

feasibility of this approach, with sporadic clinical activity. Pharmacodynamic studies have shown specific reductions in Bcl-2 protein and/or mRNA levels after treatment, either in tumour tissue or peripheral blood mononuclear cells, in a proportion of patients, although the degree of Bcl-2 down-regulation is variable. Increased rates of apoptosis in melanoma biopsies have been reported following treatment, indicating a possible down-stream effect. In limited studies, we observed PARP cleavage suggestive of caspase activation in tumour samples from NHL patients treated with GS in a phase I trial (Waters et al. JCO 2000; 18: 1812-1823). Phase III trials investigating GS-mediated chemosensitisation have been completed in advanced melanoma, CLL and multiple myeloma. The first to be reported compared DTIC±GS in patients with stage IV melanoma. Although a significantly higher response rate (11.7% vs. 6.8%; $p = 0.019$) and median progression free survival (74 days vs. 49 days; $p = 0.0003$) were observed favouring the GS arm, the trial failed to reach its primary endpoint of overall survival benefit, and GS increased toxicity. Efforts to increase the efficacy of antisense ONTs have focussed on chemical modifications that increase stability and hybridisation affinity. 2'-O-methoxy-ethyl modification of a proportion of the nucleotides achieves this aim. Such an ONT complementary to a region of homology between *bcl-2* and *bcl-x_L* produced activity *in vitro* in lung cancer, breast cancer and melanoma cell lines, with down-regulation of both target proteins without affecting pro-apoptotic members of the *bcl-2* family.

Conclusions: Antisense targeting of Bcl-2 is feasible in the clinical arena and combination with apoptosis-inducing agents appears the most promising strategy. New oligonucleotide chemistries may enhance activity, but clinical results are awaited.

Friday 1 October

14:00–15:45

PLENARY SESSION 10

Stroma as a target

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INVITED

Antibody therapies targeting the stromal compartment

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One avenue towards the development of more selective, better anti-cancer drugs consists in the targeted delivery of bioactive molecules (drugs, cytokines, procoagulant factors, photosensitizers, radionuclides, etc.) to the tumor environment by means of binding molecules (e.g., human antibodies) specific for tumor-associated markers. Angiogenesis, i.e., the proliferation of new blood vessels from pre-existing ones, is an underlying process in many human diseases, including cancer, blinding ocular disorders and rheumatoid arthritis. The ability to selectively target and occlude neovasculature will be potentially useful in diagnosis and treatment of angiogenesis-related diseases. A good-quality marker for both tumoural and non-tumoural neovasculature is the extra-domain B (ED-B) of fibronectin, a sequence of 91 aminoacids that can be inserted into the fibronectin molecule by a mechanism of alternative splicing. To date, the production of monoclonal antibodies directly recognising the ED-B domain has not been possible using hybridoma technology, because of tolerance. In collaboration with Prof. Dr. Luciano Zardi (Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy), we have overcome this problem using large synthetic antibody phage libraries, generating several high-affinity antibody fragments specific for fibronectin containing the ED-B domain, and mapping their epitope onto the three-dimensional structure of the antigen. These antibodies stain vascular structures in tumour sections and selectively target tumour neovasculature, as shown in tumour-bearing mice using infrared fluorescence and radioactive techniques. Increased binding affinity leads to improved targeting of tumoural angiogenesis, as demonstrated by biodistributions studies performed using the L19 antibody fragment with affinity for the ED-B domain in the picomolar range and L19 mutants with reduced affinity. The ability of radiolabeled L19 to target tumors in patients with cancer has recently been demonstrated using scintigraphic detection methods. A number of derivatives of the L19 antibody (fusions to cytokines, pro-coagulant factors, photosensitizers, drugs, radionuclides, etc.) have been studied in animal models. The results obtained are of therapeutic relevance, since the ED-B domain of fibronectin, a naturally-occurring marker of angiogenesis identical in mouse and man, is expressed in the majority of aggressive solid tumours, but is undetectable in normal vessels and tissues. In the last part of my presentation, I will show how we are using the *in vivo* biotinylation of tumor bearing mice by terminal perfusion, followed by proteomic analysis of tumor specimens and normal organs, for the discovery of novel tumor-associated vascular targets.

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INVITED

Stromal compartment influences on response to therapy

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Tumor metastasis, the spread of cancer from a primary tumor to distant sites, is the major cause of treatment failure and death in cancer patients. Metastasis depends, first, on delivery of cancer cells to secondary organs via the blood or lymphatic circulation, and second, on successful growth of a subset of these cells. Only a small subset of cells initiate metastatic growth, and a subset of micrometastases persist in growth to form clinically relevant macroscopic metastases. Molecular interactions between cancer cells and the new organ environment affect what proportion of cells begin and maintain metastatic growth. Thus, growth regulation in a secondary site is a key determinant of metastasis and patient outcome, and presents a temporally broad, clinically accessible therapeutic target. While it has been believed that it may be too late to treat patients in whom the metastatic process had already started, this concept suggests instead that there are clinical treatment opportunities at several stages of the metastatic process, and that metastatic growth may be vulnerable to several routes of attack. As an illustration of how growth in a secondary site may be affected by molecular factors and therapeutic strategies, we studied the role of post-surgical dietary intervention in a model of breast cancer metastasis. The anticancer activity of genistein, an isoflavone found in soy, has been shown previously *in vitro* and *in vivo*. We have begun to assess the utility of genistein in the metastatic setting. We tested the hypothesis that genistein could limit growth of potentially metastatic cells seeded prior to primary cancer surgery. Primary tumors were created by injection of human breast cancer cells into mammary fat pads of female nude mice. When primary tumors formed, they were surgically removed. Mice were then randomized into two diet groups: the control soy-free diet vs. a diet supplemented with genistein. Five weeks later, metastatic burden was assessed. A significantly higher proportion of animals that were fed the genistein-supplemented diet following surgery were free of metastases in lymph nodes or lungs. These results indicate that dietary intervention following cancer surgery can affect the growth ability of previously seeded, potentially metastatic cells. A better understanding of the interactions of cancer cells with the microenvironment in secondary sites may lead to improved treatment approaches for metastatic disease.

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INVITED

Brain metastases: molecular analysis and therapeutic options

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Approximately 15% of breast cancer patients develop brain metastases; mean survival is approximately one year. The incidence of brain metastases is expected to rise as systemic control improves and tumor cells colonize the brain as a sanctuary site. The brain microenvironment and the blood:brain barrier are hypothesized to provide distinct selection pressures for metastatic growth. To analyze the gene expression of breast cancer brain metastases, we have examined 16 surgically resected brain metastases of breast cancer and compared them to a cohort of unrelated primary breast carcinomas, matched for histology, hormone receptor status, and TNM stage. RNA isolated from laser captured microdissected tumor cells was amplified using a T7-based amplification protocol prior to cDNA microarray analysis on a 30K microarray. Comparison of brain metastases and primary tumors revealed differential gene expression of estrogen receptor, Hif1, suppressor genes, the TAP1 transporter, etc. Overexpression of Her-2/neu mRNA was more frequent in brain metastases than primary tumors, and this trend was confirmed at the DNA amplification level by FISH. Subset analysis of Her-2 amplified and unamplified brain metastases showed a robust gene expression signature with minimal overlap to Her-2 signatures previously published using either cell lines or primary tumors. Similarly, analysis of estrogen receptor-negative versus -positive brain metastases showed a robust gene expression signature largely independent of published analyses. The data indicate that gene expression patterns of metastases may vary from primary tumors or cell lines. Therapeutic targets expressed in brain metastases include Her-2, EGF receptor and histone deacetylases and will be discussed.