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Preclinical and clinical toxicity correlations for cancer drugs developed by the NCI

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The development of therapeutic agents for the effective treatment of cancer has proven to be a challenging and difficult process. Currently, anticancer drugs are used clinically at or near the maximum tolerated dose (MTD) and, frequently, with significant toxicity. Therefore, preclinical toxicity studies are extremely important to establish the safety of the clinical starting dose and to identify the spectrum of drug-induced toxicities in appropriate animal models. In the US, the clinical starting dose for Phase I studies for cancer drugs is based on the MTD determined in the most sensitive of two preclinical animal models (one rodent and one non-rodent). The purpose of this analysis was to: 1) evaluate the "safety" of the clinical starting dose based on preclinical data; 2) to compare the resultant clinical and preclinical MTDs; and 3) to evaluate the accuracy of the dose-limiting toxicity (DLT) prediction based on preclinical animal models. This study includes the evaluation of the results for 38 small molecular weight compounds that the NCI has been involved in the preclinical and clinical development between 1983 and 2002. Biological modifying agents were not included in this analysis. This group of compounds included various mechanisms of action and dosing schedules. The clinical starting doses for all the compounds included in this analysis were safe, except for one clinical trial (fazarabine). Therefore, clinical starting doses based on the most sensitive of two species were safe for 98% of the clinical trials. However, for 49% of the clinical trials included on this study, the starting dose was too low resulting in an excessive number of dose escalations in the clinic. When the clinical and the preclinical MTDs were compared, neither rodents nor non-rodents predicted the MTD more accurately. However, the clinical DLT was predicted in at least one preclinical animal model for 70% of the drugs evaluated. The most common DLT was myelotoxicity (43%) followed by gastrointestinal toxicity (24%). Some types of toxicities weren't well predicted in either of the two preclinical animal models. In conclusion, preclinical toxicology studies provide a very high level of safety for cancer drugs entering clinical trials. However, this analysis suggests that additional types of studies may be necessary in order to balance safety and predictability while minimizing the length of clinical studies.

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WORKSHOP

Molecular imaging

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Measuring response to therapy with molecular imaging

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Molecular Imaging in oncology is defined as the non-invasive imaging of the key biomolecules that are important to cancer biology. Cancer researchers have discovered diverse genes and gene products, critical to cancer development and maintenance, and many of these have now been selectively imaged. Some of these molecules are themselves targets for the action of specific anti-cancer drugs, whereas other imaged molecules reflect more general biologic features of the cancer cell phenotype.
Molecular imaging is being used to monitor the response of prostate cancer to specific anti-cancer therapies. Androgen receptor (AR) is a key biomolecule that is important to the biology of the cancer. We have begun to study the expression of AR using 16b-[18F] fluoro-5a-dihydrotestosterone (FDHT). In a group of patients with progressive androgen independent prostate cancer, we have discovered that the majority of active lesions can be visualized with FDHT using PET imaging. The impact of the expression of the androgen receptor on response of individual lesions to AR targeted anti-cancer drug therapies is being explored. Most cancer cells have an accelerated glycolysis in comparison to the tissues from which they arise. The activity of hexokinase in the cancer cell is a rate-controlling enzyme in glycolysis. [18F] 2-fluoro-2-deoxy-D-glucose (FDG), is a tracer that is widely use to image the activity of hexokinase enzyme. We have found that FDG uptake, and by inference, glycolysis, is greatly increased in androgen independent prostate cancer. We are currently using FDG and PET imaging, to monitor the effect of hormono-chemotherapies on glycolysis of individual metastatic lesions in advanced patients with prostate cancer. Technical features of the imaging methods used includes measures of regional metabolism such as Standardized uptake values or SUV, as well as combinations of SUV with volume differences, a new functional imaging parameter which we have dubbed the "total lesion glycolysis" or TLG. Work performed to date supports the hypothesis that changes in FDG uptake are an early indicator of clinical response to anti-cancer regimens.
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What new pharmacokinetic information can molecular imaging provide us

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Poor pharmacokinetics is a major cause of drug failure and thus early assessment in the drug development process can reduce cost. Drugs labelled with long-lived isotopes such as carbon-14 have been used in the past to provide toxicokinetic information on new drugs in animals and humans. Such studies are based almost exclusively on analysis of blood and urine samples. Isotopic substitution with a positron emitting atom enables the pharmacokinetics in tumours and normal tissues to be studied by positron emission tomography (PET).
PET studies can be performed prior to conventional Phase I clinical trial of a compound. Chemical identity and purity of the labelled compound, as well as animal safety and dosimetry have to be established prior to these 'microdosing' studies in humans. The PET studies can also be performed at other phases of drug development. The following information can be obtained:

- First proof of hitting intended target
- First proof of intended mechanism of action
- Effect of biochemical & physiological modulators
- Early opportunity to change pharmacophore and evaluate selective accumulation of drugs into tumours (SAR)

PET has been used within the Cancer Research UK PET oncology group to study the tissue pharmacokinetics of temozolomide, DACA and 5-fluorouracil. In the case of temozolomide, drug delivery to brain tumours has been established and proof of mechanism of ring opening has been provided. A number of translational research questions were investigated during the Pre-Phase I trial of carbon-II-radiolabelled DACA. Tumour drug delivery and delivery to normal brain, myocardium and bone marrow were assessed. PET studies have also been performed during two Phase I trials to assess the effect of different schedules on tissue pharmacokinetics. PET has been used to demonstrate proof of principle of mechanism of action of eniluracil, an inactivator of dihydropyrimidine dehydrogenase. Eniluracil decreased hepatic and renal exposure of fluorine-18-radiolabelled 5-fluorouracil, and increased tumour exposure. Modulation of 5-fluorouracil tissue pharmacokinetics by biochemical modulators (interferon, PALA and folinic acid) and physiological modulators (carbogen/nicotinamide) have been studied in cancer patients.
PET pharmacokinetic studies can provide scientific feedback on the delivery and mechanism of action of new compounds, and thus, provide an early indication of weather a drug may be ineffective in tumours. Such studies can also predict for normal tissue toxicity.

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Molecular imaging of endogenous gene expression

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Molecular imaging has its roots in molecular and cell biology, as well as in imaging technology. These disciplines have now converged to provide a well-established foundation for exciting new research opportunities and for translation into clinical applications. The development of sensitive imaging-based assays to monitor molecular-genetic and cellular processes in vivo will be of value in the study of animal models of human disease (including transgenic animals), as well as for studies in human subjects.Three different noninvasive imaging technologies developed more or less in parallel: 1) magnetic resonance imaging; 2) nuclear imaging (QAR, gamma camera and PET); 3) in vivo optical imaging of small animals. Two imaging strategies - "direct" and "indirect" – are currently most widely used. "Direct molecular imaging" can be defined in terms of a probe-target interaction, whereby

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

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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

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WORKSHOP

Combinatorial chemistry

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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.

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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.