the resultant image of probe localization and magnitude (image intensity) is directly related to its interaction with a specific target (e.g., mRNA, receptor, protein or enzyme). "Indirect molecular imaging" is a little more complex, but it is currently the most widely used strategy. Most indirect molecular imaging paradigms involve the use of reporter-transgene technology and specific probes to produce an image that reflects reporter gene expression. The reporter gene is placed under the control of upstream promoter/enhancer elements. These promoter/enhancer elements can be "always turned on" with constitutive promoters (e.g., LTR, RSV, CMV), or they can be "sensitive" to activation by specific endogenous transcription factors (factors that bind to and activate specific promoter-enhancer elements). Several non-invasive imaging paradigms will be described that illustrate transcriptional regulation of endogenous (host tissue) gene expression.Non-invasive imaging of molecular-genetic and cellular processes will compliment established ex vivo molecular-biological assays. Imaging can provide a spatial as well as a temporal dimension to our understanding of various diseases. It is now possible to serially monitor molecular-genetic processes over time in the same subject, to assess such processes before and after a specific experimental intervention, to assess the effects and time-course of specific genetic alterations in transgenic animals, and to better assess treatment effects of new molecular-based therapies and drugs targeted to specific molecular or signal transduction steps.

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What the pharmaceutical industry wants from new imaging technologies for drug development

S. Galbraith, *Bristol-Myers Squibb, Clinical Discovery, Princeton NJ. USA.*

New imaging technologies offer exciting opportunities to learn about a drug's characteristics at earlier stages of development, saving both time and development costs. The 3 most frequent reasons for a drug to fail in development are - efficacy, safety and pharmacokinetics. Imaging technologies can provide information in all of these areas, which should enable earlier Go/No Go decisions to be made. Currently anatomical imaging measurements are used in late phase trials - e.g. MRI appearance of multiple sclerosis lesions or definition of tumor response in oncology, used as a surrogate for the clinical endpoint of change in overall survival. However there is great potential to affect decision making in early phase I and II trials, where the focus is more likely to be imaging of function, molecular mechanisms and pharmacokinetics. Demonstration that the drug does not hit its target or reach the target tissue would be a clear No Go for example. Another example is in the development of cytostatic drugs in oncology. The maximum tolerated dose may not be the optimal dose for Phase II so measurement of the change in tumor microvasculature, metabolism or proliferation could be used in dose and schedule selection. Response rates in Phase II have been used with cytotoxic agents as an indicator of efficacy, but if lack of progression rather than tumor shrinkage is expected from the mechanism of action then such measurements could also provide an alternative efficacy indicator, assessed earlier and with fewer patients than time to progression. In order for this potential to be realized several hurdles need to be overcome. Ideally the same techniques planned for early phase clinical trials should be used in pre-clinical models to compare dose response and time course of the imaging endpoint with dose response for anti-tumor efficacy. The more novel techniques are by their nature less standardized, with significant differences in methodology between centers even for such a widespread technique as FDG PET. There is frequently a lack of data on reproducibility between and within patients and sites and over the timepoints of interest. Image analysis methodology needs validation, with quality control of initial image acquisition. If data are to be shared across multiple sites there is a need for a centralized database, compatible with the different hardware and software at each site. Industry needs to work with academia to develop acceptable standards

Wednesday 20 November

WORKSHOP

Combinatorial chemistry

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Combinatorial methods for identifying antitumour kinase inhibitors

R.J. Griffin, *University of Newcastle upon Tyne, Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom*

Protein kinases have emerged as attractive targets for the chemotherapy of cancer, and targeting the ATP-binding site of kinases with small molecule competitive inhibitors has proven to be a viable therapeutic approach. However, a number of problems are associated with ATP site-directed inhibitors, including cellular permeability and selectivity for the target kinase. Fortunately, although the ATP-binding domain is highly conserved among protein kinases, structural variations in regions adjacent to this site offer opportunities for the design of kinase-selective inhibitors. The availability of high-resolution crystal structures of a large number of protein kinases has also enabled a structure-based approach to inhibitor design. The presentation will focus on the development of inhibitors of two classes of serine-threonine kinases, the cyclin-dependent kinases (CDKs) and DNA-dependent protein kinase (DNA-PK). The CDKs control cell cycle progression in proliferating eukaryotic cells and are therapeutic targets in cancer therapy. However, first-generation inhibitors lack CDK specificity and selectivity for individual members within the CDK family, and also potency against tumour cells both in vitro and in vivo. We have identified the O6-alkylguanines, exemplified by O6-cyclohexylmethylguanine (NU2058), as a novel structural class. An iterative crystal structure-based design approach, utilising fully activated CDK2/cyclin A, was used to identify NU6102 (O6-cyclohexylmethyl-2-[4'-sulphamoylanilino]purine) which is 1000-fold more potent than the parent compound NU2058. In addition to identifying optimal conditions for the preparation of this inhibitor class, multiple-parallel synthesis approaches have enabled a systematic variation of the substitution pattern on the 2-phenylamino group, with a view to optimizing physicochemical and biological properties. DNA-dependent protein kinase (DNA-PK) recognises and initiates repair of DNA double strand breaks produced by ionising radiation and certain drugs, and inhibitors may, therefore, have clinical utility in the treatment of cancer. A pharmacophore mapping approach has been employed to identify novel inhibitors which are more potent and selective than the benchmark PI-3 K inhibitor LY294002. The rapid development of structure-activity relationships for these new templates has been achieved by employing a multiple-parallel synthesis approach to prepare compound libraries bearing a diverse range of substituents.

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Bleomycin combinatorial libraries: a strategy for identifying mechanism of action and improved analogues

S.M. Hecht, *University of Virginia, Department of Chemistry, Charlottesville, USA*

The bleomycins are antitumor antibiotics used clinically for the treatment of several neoplasms, including squamous cell carcinomas and malignant lymphomas. The mechanism of antitumor action of bleomycin is believed to involve the cleavage of DNA, and possibly RNA. DNA cleavage requires the participation of oxygen and a redox-active metal such as Fe2+; oxidative cleavage occurs in a sequence-selective fashion. RNA cleavage is more highly selective than that of DNA and involves the recognition both of RNA sequence and three dimensional structure. In addition to oxidative cleavage in the presence of Fe2+ and O2, metal-free bleomycin can also mediate sequence-selective RNA cleavage by a mechanism involving phosphoryl transfer, i.e. a "hydrolytic" mechanism.
While the bleomycins are useful in the treatment of cancers, they do exhibit dose-limiting toxicities. In an effort to identify more effective, less toxic bleomycin analogues, we have devised a robust, solid phase synthesis of bleomycin that permits analogues to be prepared with remarkable facility. The analogues so prepared can be characterized for their polynucleotide cleavage properties prior to removal from the resin, consistent with the eventual preparation and assay of mix-and-split combinatorial libraries of bleomycins.

Presently, we describe the parallel synthesis of a library containing 108 analogues of deglycobleomycin and the results of an ongoing biochemical evaluation of that library. Also described is the solid phase synthesis of bleomycin A5 itself, as well as three bleomycin analogues altered within the carbohydrate moiety. The ability of these species to mediate DNA and RNA cleavage will be discussed.

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Natural product-based phosphatase and tubulin-polymerisation inhibitors

P. Wipf, *University of Pittsburgh, Department of Chemistry, Pittsburgh, PA, USA*

A major goal of our work is to demonstrate the potential of complex natural products to serve as biological probes of cell cycle events and as lead structures for anticancer drug development. Complex natural products-derived targets pose significant challenges for analog synthesis due to their structural diversity and the requirement for multi-step syntheses. In a collaborative effort, we have made significant progress in the application of combined solid phase - solution phase synthetic strategies for the development of biologically relevant Cdc25 dual-specificity phosphatase inhibitors and antimitotic agents. After several stages of iterative optimizations, we have identified submicromolar inhibitors in each series that exceed the potency and selectivity of the natural product lead structures that inspired the combinatorial chemistry library development. This talk will present our interdisciplinary approach in both areas with a focus on synthetic methods and summarize the new perspectives that we have gained in the attempt to condense distinct functionalities of the structurally diverse natural product leads.

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A combinatorial chemistry approach to gene targeting agents

D.E. Thurston, *University of London School of Pharmacy, CRC GTDD Research GRP, London, United Kingdom*

The ability to modify gene expression using small molecules should lead to novel therapeutic agents as well as providing the tools to carry out carry out functional genomics studies. For example, the down-regulation of key genes or families of genes in cancer, bacterial, viral or parasite cells should lead to novel anticancer, antibacterial, antiviral and antiparasitics agents.
In principle, gene down-regulation can be achieved by intervening at the DNA, RNA or protein level, and many marketed drugs work by interacting with a specific protein. More recently, effort has been put into targeting RNA with macromolecules, and some success has been achieved with antisense, ribozyme and RNAi approaches. However, there are significant difficulties in translating macromolecules with in vitro activity into therapeutic agents. For these reasons attention has been given to targeting the DNA template itself.
Targeting DNA to regulate gene expression has a number of advantages. The most significant advantage is that most cells contain only two copies of a given gene and successful blocking of transcription ensures that no further RNA transcripts are produced. This is an inherently more sensitive and efficient approach compared to antisense-type technologies where drug molecules and RNA transcripts need to be present in stoichiometric amounts for maximum down-regulation efficiency. Furthermore, the DNA template is left intact and is capable of producing more RNA transcripts.
Some success with gene targeting at the DNA level has been achieved with nucleic acids, proteins and small molecules. The development of small molecules for gene targeting has created much interest because, unlike macromolecules, they can have favourable cellular permeation and pharmacokinetic properties and can be developed as therapeutic agents.
This presentation will review recent advances in targeting DNA using small molecules and will include recent data from the author's own laboratory which uses a combinatorial chemistry approach to produce libraries of DNA-interactive agents.

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WORKSHOP

Altering the threshold of apoptosis

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Targeting mitochondria for apoptosis induction

G. Kroemer, *Institut Gustave Roussy, CNRS-UMR1599, Villejuif, France*

The supreme goal of anti-neoplastic chemotherapy is the selective eradication of cancer cells, which appears to depend on the induction of apoptosis, the cell's intrinsic death program. One major critical event (checkpoint) intergrating several apoptosis pathways is mitochondrial membrane permeabilization (MMP). MMP largely determines the point-of-no-return of the death process, and is triggered by chemotherapy, both *in vitro* and *in vivo*. MMP is subject to a complex regulation, and local alterations in the composition of mitochondrial membranes, as well as alterations in pre-mitochondrial signal-transducing events, can determine chemotherapy resistance. Detecting MMP may be useful for detecting chmotherapy responses *in vivo*. Moroever, chemotherapeutic agents may be designed to induce MMP by local effects on mitochondria. An alternative strategy for cell death induction consists in misdirecting apoptosis effectors normally sequestered in mitochondria (and normally only release after MMP) to the extra-mitochondrial compartment. Thus, for instance overexpression of AIF (apoptosis inducing factor) can enforce the induction of apoptosis in cells which are resistant to MMP.

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Novel small molecule inhibitors of Bcl-xL anti-apoptotic proteins

D. Hockenbery, *USA*

Abstract not received.

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Regulation of Bcl-2 family members during drug-induced apoptosis

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Proteins of the Bcl-2 family are critical determinants of the cellular threshold for apoptosis [1]. The importance of the pro-apoptotic proteins Bak and Bax to the engagement of apoptosis after drug-induced damage was demonstrated by the drug resistance of Bak/Bax double knock out cells [2]. Bax and Bak proteins require activation by cell damage-induced signals to trigger apoptosis. This process is thought to involve the participation of BH-3 only members of the Bcl-2 family such as Bid, Bim and Bad and is countered by the anti-apoptotic proteins of the family such as Bcl-2 and Bcl-xL.
We examined Bax activation following drug-induced damage in SH-EP 1 (glial-like) and SH-SY5Y (neurone-like) neuroblastoma (NB) cells are derived from the parental line SK-N-SH. In a clonogenic assay, both cell lines are sensitive to cisplatin, but only SH-EP1 cells are sensitive to Taxol. In SH-EP1 cells, Bax undergoes three changes prior to cytochrome c release and apoptosis induced by either cisplatin or Taxol. Step 1 is a conformational change at the N-terminus of Bax, Step 2 is the translocation of Bax from cytosol to mitochondria and Step 3 is Bax dimerisation at the mitochondrial surface [3]. Steps 1-3 of Bax activation also occur in SH-SY5Y cells after either drug yet cytochrome c is released from mitochondria only after cisplatin treatment and not after Taxol. We are currently investigating why Taxol resistant SH-SY5Y neuroblastoma (NB) cells fail to fully activate the pro-apoptotic protein Bax after Taxol treatment and which of the BH-3 only proteins play a role in Taxol and cisplatin induced apoptosis.
Bcl-2 family proteins also respond to signals derived within the cellular microenvironment. Our recent data show that 5 pro-apoptotic Bcl-2 family proteins (Bax, Bid, Bad, Nip3 and Bim) are down-regulated in several cancer cell lines under conditions of tumour hypoxia and that this correlates with