454 INVITED

Telomere targeting agents – clinical development candidates

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One of the recognised acquired capabilities of tumours is that of a limitless replicative potential. This property is linked to the maintenance of telomeres, specialised structures on the ends of eukaryotic chromosomes that comprise repetitive sequences of DNA (TTAGGG) and a variety of associated proteins. Included among these is the reverse transcriptase enzyme telomerase, which is capable of extending telomeres. Telomeres and telomerase represent attractive cancer drug targets since, in comparison to normal tissues, tumours possess telomeres that are generally critically shortened and where, in the majority of cases, telomerase is activated. Some drug development initiatives have targeted telomerase directly (e.g., the antisense molecule GRN163L) where, in general, biological effects are related to initial telomere length which can lead to a significant lag before antitumour activity is achieved. By contrast, a class of agents that directly target telomeres (telomere targeting agents, TTAs) has emerged and include the natural product telomestatin, cationic porphyrins, trisubstituted acridines and pentacyclic acridines. These induce the G-rich single-stranded overhang of telomeres to fold into 4-stranded G-quadruplex structures. Such folding is incompatible with telomerase function and leads to telomere uncapping and then to relatively rapid onset cell senescence or death. Numerous in vitro studies with TTAs using cancer cell lines representative of many different tumour types (including melanoma, prostate and breast) have shown senescence and or apoptosis at concentrations that did not cause acute non-specific cytotoxicity. Extensive end-to-end chromosomal fusions and telophase bridges, consistent with telomere uncapping, have also been reported. Studies with the trisubstituted acridine (BRACO19) have demonstrated single agent activity in mice bearing human tumour xenografts of uterus or prostate cancer and furthermore, synergy in vivo when used in combination with either paclitaxel (vulval tumour) or cisplatin (non small cell lung tumour). Studies with RHPS4 a pentacyclic acridine have demonstrated synergy in vitro with paclitaxel, doxorubicin, camptothecins and the HSP90 inhibitor, 17AAG. Furthermore, RHPS4 has shown promising single agent activity against a human breast cancer xenograft. Similarly to BRACO19, antitumour effects in xenografts were apparent after relatively short treatment periods (7 to 14 days) and suggest that a more intermittent rather than chronic dosing regime may be applicable in man. RHPS4 is currently undergoing preclinical development in anticipation of beginning Phase I trials.

455 INVITED

Discovering novel targets within the autophagy pathway: can we eat ourselves to death?

W.N. Hait. *R.W. Johnson University Hospital, Cancer Institute of New Jersey, New Brunswick, USA*

Background: Autophagy is a highly conserved survival mechanism activated in response to nutrient and growth factor deprivation and other forms of cellular stress. Yet, autophagy may also result in terminal self-consumption under circumstances of prolonged starvation. Since the initial response to nutrient or growth factor deprivation is autophagic cell survival, we have searched for targets that would abrogate this survival mechanism and accelerate more permanent forms of cell death.
Results: Eukaryotic elongation factor-2 kinase terminates protein elongation and thereby conserves cellular energy in time of deprivation. eEF-2 kinase is structurally unusual as it shares sequence homology with prokaryotic histidine kinases. We screened a series of histidine kinase inhibitors and identified NH125, an imidazolium derivative, as a potent and selective inhibitor. NH125 was effective against a variety of human and murine cancer cell lines in vitro and in vivo. Cell lines deprived of nutrients activated autophagy as measured by the formation of LC3-II from LC3–1, Acidic Vesicle Organelles, and electron microscopy. Under these conditions, the activity of eEF-2 kinase was rapidly increased as measured by the phosphorylation of eEF-2. Cell lines depleted of eEF-2 kinase by RNAi (siRNA or stable shRNA cell lines), failed to undergo autophagy and died within hours of nutrient depletion. In contrast, control cells were able to survive for several days.
Discussion: These studies suggest that eEF-2 kinase is activated under condtions that promote autophagic cell survival and that eEF-2 kinase inhibitors may represent a novel class of anticancer agents.

456 INVITED

The importance of epigenetics in drug discovery and development

P.A. Jones. *University of Southern California, Norris Comprehensive Cancer Center, Keck School of Medicine, MC-9181, Los Angeles, USA*

Epigenetic silencing of tumor suppressor genes and non-coding RNAs plays a major role in human carcinogenesis. CpG islands, which are frequently located at the transcription start sites of human genes, can become abnormally methylated resulting in heritable silencing. This methylation of cytosine residues is associated with alterations in chromatin structure including the binding of methylated DNA binding proteins and changes in the state of covalent modification of histone residues in nucleosomes. Until now, most of the focus has been on the interplay between covalent histone modifications and DNA cytosine methylation. However, it is becoming increasingly apparent that nucleosomal occupancy plays a substantial role in controlling the accessibility of the promoter to transcriptional factors. We have developed a new assay to examine CpG islands for nucleosomal occupancy and found that several human CpG islands are missing nucleosomes in the regions just upstream of the start sites. Cancer cells with epigenetically silenced promoters showed not only extensive DNA cytosine methylation and deacetylated histones but also have extra nucleosomes in the promoter. Strikingly activation of the silenced promoters by DNA demethylating drugs involves nucleosomal eviction in addition to changes in cytosine methylation and covalent histone modifications. These observations have important implications for epigenetic therapy in which at least three interacting biological processes have to be considered in the silencing and reactivation of promoters by chromatin modifying drugs.

Friday 10 November 14:00–15:45

PLENARY SESSION 10

DNA repair modulators in clinical trials

457 INVITED

Alkylguanine-DNA alkyltransferase inhibitors: current status

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The presence of the DNA repair protein O6-alkylguanine-DNA alkyltransferase (AGT) renders cells resistant to the biological effects of methylating and chloroethylating agents. O6-Benzylguanine (BG) is a low molecular weight substrate of AGT developed to sensitize tumor cells to the cytotoxic and antitumor effects of these agents. Presently, chemotherapy regimens of BG in combination with BCNU, temozolomide (TMZ) and Gliadel are in phase II/III clinical development. The potential long-term toxicities and mutagenicity associated with this combination are unknown; however, animal studies reveal a significantly greater mutation frequency in AGT −/− mice compared to vehicle treated mice following treatment with TMZ. In contrast, the mutation frequency of AGT −/− and AGT +/+ mice treated with BCNU was the same regardless of AGT status. BG does increase lung toxicity of BCNU when used in combination in mice. Ongoing clinical trials attempting to protect from enhanced toxicity and mutagenicity in bone marrow stem cells include expression of mutant AGT proteins that confer resistance to BG in the bone marrow. BG has also been found to enhance the cytotoxicity of agents that do not form adducts at the O6-position of DNA, including platinating agents. BG's mechanism of enhancement with these agents is not fully understood; however, it is independent of AGT inactivation and may be related to platinating agent transport. A better understanding of the effects of BG will contribute to its clinical usefulness and the design of better analogs to further improve cancer chemotherapy.
This work was supported by CA81485.

458 INVITED

Base excision repair inhibition: a new target for cancer therapy

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Base Excision Repair [BER] is an efficient DNA repair pathway for removal of single abnormal bases such as uracil, N7methylguanine, N3methyladenine and 8-oxoguanine. The first step of removal of these single base lesions is performed by specific glycosylases forming an abasic site, followed by the action of AP endonuclease to cleave the backbone,

polymerase beta to remove the sugar and insert the proper base, and DNA ligase. Chemotherapeutic methylating agents such as Temozolomide produce high proportions of N7methylguanine, and as we have recently shown [AACR, 2005], Flutarabine induces formation of abasic sites. For this reason, we evaluated Methoxyamine, an agent that binds to the aldehyde form of the deoxyribose, as an agent that could block processing by base excision repair and potentiate cell death by either Temozolomide or Fludarabine. In both in vitro models of various human cancer cell lines – colon cancer, leukemia and gliomas – and in xenograft models of these tumor types, concurrent Methoxyamine potentiated and synergized the efficacy of Temozolomide or fludarabine. Since Methoxyamine has a short plasma halflife, continuous infusion was also an effective approach to therapeutic synergy. In addition, although some cell lines were resistant to Temozolomide due to defects in mismatch repair [MMR], they were sensitized by Methoxyamine. This indicates that the BER pathway is independent of the MMR pathway. Thus, Methoxyamine is a promising agent that is now entering clinical trial to potentiate chemotherapeutic agents that induce DNA damage, in particular, abasic sites, recognized by the BER pathway. In addition, other agents targeting this pathway may be useful to provide another mechanism based approach to cancer therapeutics.

459 INVITED

Poly(ADPribose)polymerase inhibitors – the current clinical status

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Poly(ADP-ribose) polymerase 1 (PARP-1) is a nuclear enzyme involved in base excision repair, the DNA repair pathway recognising single strand breaks. PARP-1 is activated by binding to damaged DNA; polymers of poly(ADP-ribose) are formed on acceptor proteins, including PARP-1 – signalling recruitment of other BER proteins. A number of inhibitors of PARP-1 are in early clinical development for a range of indications, both within oncology and in wider medical practice.

There are preclinical models demonstrating both radio- and chemopotentiation, particularly of alkylating agents and topoisomerase I poisons, by PARP inhibition. The first PARP inhibitor entered early oncology trials investigating this. AG014699, a potent tricyclic indole PARP inhibitor (Pfizer GD), has completed phase I and II evaluation in combination with temozolomide. The phase I study was driven by a pharmacodynamic endpoint, and a PARP Inhibitory Dose defined. Profound PARP inhibition was shown both in peripheral blood lymphocytes (PBL) and tumour biopsies. A phase II study of the combination in metastatic melanoma demonstrated an 18% confirmed CR/PR rate with 40% of patients remaining on treatment for 6 months or more. No PARP inhibitor specific toxicity was seen in either study, however it was clear that temozolomide-induced myelosuppression was enhanced, and a significant number of patients on the phase II study required a dose reduction. There are a number of other PARP inhibitors in late pre-clinical development or early clinical development centred on the use of temozolomide in malignant glioma.

There is recent compelling pre-clinical data demonstrating single agent activity in cancer cell lines which are defective in DNA double strand break repair. This has most conclusively been demonstrated in BRCA1 or BRCA 2 homozygous cell lines. It is this potentially exciting single agent application that is the focus of development of the oral agent KU-0059436 (KuDOS/AstraZeneca). Phase I data reported at ASCO 2006 demonstrated PBL PARP inhibition and activity in a patient with familial ovarian cancer and plans are established to perform the definitive phase II in this indication with AG014699

In addition to the potential applications in oncology the ability of PARP inhibition to reduce cell necrosis in situations of extensive DNA damage such as septic shock, burns and ischaemia means that range of application could be vast, however at present development in oncology leads the field.

460 INVITED

ATM and DNA dependent protein kinase inhibitors

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An important determinant of the susceptibility of cancer cells to DNA damaging anti-cancer therapeutics is the ability of the cells to repair the DNA damage inflicted upon them. It has therefore been proposed that inhibition of DNA repair processes could lead to the potential therapeutic endpoints of radio- and chemosensitization. Since tumor cells in general are genomically unstable and have defects in the responses to DNA damage it has been argued (and proven in certain cases) that targeting DNA repair pathways may lead to a therapeutic index in tumor cells over "normal" cells. Two key kinases involved in the detection, signaling and repair of DNA double strand breaks (DSBs) are ATM (ataxia-telangiectasia mutated) and DNA-PK (DNA-dependent protein kinase). The serine/threonine protein kinase ATM responds to DNA DSB damage by signaling, *via* phosphorylation events, to key cell cycle and DNA-repair components. Mutation of *ATM* occurs in the human autosomal recessive disorder ataxia-telangiectasia (A-T), which is characterised by a hypersensitivity to ionising radiation (IR) and aberrant cell cycle control. The structurally related enzyme DNA-PK also responds to DNA DSBs and is intimately involved in the repair of DNA DSBs by the process of non-homologous end joining (NHEJ). Inhibition of ATM or DNA-PK activity could therefore lead to cellular radio- and chemo-sensitisation. Screening of a combinatorial library based on the non-specific PI 3-kinase inhibitor LY294002, has identified a small molecule ATP competitive inhibitor of ATM, 2-morpholin-4-yl-6-thianthren-1-yl-pyran-4-one (KU-55933) and an ATP competitive inhibitor of DNA-PK, termed NU7441. Both molecules show low nanomolar activity and are highly specific for the respective kinases. KU-55933 inhibits DNA damage signalling pathways and potentiates the cytotoxic effects of ionizing radiation and other classes of DNA DSB inducing agents. Similarly, the DNA-PK inhibitor also acts to potentiate the effects of IR and topoisomerase II inhibitors *in vitro* and *in vivo*. Our results to date support the further *in vivo* evaluation of these novel classes of molecules as chemo- or radiosensitizers prior to clinical evaluation.

Friday 10 November

Poster Sessions

Antimetabolites

461 POSTER

Early phase experience with pralatrexate (10-propargyl-10-deazaaminopterin [PRX]), a novel antifolate with high affinity for the reduced folate carrier, in patients with chemotherapy refractory lymphoproliferative malignancies

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Pralatrexate is an antimetabolite designed to have a much greater affinity for RFC-1. RFC-1 is the major transporter of both natural folates and folate analogs, and is an oncofetal protein whose expression is markedly increased by oncogenes like H-ras and c-myc, theoretically improving its therapeutic index. A single agent phase 1 and 2 study of pralatrexate has been conducted since 2001. Based on data in lung cancer, a dose of 135 mg/m^2 given on an every other week (w) schedule was used to treat 16 patients, including patients with Hodgkin's Disease (HD; n = 5), aggressive B-cell lymphoma (LBCL; n = 8), mantle cell lymphoma (n = 2) and one patient with peripheral T-cell lymphoma (TCL). A higher incidence of stomatitis (6 of 16 patients with Grade 3 or 4 stomatitis) was seen in patients with marked elevations in pre-treatment homocysteine (Hcy) and methylmalonic acid (MMA). Comparatively little to no increase in stomatitis occurred in patients with Hcy and MMA less than 10 microM and 200 nM respectively. Patients with elevated Hcy and MMA who developed stomatitis with pralatrexate did not develop advanced grade stomatitis after normalization of their Hcy and MMA with folic acid and vitamin B_{12} supplementation. In this experience, none of 15 patients with B-cell lymphoma experienced a remission, while one of 16 patients with T-cell lymphoma attained a PET negative CR after one dose. Population pharmacokinetic (PK) modeling established the importance of the nutritional covariates (Hcy and MMA) and PK parameters on toxicity, leading to a weekly phase I study. The dose escalation study started at 30 mg/m^2 weekly (w) $\times$3 every 4 w; progressing to 30 mg/m^2 w $\times$ 6 every 7 w, then increasing by 15 mg/m^2 on the 7 week schedule. The MTD was defined as 30 mg/m^2 w $\times$6 weeks on an every 7 week basis. To date, 31 patients have been accrued to the amended study, including patients with HD (n = 3); DLBCL (n = 6) and 22 patients with various sub-types of TCL. In total, including patients from the every other week and weekly treatment schedules, 42 patients with relapsed or refractory lymphoma have been treated. Of these, 8 complete remissions (CR) and one partial remission (PR) have been recorded, with all 8 CR occurring in patients with TCL (of which 11 of 22 are evaluable at this time [RR= 72%]), and the one PR in a patient with DLBCL (RR= 4%). The longest durations of response for the TCL patients have been 9 (panniculitic), 12 (T-cell ALL) and 16+ months (HTLV-1 ATLL). Normalization of Hcy and MMA pre-treatment with vitamins has completely abrogated the stomatitis, the major dose limiting toxicity (DLT). Little hematological toxicity (grade 2 thrombocytopenia in 2