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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.

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

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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.

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The importance of epigenetics in drug discovery and development

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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.
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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,