies, the application to a broad range of tumor types, the potential for combinations with most other therapeutic approaches including the newer and safer molecular targeted anti-tumor therapies and the potential for use in the adjuvant as well as advanced disease setting. Challenges for the future include determining the patient populations most likely to respond to anti-VEGF or other anti-angiogenic therapies, the best molecular markers for patient stratification, the stage of disease most responsive to therapy, the best surrogate markers for predicting patient response and the safest and most effective combination therapies. It is an exciting new era in cancer therapy.

459 INVITED

Targeting VEGF with Avastin

N. Ferrera. *USA*

Abstract not received.

460 INVITED

Clinical development of AVE 8062 and ZD6126

P. LoRusso. *USA*

Abstract not received.

461 INVITED

Tyrosine kinase inhibitors that target more than the VEGF-R: SU 11248 and ZD6474

S.G. Eckhardt. *University of Colorado, Division of Oncology B171, Aurora, USA*

Targeting the VEGF pathway has historically been considered the most direct method of inhibiting tumor angiogenesis. The recent approval of bevacizumab has benchmarked this approach in the clinic for colorectal cancer, whereas data with this antibody also appears promising in renal cell cancer and non-small cell lung cancer. Among the VEGF-R directed small molecule tyrosine kinase inhibitors, most are only relatively specific for the VEGF-R, and at least two of these, SU11248 and ZD6474, are capable of inhibiting a spectrum of other growth factor receptors that are thought to be relevant in the treatment of cancer. ZD6474 is an anilinoquinazoline with in vitro inhibitory activity against both the VEGFR2 and EGFR. Although there is a 10-fold difference in sensitivity to ZD6474 between the two receptors (IC50 of 40 and 500 nM, respectively), these concentrations are clearly achievable in the plasma of cancer patients at tolerable doses. Interestingly, toxicities of ZD6474 in phase I were partly mechanism-based and included mild hypertension, diarrhea, and skin rash. Objective responses were observed in patients with non-small cell lung cancer. SU11248, an indolinone, demonstrates sub-micromolar inhibitory activity against the VEGFR2, c-KIT, Flt-3, PDGFR-beta and FGFR, in decreasing order of potency. In phase I studies of SU11248, tumor regressions were observed in patients with renal cell cancer, neuroendocrine tumors, and imatinib-refractory GIST. Several observations can be made regarding these less specific inhibitors. The preclinical studies generally demonstrate tumor regression in addition to inhibition of tumor growth, and efficacy is also observed in larger, well-established tumors. In the clinic, the spectrum of toxicities is a bit more complex than more specific tyrosine kinase inhibitors, whereas tumor regressions are more common. As with all biologically-targeted compounds, the challenge will be to improve patient selection, which may be more difficult with agents that modulate more than one aberrant signaling pathway.

Friday 1 October 10:15–12:00

PLENARY SESSION 9

Apoptosis pathway targeting agents

462 INVITED

Targeting apoptosis in cancer with APO2L/TRAIL

A. Ashkenazi. *Molecular Oncology, Genentech, Inc., South San Francisco, CA, USA*

Apo2L/TRAIL is a recently discovered member of the tumor necrosis factor (TNF) gene superfamily that triggers apoptosis through engagement of two specific death receptors: DR4 and DR5. Upon engaging DR4 and/or DR5, Apo2L/TRAIL assembles a death-inducing signaling complex (DISC) that activates the apoptosis-initiating proteases caspase-8 and caspase-10 through the adaptor molecule Fas-associated death domain (FADD). As a soluble, zinc-coordinated trimer, Apo2L/TRAIL selectively induces apoptosis in many types of tumor cells but not in most normal cells, suggesting that it may be useful for cancer treatment. Unlike most conventional cancer therapeutic agents, Apo2L/TRAIL activates the apoptotic caspase cascade independently of the p53 tumor suppressor gene. In several cancer xenograft models, based upon established tumor cell lines or patient-derived tumors, Apo2L/TRAIL has demonstrated single agent anti-tumor efficacy as well as synergy with various types of chemotherapy. Thus, Apo2L/TRAIL might be effective not only for the second-line treatment of tumors that have acquired resistance to conventional therapy, but also for augmenting the efficacy of current first-line treatment in several types of cancer.

463 INVITED

Translation targeting TRAIL receptors to the clinic

A. Tolcher. *Institute for Drug Development, Cancer Therapy and Research Center, San Antonio, USA*

Apoptosis is a biochemical process of serial activation of upstream initiator cysteine aspartyl specific proteases (Caspases) that recruit downstream effector caspases, mediate proteolysis and ultimately result in cell death.[1] Effector (or *executioner*) caspases induce selective cleavage

at specific aspartate residues leading to the degradation of critical cellular housekeeping proteins required for cellular viability including signal transduction protein kinases, cytoskeletal proteins, chromatin modifying proteins, and DNA repair proteins.[2] Since the activation of effector caspases irreversibly commits the cell to apoptosis, the activation of the early caspases is a tightly regulated process mediated by at least two interrelated pathways of apoptosis–the extrinsic pathway (mediated by the tumor necrosis factor receptor protein family) and the intrinsic pathway mediated by the Bcl-2 family of proteins.[3,4]

The Role of the Apoptotic Pathways is regulation of Caspases. There is a hierarchical organization to the pathways of cellular apoptosis. The final common biochemical pathway that executes the process of apoptosis requires the activation of upstream and downstream caspases. As mentioned previously, caspases are a family of tightly-regulated intracellular cysteine proteases that cleave cellular proteins, including cleavage and activation of other caspase family members, specifically at aspartic acid residues. These proteases exist as inactive zymogens that, with activation upstream, lead to a cascade of proteolysis downstream with autoactivation of other caspase family members.[4] Not all caspases are involved in apoptosis. Principally, caspase-3, -6, -7, -8, and -9 have well described functions in cell death pathways. The downstream caspases mediate cellular destruction of "housekeeping" cellular functions through cleavage of protein kinases and other signal transduction proteins, cytoskeletal proteins, chromatin modifying proteins (e.g. polyADP ribosyl polymerase, PARP) DNA repair proteins, and inhibitory subunits of endonucleases (CIDE family of proteins).[2,4] This process is both rapid and methodical eliminating proteins necessary for cell viability. The activation of the upstream caspases is mediated by at least two known pathways that have been termed the Intrinsic and the Extrinsic pathway of apoptosis. Each pathway utilizes a separate upstream caspase family member to activate caspase-3 activation and subsequent downstream caspase member's activation. Although these pathways are frequently described separately, this separation is both simplistic and arbitrary since several lines of evidence indicate important links between the two pathways that further regulate the pro-apoptotic/anti-apoptotic homeostasis.[5,6]

Targeting The Extrinsic Pathway. The extrinsic pathway is composed of the death receptors, their ligands, and early initiating caspases. Death receptors (DR) are members of the tumor necrosis factor receptor (TNF-R) superfamily and include members that have both functional receptors that possess a functional intracellular death domain (DD), and decoy receptors that lack this functional domain. The TNF superfamily includes TNF-R1, FAS/APO1, DR3, TRAIL-R1 (DR4), TRAIL-R2 (DR5), TRAIL-R3 & -R4 (DcR-1 and -2) and DR6 and members of this family (e.g. TNF-α receptor, CD95/FasL/APO-1L receptor, and TRAIL/APO-2L receptor) regulate cell metabolism, cytokine production and apoptosis, while others (eg, TRAIL-R3, OPG) may be involved in cell proliferation and survival.[7,8] Death-domain containing receptors exist as trimers in the inactive state, and are activated upon ligand binding. Each receptor has distinct ligand specificity (e.g. TRAIL-R1, and R2 will only bind TRAIL). Following interaction of ligands with their receptors, "Fas associated death domain" (FADD) adaptor protein (Ashkenazi and Dixit, 1998) is recruited to the cytoplasmic tail of the receptor, which in turn recruits procaspase 8 and 10 and determines the formation of death inducing signaling complex (DISC), and leads to auto-cleavage and activation of the two caspases.[9–11] Caspases 8 and 10 activate downstream caspase "executioner" 3, 6, 7 which cleave the cellular death substrates and lead to apoptosis. The principal negative regulator of TRAIL-induced apoptosis is FLICE-FADD Like Inhibitory Protein (c-FLIP), which binds to and inhibits DISC. Recruitment of FADD to DISC and activation of caspase 8 and 10 appear to be necessary for TRAIL-mediated apoptosis, and deficiency or mutations in caspase 8 and 10 or FADD genes could contribute to the resistance to TRAIL.[10,12,13]. The potential for induction of antitumor activity, by what is now termed the extrinsic pathway, was first described over a century ago when tumor regressions were observed in the presence of an infectious process. Refinement of this observation over the ensuing years included the identification of gram-negative bacterial as the responsible precipitating infectious etiology and that an extract of the lipopolysaccharide membrane (Colley's Toxin) was the active component for TNF-α induction. Several alternative therapeutic strategies have developed to induce apoptosis in tumors by activating the receptors extrinsic pathway. These include the development of agents that mediate TRAIL release with autocrine and paracrine induction of apoptosis, recombinant-TRAIL, the synthesis of small molecules and peptides that interact with the TRAIL receptors, and human monoclonal antibodies that act as agonists of the TRAIL receptors. All-trans-retinoic acid (ATRA), indicated for the treatment of acute promyelocytic leukemia (PML), appears to mediate antitumor activity through apoptotic events of the extrinsic pathway with expression and release of TRAIL.[14] In elegant studies, an experimental PML cell line exposed to ATRA underwent a sequential process of initial differentiation and anti-apoptotic events mediated via the NF-κB pathway, followed by caspase pathway activation and apoptosis secondary to expression of membrane associated TRAIL acting on the DR4 and/or DR5 receptors. Interestingly, in co-culture experiments, the apoptotic events not only occurred in ATRA treated PML cells but also adjacent retinoic acid receptor α mutant cells (non-ATRA responsive) and non-PML cells (breast cancer MCF-7 cells) demonstrating true paracrine effects as well as implicating TRAIL as a therapeutic strategy in other malignancies beyond ATRA and PML.[14]

Tumor necrosis factor-α is the prototypic ligand for the TNFR family and has the potential to induce apoptosis in tumor cells as well as activation of components of the inflammatory processes.[15–18] TNF-α and Fas ligand are not candidates for drug therapy due to the non-specific activation of multiple TNF receptors and the observation of septic shock with fulminant hepatic failure in animals.[16] In contrast, recombinant soluble human TRAIL (rhu-TRAIL) is a candidate for clinical development based on the potential to induce apoptosis in a broad spectrum of human cancer cell lines but not in normal cells *in vitro*.[19–21] Moreover, in xenograft models antitumor activity has been observed without normal tissue toxicity.[19–21] The selectivity of TRAIL to mediate apoptosis in tumor cells, but not normal non-malignant cells, has not been fully elucidated but represents the key element to a therapeutic index for this class of agents. Overexpression of death receptors and/or a relative absence of decoy receptors in tumor cells have been postulated as the mechanism most likely responsible.[22–26] Preclinical studies demonstrating apoptosis in human hepatic cells *in vitro* raised concerns for clinical studies however recent evidence indicates that different versions of rhu-Trail may exhibit a different propensity for hepatocyte toxicity.[27,28] Currently rhu-TRAIL is undergoing late stage preclinical and toxicologic testing prior to entry into the clinic. [28]

Monoclonal antibodies with agonist-like properties at the DR4 and DR5 sites represent an alternate strategy for the induction of apoptosis via the extrinsic pathway. Following antibody-antigen complex formation, caspase activation and apoptosis induction occurs resulting in tumor regressions in some xenograft tumors.[29,30] The affinity for DR4 and DR 5 binding appears to be less important for agonist activity than the specific binding site on the receptor.[30] This implies that that specific agonist sites exist within the receptor and suggests that widely divergent results may be obtained with different antibodies ostensibly directed to the same target DR4. Human monoclonal antibodies targeting the TRAIL receptors DR 4 and DR5 entered clinical development in 2003. HGS-ETR1 (TRM-1) is a fully human monoclonal antibody targeting the TRAIL-R1 molecule. Preclinical studies indicate that HGS-ETR1 not only induces both tumor growth inhibition but also tumor regressions in xenograft tumors derived from human colon and non-small lung carcinoma cell lines providing the impetus for clinical development. Three phase I studies of HGS-ETR1 are near completion.[31,32] In preclinical toxicology studies modest hepatocyte abnormalities were observed in human co-culture experiments, but not in primate studies. Based, at least in part, on the potential for hepatocyte toxicity observed reported for TRAIL in other toxicologic studies, a very conservative dose escalation scheme was employed with a starting dose of 0.01 mg/kg administered once intravenously. In the three studies more than 60 patients have safely been treated with HGS-ETR1 and successful dose escalation over a 1000-fold dose-range to 10 mg/kg IV administered every 28 days has been achieved without evidence of dose-limiting toxicity. The clearance of HGS-ETR1 is compatible with other therapeutic human monoclonal antibodies, with an elimination half-life of approximately 14–21 days. Plasma concentrations are attainable that portend antitumor activity in preclinical models. Preliminary biologic activity has been observed with durable stable disease in some patients as well as minor tumor regressions in one patient.[31,32] More recently, HGS-ETR2, a high-affinity fully human monoclonal antibody directed to DR5 entered clinical evaluation in phase I studies. Preclinical toxicologic studies in cynomolgus monkeys, which share 90% of the amino acid sequence identity with the Human TRAIL-R2, demonstrate no hepatic toxicity. From an initial dose of 0.1 mg/kg in the human studies, dose-escalation is continuing at 0. 3mgk/kg administered utilizing schedules of administration of either every 14 or 21 days. The preliminary pharmacokinetic profile indicates both a prolonged elimination half-life as well as a volume of distribution that exceeds the plasma volume.

Challenges in the Development of TRAIL Receptor Targeting Therapies. TRAIL-R targeting therapies represent an emerging and novel strategy in the field of oncology drug development. Preclinical studies indicate that TRAIL-R targeting agents may have the potential for both single agent activity as well as the potential to enhance chemotherapy-induced apoptosis. Success in the latter strategy, enhancing the effectiveness of current chemotherapy, has so far remained elusive for other apoptosis-enhancing agents. Currently there are no identified predictive biomarkers that indicate which tumors are susceptible to TRAIL-R targeting therapies. The presence of the receptor appears to be necessary for activity in preclinical models however, no clear relationship between the magnitude of TRAIL-R expression and sensitivity to TRAIL or the agonist antibodies has so far been discerned. To adequately address this, and therefore identify patient subsets that can further enrich pivotal

studies of these agents, carefully conducted phase II studies will need to be performed to identify predictive biomarkers, direct subsequent pivotal studies to the population that has tumors that express these biomarkers, and thereby enhance the likelihood that these agents will be successfully developed.

References

[1] Thornberry NA, Lazebnik Y: Caspases: enemies within. Science 281:1312–6., 1998

[2] Thornberry NA: Caspases: a decade of death research. Cell Death Differ 6:1023–7., 1999

[3] Keane MM, Ettenberg SA, Nau MM, et al.: Chemotherapy augments TRAIL-induced apoptosis in breast cell lines. Cancer Research 59:734–41, 1999

[4] Sun SY, Yue P, Zhou JY, et al: Overexpression of BCL2 blocks TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in human lung cancer cells. Biochemical & Biophysical Research Communications 280:788–97, 2001

[5] Tsujimoto Y, Cossman J, Jaffe E, et al: Involvement of the bcl-2 gene in human follicular lymphoma. Science 228:1440–3, 1985

[6] Tsujimoto Y, Ikegaki N, Croce CM: Characterization of the protein product of bcl-2, the gene involved in human follicular lymphoma. Oncogene 2:3–7, 1987

[7] Pitti RM, Marsters SA, Lawrence DA, et al: Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. Nature. 396:699–703, 1998

[8] Wiley SR, Schooley K, Smolak PJ, et al: Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity. 3:673–82, 1995

[9] Ashkenazi A, Dixit VM: Apoptosis control by death and decoy receptors. Current Opinion in Cell Biology. 11:255–60, 1999

[10] Suliman A, Lam A, Datta R, et al: Intracellular mechanisms of TRAIL: apoptosis through mitochondrial-dependent and -independent pathways. Oncogene. 20:2122–33, 2001

[11] Wesselborg S, Engels IH, Rossmann E, et al: Anticancer drugs induce caspase-8/FLICE activation and apoptosis in the absence of CD95 receptor/ligand interaction. Blood. 93:3053–63, 1999

[12] Kischkel FC, Lawrence DA, Chuntharapai A, et al: Apo2L/TRAIL-dependent recruitment of endogenous FADD and caspase-8 to death receptors 4 and 5. Immunity 12:611–20., 2000

[13] Kischkel FC, Lawrence DA, Tinel A, et al: Death receptor recruitment of endogenous caspase-10 and apoptosis initiation in the absence of caspase-8. Journal of Biological Chemistry 276:46639–46, 2001

[14] Altucci L, Rossin A, Raffelsberger W, et al: Retinoic acid-induced apoptosis in leukemia cells is mediated by paracrine action of tumor-selective death ligand TRAIL. Nature Medicine 7:680–6, 2001

[15] Fiers W: Tumor necrosis factor. Characterization at the molecular, cellular and in vivo level. FEBS Lett 285:199–212, 1991

[16] Havell EA, Fiers W, North RJ: The antitumor function of tumor necrosis factor (TNF), I. Therapeutic action of TNF against an established murine sarcoma is indirect, immunologically dependent, and limited by severe toxicity. J Exp Med 167:1067–85, 1988

[17] Havell EA: Evidence that tumor necrosis factor has an important role in antibacterial resistance. J Immunol 143:2894–9., 1989

[18] Ogasawara J, Watanabe-Fukunaga R, Adachi M, et al: Lethal effect of the anti-Fas antibody in mice. Nature 364:806–9., 1993

[19] Walczak H, Miller RE, Ariail K, et al: Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. Nature Medicine 5:157–63, 1999

[20] Chinnaiyan AM, Prasad U, Shankar S, et al: Combined effect of tumor necrosis factor-related apoptosis-inducing ligand and ionizing radiation in breast cancer therapy. Proceedings of the National Academy of Sciences of the United States of America 97:1754–9, 2000

[21] Ashkenazi A, Pai RC, Fong S, et al: Safety and antitumor activity of recombinant soluble Apo2 ligand. Journal of Clinical Investigation 104:155–62, 1999

[22] Pan G, Ni J, Wei YF, et al: An antagonist decoy receptor and a death domain-containing receptor for TRAIL. Science 277:815–8, 1997

[23] Pan G, O'Rourke K, Chinnaiyan AM, et al: The receptor for the cytotoxic ligand TRAIL. Science 276:111–3., 1997

[24] Gibson SB, Oyer R, Spalding AC, et al: Increased expression of death receptors 4 and 5 synergizes the apoptosis response to combined treatment with etoposide and TRAIL. Molecular & Cellular Biology 20:205–12, 2000

[25] Ibrahim SM, Ringel J, Schmidt C, et al: Pancreatic adenocarcinoma cell lines show variable susceptibility to TRAIL-mediated cell death. Pancreas 23:72–9, 2001

[26] Kim K, Fisher MJ, Xu SQ, et al: Molecular determinants of response to TRAIL in killing of normal and cancer cells. Clinical Cancer Research 6:335–46, 2000

[27] Jo M, Kim TH, Seol DW, et al: Apoptosis induced in normal human hepatocytes by tumor necrosis factor-related apoptosis-inducing ligand. Nature Medicine 6:564–7, 2000

[28] Lawrence D, Shahrokh Z, Marsters S, et al: Differential hepatocyte toxicity of recombinant Apo2L/TRAIL versions. Nat Med 7:383–5., 2001

[29] Chuntharapai A, Dodge K, Grimmer K, et al: Isotype-dependent inhibition of tumor growth in vivo by monoclonal antibodies to death receptor 4. J Immunol 166:4891–8., 2001

[30] Ichikawa K, Liu W, Zhao L, et al: Tumoricidal activity of a novel anti-human DR5 monoclonal antibody without hepatocyte cytotoxicity. Nature Medicine 7:954–60, 2001

[31] Tolcher AW, Mita M, Patnaik A, et al: A Phase I and pharmacokinetic study of HGS-ETR1 (TRM-1), a human monoclonal agonist antibody to TRAIL R1 in patients with advanced malignancies. Proceedings of the American Society of Oncology 22:210s, 2004

[32] Le LH, Hirte HW, Hotte SJ, et al: Phase I study of a fully human monoclonal antibody to the tumor necrosis factor-related apoptosis-inducing ligand death receptor 4 (TRAIL-R1) in subjects with advanced solid malignancies or non-Hodgkins lymphoma. Proceedings of the American Society of Oncology 22:171s, 2004

464 INVITED

Regulation of apoptosis by synthetic helices of the BCL-2 family

L. Walensky[1,2], A. Kung[2,3], I. Escher[4], T. Malia[5], S. Barbuto[1], R. Wright[3], G. Wagner[5], G. Verdine[3], S. Korsmeyer[1]. *[1]Dana-Farber Cancer Institute SM-758, Howard Hughes Medical Institute, Boston, USA; [2]Dana-Farber Cancer Institute, Department of Pediatric Hematology/Oncology, Boston, USA; [3]Dana-Farber Cancer Institute, Department of Cancer Biology, Boston, USA; [4]Harvard University, Department of Chemistry and Chemical Biology, Cambridge, USA; [5]Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology, Boston, USA*

Defects in "apoptosis" or programmed cell death are a hallmark of cancer. The BCL-2 family of pro- and anti-apoptotic intracellular proteins constitutes a critical decisional control point in the intrinsic cell death pathway. Protein interaction between BCL-2 members is a prominent mechanism of regulation and is mediated through the amphipathic alpha-helical BH3 segment, which functions as an essential death domain. The manufacture of small molecules to activate cell death pathways has been complicated by the extensive, shallow and hydrophobic interface of apoptotic protein targets. The *in vivo* utility of specific peptides to inhibit or activate these signaling pathways has been compromised by their lack of secondary structure, susceptibility to proteolytic degradation, and difficulty penetrating cells. We developed a chemical strategy, termed hydrocarbon stapling, to generate BH3 peptides with dramatically improved pharmacologic properties. The stapled peptides, entitled "Stabilized Alpha-Helix of BCL-2 domains" or SAHBs, proved to be helical, protease resistant, and cell permeable molecules that bound with increased affinity to multidomain BCL-2 member pockets. A SAHB of the BH3 domain from BID, for example, activated the genetic pathway of apoptosis to kill leukemia cells. In addition, SAHB effectively inhibited human leukemia xenografts *in vivo*. Synthetic approaches such as hydrocarbon stapling that reinforce native peptide sequences provide an alternative strategy to manipulate protein-protein interactions and target cell death pathways in cancer.

465 INVITED

Targeting Bcl-2 using antisense molecules

J. Waters. *The Institute of Cancer Research and Royal Marsden NHS Trust, Department of Medicine, Sutton, UK*

Abnormal expression of Bcl-2 protects malignant cells from apoptosis, and is implicated in the aetiology of non-Hodgkin's lymphoma and in chemoresistance of several tumour types. Oligonucleotides (ONT) complementary to a region of the *bcl-2* mRNA can specifically down-regulate Bcl-2 expression, leading *in vitro* to increased rates of apoptosis and enhanced chemosensitivity. Oblimersen (Genasense, GS), the lead Bcl-2-targeted antisense ONT is an 18-base phosphorothioate ONT targeting the first 6 codons of the Bcl-2 open reading frame. Clinical trials of GS as a single agent or with chemotherapy have demonstrated the