have low PTEN levels and an activating *PIK3CA* mutation, and IGROV-1 cells are null PTEN. Both cell lines express a high basal level of phosphorylated AKT (p-AKT). Exposure to IC50 concentrations of PI103 (0.47 μM, 0.14 μM respectively) led to a reduction in p-AKT, p-GSK3β and p-S6 ribosomal protein at 2h and/or 12h. Paclitaxel or carboplatin, which are commonly used drugs in OC, were combined with PI103 at a fixed ratio of their IC50 and growth inhibition measured after continuous exposure using an MTT assay, and the data analysed using the median-effect equation of Chou and Talalay. In SKOV-3 cells, PI103 was synergistic when combined with paclitaxel with a combination index (CI) @ fraction unaffected (fu)0.5 of 0.42 ± 0.16, demonstrating that <50% of each drug is required to give the same effect as that predicted from additivity. In contrast, PI103 was strongly antagonistic when combined with carboplatin (CI@fu0.5 = 2.8 ± 1.1). Reverse results were obtained with IGROV-1 cells with CI@fu0.5 values of 2.4 ± 0.37 and 0.67 ± 0.11 for paclitaxel and carboplatin respectively. Investigations were extended to the CHI and HX62 OC lines (PI103 IC50 = 1.4 and 0.71 μM respectively) in which the PI3K/AKT pathway appears less deregulated. CH1 is the most sensitive of all the lines to the cytotoxic agents and synergy was observed when PI103 was combined with paclitaxel (CI@fu0.5 = 0.72 ± 0.16) and particularly with carboplatin (CI@fu0.5 = 0.48 ± 0.08). In contrast, the combinations in HX62 cells were close to additive, consistent with their independent drug actions. The two examples of antagonism were investigated further and sequencing the cytotoxic agent 24h prior to PI103 led to additivity. These data suggest that it may be useful to combine inhibitors of PI3K/AKT/mTOR signalling with paclitaxel or carboplatin in patients with ovarian cancer although the preferred molecular context for synergy requires further analysis.

252 ORAL

Identification of responders and non-responders to imatinib prior to treatment

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Background: Chronic myelogenous leukemia (CML) is characterized by a chromosomal translocation resulting in a shortened chromosome 22 termed the Philadelphia chromosome. This aberration leads to the fusion of the *BCR* and *ABL* genes and the production of a chimeric protein with a deregulated tyrosine kinase (TK) activity. Imatinib, a potent and specific TK inhibitor, has emerged as the first line treatment for CML. While imatinib induces complete cytogenetic remission (CCR) in the majority of patients, some individuals do not respond and would benefit from alternative therapies earlier in the course of disease. The goal of this work was to develop an *in vitro* assay to reliably predict response to imatinib therapy prior to treatment.
Human aspartyl (asparaginyl) β-hydroxylase (*ASPH*) is a highly specific biomarker for cancer. Increased *ASPH* expression has been detected at the protein and mRNA levels specifically in tumor cells. Over-expression of ASPH results in its translocation to the cellular surface where it is a potential target for antibody-based cancer therapy.
Methods: Leukocytes from 39 patients were isolated from fresh whole blood prior to the initiation of therapy and cultured for 24 hours in the presence or absence of 1 μM imatinib. *ASPH* and *BCR*/*ABL* transcript levels were determined by real-time quantitative polymerase chain reaction (RQ-PCR) analysis. Patients were treated with imatinib and there response status was assessed vis-a-vis complete molecular remission (CMR) by RQ-PCR of the *BCR*/*ABL* fusion gene.
Results: Prior to treatment, all patient samples had increased expression of the *ASPH* transcript (~5-fold). The leukocytes of 27 patients displayed a 30–75% decrease in *ASPH* expression after culture in the presence of imatinib. All of the corresponding patients achieved CMR after drug therapy. The leukocytes of the 12 other patients displayed less than a 25% reduction in *ASPH* transcript levels and these patients proved to be non-responders to drug treatment. Transcript levels of either the *BCR*/*ABL* gene itself or a control gene, *Ki67*, did not correlate with drug response.
Conclusions: Decreased levels of expression of the *ASPH* transcript after a 24 hour *in vitro* exposure of primary leukocytes to imatinib is a simple and sensitive assay for the determination of likely response to imatinib prior to the initiation of treatment. As well, this assay may represent a quick and simple approach to high throughput screening for new drug candidates against CML.

252A ORAL

Relapse in children with acute lymphoblastic leukaemia is associated with selection of a pre-existing drug resistance subclone

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Acute Lymphoblastic Leukaemia (ALL) is the most common cancer in children. Although virtually all patients achieve clinical remission following induction chemotherapy, one in four patients will subsequently relapse. We have previously shown that these relapses are due to small numbers of surviving and highly drug refractory cells (J Clin Oncol 21:704–9, 2003). It is unclear, however, whether relapse results from acquisition of a therapy-induced resistant phenotype or from selection of an intrinsically resistant subpopulation of cells. To investigate this process, 25 matched diagnosis and relapse ALL samples were analysed for the presence of clonal populations based on rearrangements of over 30 antigen receptor chain genes (Blood 103:3905−14, 2004). These unique clonal markers revealed the emergence or evolution of new clonal populations at the time of relapse in 13 patients. In eight of these samples, highly sensitive clone-specific PCR demonstrated the presence of this 'new' relapse clone at low levels in the matched diagnosis sample, indicating selection of a pre-existing subpopulation of cells as mechanism of relapse. Kaplan-Meier survival analysis demonstrated a significant relationship between the presence of the relapse clone at diagnosis and the length of first clinical remission (CR1). Furthermore, the quantity of the relapse clone at diagnosis was strongly inversely correlated with CR1. In particular, this pattern of clonal evolution could be replicated following engraftment and passaging of primary leukaemia samples in an in vivo model of ALL, using non-obese diabetic/severe combined immunodeficient mice. These data therefore indicate that early relapse in ALL patients commonly results from the selection of an intrinsically resistant subclone that is present at diagnosis but undetectable using routine methods. Using current treatment protocols, relapse appears inevitable for these patients and this research highlights the need to identify such patients early during treatment and to explore alternate therapies for them.

Thursday 9 November 16:30–18:15

PLENARY SESSION 7

The tumour microenvironment and metastasis

253 INVITED

The KISS1 metastasis suppressor regulates tumor cell response to microenvironmental signals and growth at secondary sites

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The primary obstacle to cancer cure is development of distant metastases. At diagnosis, tumors have already shed many cells into the circulation rendering anti-metastatic therapies moot since the cells have already seeded other tissues. Recent findings with metastasis suppressors indicate that tumor cells expressing KISS1 are able to complete every step of the metastatic cascade except proliferation at the secondary site. Tumor cells inoculated at orthotopic sites (i.e., intradermal) form progressively growing tumors that shed cells which remain dormant in the lungs. Tumor cells inoculated intravenously seed the lungs, but fail to establish macroscopic lesions. The nascent KISS1 protein (~17 kDa) has never been detected while a secreted internal 54 amino acid polypeptide [termed metastin or kisspeptin-54 (KP54)] had previously been isolated from placenta. To assess whether KISS1 or KP54 secretion is necessary for metastasis suppression, an internally FLAG-tagged KISS1 was constructed with (designated KFM) or without (designated ΔSS) the putative signal peptide, transfected into metastatic human C8161.9 melanoma cells and evaluated for tumor growth and metastasis following orthotopic or intravascular injection. ΔSS expressing cells no longer produced detectable KISS1 or KP54 in culture media. Moreover, ΔSS-transfected cells were as metastatic as parental cells, while KFM-transfected cells were suppressed for metastasis. Both still allowed local tumor growth. Media was isolated from cells expressing KFM, immunoprecipitated using anti-KISS1 and anti-FLAG antibodies, analyzed by mass spectrometry and internal sequencing and verified to be polypeptides derived from KISS1. Therefore, secretion of KISS1 is necessary for its anti-metastatic effects. As a secreted molecule

that impacts metastasis, KISS1 (products) are likely drug-able and may be useful for inhibiting colonization of tumors at secondary sites.
Support: CA87728, National Foundation for Cancer Research Center for Metastasis Research, DAMD17-02-1-0541, NIH Medical Scientist Training Program Grant T32 GM08361

254 INVITED

Tumour vascular diversity and its translation into targeted therapeutics

R. Pasqualini, W. Arap. *USA*

Abstract not received.

255 INVITED

Targeting the invasion-association integrin alphavbeta6 as an anti-carcinoma strategy

I. Hart, D. Dicara, J.F. Marshall. *Queen Mary's School of Medicine and Dentistry at Barts and The London, Institute of Cancer & CR-UK Clinical Centre, Centre for Tumour Biology, London, United Kingdom*

The epithelial-specific integrin αvβ6 usually is not expressed in resting tissues but is upregulated in wound-healing, inflammation and in many different cancers. Indeed our own immunohistochemical analyses have shown that in oral squamous cell carcinoma, for example, around 90% of tumours express high levels of αvβ6, often at their invasive margins. Interestingly the induced expression of αvβ6 in experimental systems has been shown to increase carcinoma cell invasion, possibly explaining why strong expression of αvβ6 correlates with a 69% reduction of patient survival in colon cancer (Bates RC, et al. J Clin Invest 2005; 115: 339–347). As an integrin hetrodimer αvβ6 obviously is expressed at the cell surface and thus represents a potential target for imaging and, possibly, therapy. Characterised as the prime receptor for Foot-and-Mouth-Disease virus αvβ6 is known to recognise the Arg-Gly-Asp (RGD) motif present in the G-H loop of the VP1 structural protein. We have designed peptide probes, based around this core RGD sequence, and shown that longer (20 mer) peptides are better antagonists of αvβ6 activity than shorter peptides (lead peptide A20FMDV2; IC_{50} = 1 nM). Structural analysis by NMR has shown that efficacy of these longer peptides corresponds with the presence of a helix immediately C-terminal to this critical RGD motif. Non-adjacent residues, brought into juxtaposition as a linear array on the outer face of the helix, also appear to interact with αvβ6. Using engineered human tumour cell lines which differ only in their expression of αvβ6 we were able to demonstrate specificity of binding using biotinylated peptides. *In vivo* specificity was demonstrated using nude mice bearing αvβ6-positive and -negative xenografts which were injected with ^{18}F-FBA-A20FMDV2; positive: negative tumour ratio >4:1 with MicroPET showing selective accumulation in size-matched β6-positive tumours. These data indicate that carcinoma-specific αvβ6 may represent a suitable target for these peptides; a possibility currently under investigation in this laboratory.

256 INVITED

Targeting hypoxia as an anti-metastatic strategy

K. Williams. *University of Manchester, School of Pharmacy and Pharmaceutical Sciences, Manchester, United Kingdom*

Metastases are the primary cause of cancer-related deaths. Several pre-clinical and clinical studies have demonstrated that the development of metastatic disease is linked with the extent of hypoxia in the primary tumour. From the data that has been amassed so far, it would appear that hypoxia, through a number of discreet mechanisms, can "breed" an aggressive disease phenotype. This is problematic given the fact that hypoxia has been identified in all solid tumours analysed to date and that hypoxic cells are resistant to standard radiotherapy and many forms of chemotherapy. On the other hand hypoxia is a tumour-specific physiological abnormality and as such it can be exploited as a difference between tumours and normal tissues. The association between hypoxia and the development of metastases suggests there may be a potential therapeutic window of opportunity in targeting primary tumour hypoxia to reduce metastatic dissemination. There are two ways to approach this. The first is to target the condition of hypoxia per se, using bio-reductive agents that are selectively cytotoxic towards hypoxic cells. The second is to identify hypoxia-dependent changes in gene expression that are pivotal in the transition to a metastatic phenotype. Examples will be given where these approaches have led to a significant reduction in metastatic burden in experiments.

Thursday 9 November

Poster Sessions

Bioreductive agents

257 POSTER

Tirapazamine disrupts vascular endothelial-cadherin, suggesting a mechanism behind its ability to cause central vascular dysfunction

L. Huxham, A. Kyle, A. Minchinton. *BC Cancer Research Centre, Medical Biophysics, Vancouver, Canada*

By mapping the microregional effects in HCT-116 tumour xenografts, we have shown that the hypoxic cytotoxin tirapazamine (SR 4233: 3-amino-1,2,4-benzotriazine 1,4-dioxide) unexpectedly causes central vascular dysfunction 1 day after treatment with a progression over the following 1–3 days to necrosis. Similar effects, but with different kinetics, have been seen after treatment with known vascular targeting agents such as combretastatin A4 phosphate which is a microtubule disrupting agent and has also been shown to interfere with the endothelial cell-specific junctional molecule vascular endothelial-cadherin (VE-cadherin).
To investigate the mechanism of action behind the vascular dysfunction caused by tirapazamine we have examined human umbilical vein endothelial cells under oxic and hypoxic conditions. Cells were seeded in 4 well glass slide chambers and grown until confluent. Chamber slides were then gassed with specific levels of oxygen. The confluent monolayers were treated with 100 μM tirapazamine for 1.5 hours and stained to show hypoxia (pimonidazole), DNA double strand breaks (γH2AX), microtubule fine structure (β-tubulin), and vascular endothelial cell adhesion junctions (VE-cadherin).
Under hypoxic conditions tirapazamine treated cells showed labeling for pimonidazole and an increase in γH2AX compared to cells in an oxic environment. Microtubule disruption was not seen after exposure to tirapazamine in either the oxic or hypoxic groups. However, under hypoxic conditions tirapazamine did cause disruption of VE-cadherin as seen by an absence of pseudopodia and by fragmentation of the structured cell membrane junctions.
We propose that this activity of tirapazamine *in vivo* is related to its effect on hypoxic tumour vasculature located in the centre of tumours. The observed central vascular dysfunction may be due to disruption of the cell adhesion junctions between endothelial cells in hypoxic regions, thereby damaging the vessel and leading to cessation of perfusion along the vessel.
This research is supported by the Canadian Institutes of Health Research and the Michael Smith Foundation for Health Research. Animals were maintained in accordance with the Canadian Council on Animal Care guidelines.

258 POSTER

Tricyclic triazine 1,4-dioxides: a new class of hypoxia-selective cytotoxins with improved extravascular transport compared to tirapazamine

M.P. Hay, K.O. Hicks, B.G. Siim, F.B. Pruijn, H.H. Lee, S. Yang, K. Pchalek, A. Blaser, W.R. Wilson, W.A. Denny. *The University of Auckland, Auckland Cancer Society Research Centre, Auckland, New Zealand*

Pharmacokinetic/pharmacodynamic (PK/PD) modelling has shown the in vivo activity of tirapazamine (TPZ), a bioreductive cytotoxin currently in Phase III clinical trial in combination with chemo-radiotherapy, to be limited by poor extravascular transport (EVT). EVT limitations result from slow diffusion and high bioreductive metabolism.
We present a new class of tricyclic triazine 1,4-dioxides (TTOs) as hypoxia-selective cytotoxins with improved EVT compared to TPZ. The indanetriazine core was designed to increase EVT by increasing lipophilicity and decreasing hypoxic metabolism through lowered electron affinity. The addition of a lipophilic amine side chain, attached via the 3-NH position, provided increased solubility while maintaining EVT. Replacement of the 3-NH linker with an alkyl linker improved diffusion by increasing lipophilicity and removing H-bond donors. The 3-alkyl substituents contributed to increased rates of metabolism which were balanced by the electron-donating nature of the indanetriazine core.
TTOs were screened for in vitro hypoxic cytotoxicity (IC_{50}) and hypoxic selectivity (HCR = aerobic IC_{50}/hypoxic IC_{50}) in human HT29 colon carcinoma cells. Diffusion coefficients (D_{MCL}) were calculated from diffusion studies in HT29 multicellular layers and rates of hypoxic metabolism (k_{met}) measured in single cell suspensions. Calculation of a 1-D transport parameter (X_*) allowed comparison of EVT between TTOs. PK/PD modelling predicted the plasma AUC required for 1 log of hypoxic cell killing