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.

23

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

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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

24

Combinatorial methods for identifying antitumour kinase inhibitors

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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.

25

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

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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.