DISCOVERY AND DEVELOPMENT OF THERAPEUTIC COMPOUNDS

Organizer: Joseph Mike McCune

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In Vitro Studies Predictive of Toxicity and Carcinogenesis

X3-001 AN OVERVIEW OF IN VITRO TOXICITY ASSAYS, David W. Hobson, Battelle Memorial Institute, Columbus OH 43201-2693.

Historically, the process of discovery and development of therapeutic compounds principally employed animal models of various types for use in assessing the basic pharmacologic activity and toxicologic effects of new chemical substances and mixtures and in vitro preparations were employed principally to study biochemical an cellular aspects of the pharmacologic or toxicologic response observed in vivo. Although this is still the case for some therapeutics, increasingly, the selection of models for use in drug discovery and development studies now often includes a combination of animal and in vitro models based on the objectives of the particular evaluation, scientific relevance to the anticipated target in humans, an appreciation of the need to either evaluate or eliminate response complexity within the intact animal, cost effectiveness and expedience. Nevertheless, such in vitro procedures, while being quite commonly used to isolate and study particular aspects of the in vivo response, have only recently started to receive serious consideration as possible full or partial replacements for some of the traditional in vivo models used in drug discovery and development. Much of our present ability to view the potential contribution of in vitro models in a different fashion is in great extent the direct result of significant groundwork from past investigations conducted toward the isolation and understanding of organ-specific biochemical targets and the molecular biology of disease, toxicity, and therapeutic processes observed in the animal or human. A thorough understanding of the disease process, toxicologic mechanism, and desired therapeutic target in the animal continues to be essential for the development of successful in vitro procedures for routine use. The insertion of in vitro models of various types into pharmaceutical discovery and development programs is now rapidly expanding and may, in the near future, become a key element in reducing the time and costs required to identify promising new therapeutics. At present there is a large and growing literature on the development, evaluation and use of in vitro models for target specific activity screening, cytoxicity, histotoxicity, molecular pharmacodynamics, tissue isolated metabolism pharmacokinetics, etc., and some models are being examined for use as animal replacements in the initial toxicity assessment for some endpoints. Models available currently to evaluate, in vitro, the chemical toxicity utilize many different test systems, employ a variety of endpoints, and are at different stages of development and validation. For toxicity assessment, in vitro models are commonly used to assist in, (1) the selection or screening of in vivo test candidates, (2) the prioritization of compounds for more detailed testing or for risk assessment, (3) the investigation of activation or detoxification mechanisms, (4) the determination of tissue penetration rates or distribution characteristics. Due to increasing interest in the utilization of such models to reduce screening costs and developmental cycle time as well as for the reduction, replacement, and refinement of in vivo model utilization through the introduction of alternative in vitro procedures, the number of available models for various purposes is growing rapidly. Fortunately, a general classification of in vitro drug development models is possible on the basis of their level of biological complexity as being, (1) relational models, (2) biochemical models, (3) cellular models, (4) tissue models, or (5) perfused organ models. Further classification can be made on the basis of the type of toxicity evaluated, the class of compounds assessed, organ system affected, biomolecular targets present, etc. Specific examples of in vitro toxicity assessment models from each general class will be presented as well as examples of models used to identify and evaluate potential toxic effects from different types of therapeutics. Currently, the principal application of in vitro models to assess toxicity occurs in the discovery phase of the development process. Although proposed in the literature, in vitro models for use in preclinical toxicity evaluation have yet to receive any degree of universal acceptance by regulatory agencies. The difficulty in completely validating the relatively specific endpoints measured by in vitro models against the relatively broad range of effects possible in currently accepted animal models is a major problem in the development of in vitro models acceptable for use as animal replacements in preclinical tests. For discovery toxicity screening, however, significant reductions in the number of compounds screened can be made on the basis of validated in vitro toxicity assessment procedures.

Clinical Pharmacology and Interface Between Pre-Clinical and Early Clinical Studies

X3-002 ASSESSMENT OF BIOLOGICAL ACTIVITY OF DRUGS, Joann L. Data, The Upjohn Company, Kalamazoo, MI

The assessment of a number of biological activities of a compound is an inherent part of the drug development process. At the drug discovery phase several desired activities are used to select lead candidates for further evaluation in more complex and ideally more relevant models of the desired activity of the compound. Other biological activities are assessed to determine if the leads selected have any liabilities that might preclude ultimate utility of the compound as a drug. During this phase of the drug development process the challenge is the relevancy of the biological response assessed to the ultimate use of the compound in human disease. Receptor binding assays are useful tools for screens but can not be used alone as the sole criteria for compound selection. Pharmacologically modified preparations, from tissues to whole animals, again are useful evaluation tools but can prevent other normal physiological responses from occurring that could prevent the compound from working naturally or prevent a liability from being seen.

Animal safety and animal toxicology studies are used to assess various other biological activities, desired as well as undesired that the molecular species might have. This safety assessment assists in the clinical introduction of the identified molecular species.

Now the controversy on the use of the assessment of biological activity begins. For certain types of agents there is a tight correlation between response measured and ultimate drug utility or impending toxicity. This is particularly true for cardiovascular agents used to modify hemodynamic responses or platelet anti-aggregatory agents. For other classes of agents a given biological response has a totally unknown correlation with ultimate compound utility. Here the evaluation can give the researcher evidence of drug absorption and tissue activity which then can be correlated with kinetics as well as safety. The duration of the activity may also be useful in selection of dosage schedules in future clinical trials. Caution must be placed on over interpretation of the findings in the biological assessment. If the marker selected is not tightly correlated with the efficacy desired, incorrect dose selection or dose frequency may occur. As one progresses further in the drug development process, evaluation of biological activity may be used to determine early potential responders or non-responders, to further evaluate potential liabilities of the product or to make dosage adjustments on certain trial participants.

It is important to put the biological assessment in context. Equally important is to not incumber early safety evaluations of the compound in man with too many biological assessments. The more that is known about both the disease state to be modified as well as the characteristics of the molecular entity, the greater the use of a given biological assessment. Unfortunately, often this type of correlation does not become evident until many compounds of a given class have been evaluated or a given drug has been used for many years. Biological assessments are tools that help construct the pathway of drug development. They must be used correctly to provide relevant information as the process progresses. Appropriate use can be powerful. Misuse can be devastating. Care of the researcher to put the findings in context all during the process will provide the best information on which good decisions can be made in timely fashion. This information can provide a good understanding of the drug's potentials and liabilities as it moves to greater use and utility.

X3-003 MANAGEMENT OF EARLY DRUG STUDIES IN MAN, Robert E. Desjardins, American Cyanamid Company, Medical Research Division, Pearl River, NY 10965.

Knowledge about the relationship of the pharmacokinetic profile of a drug to its pharmacologic and toxicologic properties in preclinical studies facilitates the design and improves the efficiency of early drug studies in man. Similarly, the application of these principles to clinical evaluations of drug safety and activity facilitates progression of the drug development process. The resulting chief benefit is minimization of the risk of costly errors in design of the sequential phases of drug evaluation in man. As the clinical development process advances the costs rise substantially. It is, therefore, cost-effective to incur the expense of obtaining relevant pharmacokinetic and pharmacodynamic data early in the drug development process to reduce the risk of delays associated with errors in later phases of the process. It is also important to reduce the risk of unexpected and unwanted results in later clinical trials.

While the extent to which these principles apply may vary with the therapeutic category, it is generally true for most classes of drugs. Its applicability is intuitively obvious in the case of drugs for which there is a temporal association of plasma concentration and a measurable effect. However, where this is not the case, it is often possible to demonstrate a relationship between pharmacokinetic parameters of drug exposure and temporally distant but relevant surrogates of activity or toxicity.

Effective application of these principles is a multidisciplinary effort. A high level of cross-functional integration is required. This will occur best in an organization that actively promotes a culture of teamwork and cooperation.

New drug development in the pharmaceutical industry occurs today in a highly competitive climate. The predominant inclination of management is to reduce cycle time which often creates pressure to minimize the time and resources spent in early stages of drug evaluation and to proceed to advanced stages as quickly as possible. This may be in conflict with the objective of rational drug development guided by pharmacokinetic and pharmacodynamic principles.

Three essential elements for successful application of these principles are therefore: (1) knowledge in all relevant disciplines of the technology, (2) organizational commitment to teamwork, (3) managerial understanding and support.

X3-004 INTERSPECIES TOXICOKINETIC SCALING. Joyce Mordenti, Pharmacokinetics, Department of Safety Evaluation, Genentech, Inc., South San Francisco, CA 94080 USA

Experience with multiple biomacromolecular products indicates that the pharmacokinetic behavior of many macromolecules is predictable across species. This information permits extrapolation of preclinical safety and efficacy data to the clinical setting on the basis of pharmacokinetic equivalence rather than on a body weight (mg/kg) basis. With a better understanding of this cross-species relationship, the confidence in the safety of a therapeutic agent in initial clinical studies is increased. This presentation will use actual case studies to illustrate how allometric scaling techniques are used to establish safety factors for Phase I doses. Several routes of administration (intravenous, inhalation, and topical) will be discussed.

X3-005 CONCENTRATION DRIVEN EARLY CLINICAL STUDIES, Carl C. Peck, CDER, FDA, Rockville, MD

The primary goal of drug development is the establishment of a scientific database that supports the effectiveness and safety profiles of dosage regimen(s) proposed for use in individual patients. To this end, the efficiency and informativeness of a drug development program may be enhanced by integration of pharmacokinetics (PK) and pharmacodynamics (PD), especially in early clinical studies. The scientific rationale for integration of PK/PD early in drug development derives from the need to understand and control the sources of variability that link dosage regimens with the time course of clinical effects. PK/PD provides the unifying framework for controlling variability of response by providing the necessary scientific linkages among the key components (dose and dosage form, patient compliance, bioavailability/distribution/metabolism/ elimination, and drug concentration-effect characteristics).

Although concentration-oriented drug development was demonstrated 50 years ago in a national effort to develop new antimalarial medications, this approach seems to have been ignored in lieu of an empirical, dose-oriented drug development strategy. Only recently has a concentration-oriented approach been reemphasized.

Opportunities for integration of PK/PD in drug development have been described in a report emanating from a 1991 conference sponsored by AAPS, ASCPT, and FDA (Clin Pharm Ther 1992; 51:465-473.) Included in the report are suggestions for derivation of PK/PD data in preclinistudies and a strong emphasis on importance of Phase 1 PK/PD studies. The primary motivation for incorporation of PK/PD in early clinical studies is to enable efficient characterization of dose-response in Phase 2. PK/PD data derived in Phase 1 can be used in simulation studies to guide Phase 2 clinical trial designs.

In Vivo Studies Predictive of Efficacy-I

X3-006 PRECLINICAL EVALUATION OF IMMUNOSUPPRESSANT AGENTS, Franco E. Di Padova, Peter R. Wenner, Jean F. Borel, Preclinical Research, Sandoz Pharma Ltd, CH-4002 Basel.

The goal of immunosuppression would be to induce Ag specific tolerance i.e. high selectivity of action in the absence of side effects and maintenance treatment. At present this goal has not been achieved and different therapeutic protocols are used in various clinical conditions such as allotransplants or autoimmune diseases. Even if the introduction of Cyclosporine in the last ten years has made more selective immunosuppression possible, presently used therapeutic protocols still include drugs or procedures which are non-selective and mainly antiproliferative. To improve immunosuppressive treatments new compounds are under development by pharmaceutical companies. For the future it can be foreseen that xenotransplantation will represent another challenge for immunosuppression.

For the discovery of immunosuppressive drugs, the contribution of both in vitro and in vivo research is mandatory. Schematically, in vitro activities are focused on the screening, on the elucidation of the mechanism of action and on structure-activity relationships. High capacity screening is vital for the discovery of new compounds and the type of test systems are largely dependent on the defined target. Biological phenomena, such as T-cell or B-cell proliferation to various stimuli, offer reliable screening systems. Reporter gene assays represent further refinements of the screening process because they identify compounds specifically interfering with signal transduction pathways or gene expression. Once a novel mechanism of action is identified, a new screening approach specifically targeted to the newly discovered biochemical pathways may be developed. Moreover the search for nonconventional sources of immunosuppressive drugs and the proper handling of information are essential for the finding of new leads. Because presently available immunosuppressants range from steroids to fungal metabolites, empiricism is mainly directing the early phases of the discovery process.

Once a new lead structure is found, the elucidation of its mechanism of action and its chemical derivation with the aim to obtain more active compounds are the result of coordinated efforts among pharmacology, chemistry and drug design.

Evaluation of immunosuppressive activity, pharmacodynamics, toxicity and pharmacokinetics is done in vivo. Short-term and simple in vivo models in rodents are preferred for gross evaluation. More sophysticated and time consuming models for which particular technical skills are required (e.g. transplantation of vascularized organs) are reserved for later work phases. Short term toxicity models are crucial for revealing early potential major side effects. Monitoring of drug blood levels is helpful in defining the relationship between immunosuppressive activity and toxicity. In the future, new means for immunosuppression which may be different from traditional drugs may become available. The in vivo models, presently used, might not be suitable to study the action of such new molecules which might be species specific in their mode of action. For example, human MHC-T cell receptor interactions cannot be assessed in presently available animal models and a combination of molecular engineering and transgenic technology is crucial for the development of the appropriate models.

It is foreseeable that the refinements of our knowledge of the immune system, a combination of empiricism, rational approach, chemistry and serendipity and new high capacity screenings may allow the pharmaceutical companies to develop more effective, more specific and less toxic immunosuppressants.

X3-007 DIFFERENTIAL DRUG RESPONSES *IN VIVO*, Øystein Fodstad, Knut Breistøl, Arne T. Myklebust, Gunhild Mælandsmo, Eivind Hovig, and Inge Kjønniksen, Department of Tumor Biology, The Norwegian Radium Hospital, Oslo, Norway.

In the management of cancer patients it is well known that tumor metastases residing in different organs may differ considerably in their response to chemotherapy. To study this problem, the LOX human malignant melanoma was grown in various sites in athymic, nude rats and the response to chemotherapeutic agents with different mechanisms of actions was assessed. A significant site-dependent differential response to two alkylating agents, but not to doxorubicin and cisplatin, was found. Thus, bone metastases were relatively resistant to dacarbazine and mitozolomide, drugs that were highly active against lung and s.c. tumors. A number of possible mechanisms underlying these differences were investigated. Evidence was obtained suggesting that interaction between the tumor cells and the microenvironment in the host tissues might have influenced tumor responsiveness at the cellular level. No difference between site of growth was seen in tumor cell expression of the mdr1 or GST-π proteins, as assessed by immunostaining. Studies are under way to examine whether LOX tumor cells in the bone marrow may have been induced to express higher levels of O^6-methylguanine-DNA methyl-transferase (MGMT), as cells lacking expression of this gene are known to be very sensitive to alkylating agents. A sensitive, semi-quantitative PCR method is being developed to measure MGMT mRNA levels in LOX cells isolated from the different sites of growth. In pilot experiments, indications have been obtained that the chemosensitivity of intracranially growing LOX cells may also be affected by microenvironmental factors. Experiments have been initiated to study whether differential responses similar to those observed with LOX cells might be present for other tumors and for other drugs. It is concluded that human tumor models in athymic rodents provide new opportunities for studying *in vivo* tissue-dependent factors affecting drug activity at the cellular level.

X3-008 HISTOCULTURE AND THE IMMUNODEFICIENT MOUSE COME TO THE CANCER CLINIC, Robert M. Hoffman[§£], [£]AntiCancer, Inc. 5325 Metro Street, San Diego, California.[§]Laboratory of Cancer Biology, University of California, San Diego 0609F, La Jolla, California 92093-0609.

The 'MetaMouse' model developed by us allows direct 'onplantation' of histologically-intact patient surgical cancer specimens orthotopically to immunodeficient 'nude' and SCID mice by microsurgical techniques with subsequent high-level expression of local growth on the target organ and high metastatic potential. Nine MetaMouse human cancer models have been developed including those for the colon, bladder, lung, stomach, prostate, ovary, pancreas, breast, and head and neck. The human tumors growing and metastasizing in the mice correlate with the clinical course of the tumors in the actual patients. Studies on chemotherapy in the MetaMouse models indicate that the response of the locally-growing tumors may be different from their metastases. The MetaMouse approach should be useful for new drug evaluation on local tumor growth and on metastatic growth itself and for development of strategies for individual treatment. The MetaMouse approach thus offers a "patient-like" model for preclinical evaluation of experimental and standard cancer therapy and diagnostics.

X3-009 PRE CLINICAL MODELS OF ALZHEIMER'S DISEASE, Ivan Lieberburg, Athena Neurosciences, Inc., South San Francisco.

Alzheimer's disease (AD) is a chronic, fatal dementing illness of the elderly characterized by loss of cognition, agitation, depression and paranoia. The average patient lives for eight years after the onset of symptoms which invariably progress to a profound vegitative state followed by death. Some 3-4 million Americans and 17-25 million people worldwide are afflicted with AD. It is the fourth leading cause of death in the U.S. preceded only by heart disease, cancer and stroke. As the population ages this devastating illness promises to become a highly significant medico-economic problem. At autopsy, the brain of the AD patient is characterized by dramatic loss of neurons and synapses in association cortical areas, a substantial reduction of a wide number of neurotransmitters including but not limited to acetylcholine, norepinephine, serotonin, dopamine, somatostatin and glutamate, and large numbers of characteristic deposits called amyloid plaques and neurofibrillary tangles. Unfortunately, from the stand point of drug discovery, AD is a uniquely human disease. Even higher primates do not manifest this disorder. Thus, animal models of the disease mimic only certain discreet aspects of AD pathology or neurochemistry. The most popular and best described animal models include (1) the fimbria-fornix lesion-induced loss of forebrain cholinergic activity (2) excitatory amino acid-induced neuronal death (3) behaviorally impaired aged animals (4) transgenic and homologously recombinant mice and (5) models of amyloid production. Discussion will center on the logic of each of these various models and their utility in mimicking the disease for drug discovery purposes.

In Vivo Studies Predictive of Efficacy-II

X3-010 DEVELOPING NEW AGENTS FOR TREATMENT OF CHILDHOOD CANCER, Peter J. Houghton[1], Clinton F. Stewart[1], William H. Meyer[1], Marc E. Horowitz[1], and Janet A. Houghton[1], [1]St. Jude Children's Research Hospital, [2]Pediatric Oncology Branch, NCI.

In the past decade, relatively few new agents have been introduced into the armamentarium for treatment of childhood solid tumor. Development of aggressive therapy using ineffective agents has, in many instances, led to increased toxicity without resulting in benefit to the individual patient. Clearly, there is a need to identify new agents that have significant efficacy, and to identify new targets against which effective inhibitors can be developed. The mechanism by which new agents effective against childhood malignancies are identified, however, presents formidable problems. We have shown that classic phase II trials (against relapsed tumors) may fail to identify drugs that would have significant activity if used at diagnosis. The problem is compounded by relatively few patients, and, in many cases, potentially curative therapy that compromise the process of evaluating new agents and introducing these into clinical management. To circumvent these problems, we have tested the value of human tumor xenografts as preclinical models for identifying active agents in a retrospective and prospective manner. Models of childhood rhabdomyosarcoma (RMS, a highly chemosensitive tumor, model A) obtained at diagnosis, or at relapse (having acquired resistance, model B), and colon adenocarcinomas (intrinsically resistant, model C) have been heterografted into mice. Results show that tumors in model A are sensitive to agents known to have potentially curative activity in RMS (vincristine, actinomycin D, doxorubicin, cyclophosphamide), whereas tumors in model B are significantly less sensitive, and resemble model C tumors when clinical criteria of partial and complete regressions are used to evaluate efficacy. In a prospective mode, model A has identified melphalan and ifosfamide as highly active, and both agents have yielded >80% responses (CR + PR) as single agents in diagnosis RMS. These models have identified several new classes of antitumor agents that have high therapeutic efficacy against model A tumors. Of these, the inhibitors of topoisomerase I appear to have very significant activity, and are entering phase I evaluation in pediatric patients. The use of xenograft models together with preclinical and clinical pharmacokinetic and pharmacodynamic modeling appears to provide a novel and effective approach to introducing potentially highly effective agents into clinical trial, and allows evaluation of new single agents in an "up-front" window in previously untreated patients with advanced disease and poor prognosis.

X3-011 MODULATING ATHEROGENESIS BY MANIPULATING APOLIPOPROTEIN GENES IN MICE, Edward Rubin, M.D., Ph.D. Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720

The many genetic components contributing to atherosclerosis in humans have been difficult to untangle, in part due to the lack of genetically defined model systems. To test the suitability of the mouse as an assay system for the genetic determinants of atherosclerosis in humans, we have over-expressed several human genes involved in lipid transport [apoAI, apoE, and apoAII] in mice and assessed atherogenesis. In humans, high plasma levels of apoAI are associated with decreased atherosclerosis, although whether this is a direct or indirect association has been difficult to test. Using a quantitative assay, we observed that high levels of human apoAI dramatically decrease murine atherogenesis supporting a direct role for apoAI in preventing atherosclerosis. Due to the role of apoE as a ligand in the receptor-mediated clearance of lipoproteins, it has also been hypothesized that increasing levels might inhibit atherogenesis. To test this we compared the extent of preatherosclerotic lesion formation in transgenic mice expressing human apoE at various levels. We determined that increasing levels of human apoE was associated with decreased diet-induced atherogenesis in the apoE transgenic animals compared to the control animals.

We next investigated the less defined relationship between human apoAII and atherosclerosis. Plasma HDL is found in humans which contains both apoAI and apoAII (AI w/ AII), as well as that which contains apoAI without apoAII (AI w/o AII). Because it is difficult to study the properties of these two major forms of HDL independently in humans, little direct evidence exists as to whether the presence of apoAII affects HDL's antiatherogenic properties. Our studies compared two lines of transgenic mice with similar levels of HDL, but whose HDL differed in apolipoprotein composition. One group of animal's HDL contained primarily human apoAI, while the second group of animal's HDL contained both human apoAI and human apoAII. Our results indicate that HDL containing apoAII has significantly less antiatherogenic properties than HDL free of apoAII, providing the first direct *in vivo* demonstration that the apolipoprotein composition of HDL determines its antiatherogenic properties. These studies demonstrate that experimental mouse genetics can provide new avenues for examining the multiple genetic elements which contribute to atherosclerosis in humans.

Clinical Trials-I: Advantages, Limitations, and Applicability of Large Simple Trials

X3-012 THE POTENTIAL UTILITY OF LARGE, SIMPLE TRIALS IN AIDS DRUG DEVELOPMENT, Susan S. Ellenberg and Mary A. Foulkes, National Institute of Allergy and Infectious Diseases, Bethesda, MD.

Clinical trials in which investigators have attempted to enter unusually large number of patients, use minimally restrictive entry criteria, and collect data only on essential baseline characteristics and the outcome of primary interest have come to be known as "large, simple trials." Such trials have been extremely successful in Europe, especially in the area of coronary heart disease. In general, the purpose of large, simple trials is to establish with great credibility the public health consequence of a particular medical intervention that is, or might come to be, widely used. One needs a large trial to achieve a precise estimate of the treatment effect, ensuring that a modest but still important benefit (or harm) will be identified; one needs a simple trial so that it can be successfully carried out in the wide variety of settings necessary to obtain the required patient numbers.

We consider the potential application of this methodology to the evaluation of treatments for HIV/AIDS. With about one million individuals infected with HIV in this country alone, all or nearly all of whom are expected to succumb eventually to AIDS, modest improvements in therapeutic efficacy may well be worthwhile. Large trials may also establish the acceptability of less intensive therapeutic approaches, e.g., lower dose levels or less invasive routes of administration.

One setting in which the large, simple trial may be especially useful in evaluating therapeutic approaches for AIDS/HIV is "expanded access" or "parallel track" programs, in which patients failing on or no longer able to tolerate standard therapies are provided access to promising experimental agents prior to completion of studies addressing therapeutic efficacy. Such programs could provide access in the context of simple randomized trials comparing two or more dosage levels of the new drug. This approach would be advantageous to the pharmaceutical sponsor, in that it could serve as additional evidence in support of an application and/or as guidance with regard to optimum dose level, without any disadvantage to the community soliciting access to the drug. Such trials could be carried out with only marginally greater complexity than a non-randomized distribution program.

One concern about the use of large, simple trial is the "noise' factor; that the heterogeneity of the patient population, the lack of possibly complex procedures to confirm eligibility, endpoints, etc., the focus on objective endpoints potentially subject to more of a dilution effect (e.g., all-cause mortality), and the absence of intense monitoring procedures to ensure accuracy of data, may obscure effects that might be seen in a more traditionally conducted trial. This problem may be of special concern in studies of AIDS drugs because of potentially excessive compliance problems, the enormous variability of relevant laboratory measures, and the lack of clearly meaningful and objectively determinable endpoints other than mortality. A quantitative assessment of the potential effects of such factors on trial results will be presented.

Clinical Trials-II: Surrogate and Other Non-Ultimate End Points

X3-013 JUDGING THE EFFICACY OF ANTICANCER AGENTS - RESPONSE RATES, QUALITY OF LIFE, AND SURVIVAL, Michael A. Friedman, M.D., Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, 6130 Executive Boulevard, Room 742, Bethesda, Maryland 20892, U.S.A.

Historically, two criteria have satisfied the FDA in the demonstration of effectiveness of a new anticancer agent -- increased survival or improved quality of life (QOL). However, because of the subtlety of the disease process and biologic heterogeneity, new agents of dramatic effectiveness have not been frequently identified.

The most convincing evidence of effectiveness is long term, disease-free survival. For patients with advanced cancer, this goal has proven elusive -- the exceptions being rare, responsive diseases like testicular cancer and hairy cell leukemia (HCL). New agents inducing complete, long lasting remissions are readily approvable. Deoxycoformycin and 2-chloro-deoxyadenosine are obvious examples for HCL, as are ifosfamide and VP-16 for testicular cancer. However lacking such dramatic impact, other endpoints must be utilized.

In this regard, impermanent regressions of disease may be considered important. Complete regressions (CR) usually have considerable clinical meaning and may serve as surrogate for improved QOL. For advanced ovarian and renal cancer, such provide the basis for FDA approval. For ovarian cancer, CRs are clearly associated with superior survival time. Unless there is countervening acute toxicity, it would be counterintuitive to think otherwise. Following this line of reasoning, the value of objective partial remissions should be reevaluated. Often such (PRs) have net positive benefits -- improving physiologic or psychologic status. Decreases in the size of intracerebral neoplasms or diminished malignant ascites are highly worthwhile. However, the imprecision of measuring partial regressions and the chance for observer error complicate these considerations. Currently, there are an unprecedented number of new agents available for evaluation. In addition to cytotoxic species, there are agents which modulate, enhance or diminish physiologic effects of anticancer agents. The number of possible combinations with established and new agents), sequences, doses, routes, schedules, etc. is vast. The possibility of placebo controlled trials or of studies that prohibit so called "cross-over" therapy is small. Studies with large sample sizes to detect the modest but real contribution of a new agent to a standard therapy have obvious disadvantage (although the ultimate need for such studies is undiminished). More creative cooperation between sponsors, performing and regulators of oncology trials should lead to more efficient and accessible study endpoints.

Economic Models for Introduction of New Therapeutics

X3-014 THE RISING COST OF NEW DRUG DEVELOPMENT, Joseph A. DiMasi, Center for the Study of Drug Development, Tufts, University, Boston.

The costs, risks, and duration of new drug development are matters of concern to pharmaceutical firms, regulators, and policy makers. A recent study[1] documented how high new chemical entity (NCE) costs have become. For a stratified random sample of 93 NCEs tested in humans for the first time during 1970-1982 by 12 U.S. pharmaceutical firms, the pre-tax cost per approved NCE was estimated to be $231 million (in 1987 dollars). The cost estimate includes full allocation of the costs of research failures to the costs of the successes (approved NCEs) and capitalization of all costs to the point of marketing approval (at a 9% inflation-adjusted discount rate). In comparison to the results of a study that used a similar methodology and covered a period that is nearly a decade earlier, NCE development cost (in constant dollars) was seen to more than double. The process is lengthy as well as costly. The average time from synthesis to marketing approval was estimated to be approximately 12 years - three and one-half years in preclinical development, six years in clinical development, and two and one-half years in regulatory review.[1] Quantifying R&D costs for new drugs is useful for a number of reasons. The incentive to undertake new drug development is related to the cost of drug development. Thus, other things being equal, higher R&D costs can adversely affect the flow of new therapies available to patients. Studies of the profitability of NCE development can therefore be made more accurate with good estimates of NCE development costs. R&D cost can also affect how industrial innovation will be structured. Rising R&D costs may, for example, have contributed to a trend toward consolidation in the pharmaceutical industry. Finally, documenting that R&D costs are high and rising makes it imperative that we try to answer questions about the drivers of new drug development costs. We need to ask if high development costs are due to overly stringent regulation, to inefficiencies in the pharmaceutical industry, to a focus on more costly areas of research, or some combination of these and other possibilities.

[1]DiMasi, JA, Hansen, RW, Grabowski HG, Lasagna L, Cost of Innovation in the Pharmaceutical Industry, Journal of Health Economics 10: 107-142, 1991.

Opportunities and/or Obstacles in the Development of New Technologies

X3-015 COOPERATIVE MANAGEMENT OF REVOLUTIONARY TECHNOLOGIES: THE ANTISENSE EXPERIENCE, Stanley T. Crooke, Isis Pharmaceuticals, Carlsbad, CA

Antisense technology may represent a revolution in therapeutics. Because it focuses on an entirely unexplored class of chemicals with unique mechanisms of action, it poses particularly difficult development and regulatory issues.

As the medicinal and process chemistries of oligonucleotides are in their infancy, the drugs are costly, difficult to synthesize, precious and the number of analogs is increasing exponentially. Consequently, proof of structure, purification, determination of stability and formulation represent significant research exercises.

Antisense oligonucleotides are species specific. This creates major issues in the demonstration of in vivo activities and in the evaluation of their toxicologic profile.

As methods of purification and analysis are relatively primitive, pharmacokinetic studies are still difficult and determination of metabolic patterns virtually impossible.

Thus, the rapid progress in converting this novel technology to drugs in development is gratifying, but results in numerous issues that require prudent cooperative interactions between the sponsoring company and the FDA.

X3-016 DEPLOYMENT AND VALIDATION OF NEW TECHNOLOGIES FOR DRUG DEVELOPMENT. David W. Martin, Jr., M.D.

Over the last 15 years some remarkable technological breakthroughs have occurred in the life sciences led, of course, by the development of recombinant DNA technology. Most of these new technologies are deployed in research endeavors, including those aimed at the discovery of new pharmaceutical agents. They have contributed significantly to the quality of the potential product and its accelerated discovery. However, the deployment of novel technologies for the development of new drugs has not been so apparent, even though applications are not difficult to imagine. A major obstacle has been the necessity of validating novel technologies before they can be accepted by regulatory authorities throughout the world. For example, the use of specific transgenic animals to assay *in vivo* mutagenic properties of a drug substance or immunogenicity of biotech products offers potential advantages over the current assays. Yet the use of the novel transgenics has not been validated so as to enable a development organization to submit such data in lieu of the *in vitro* or primate data. Similarly, new techniques to detect generally toxic effects of substances on cultured human cells offer to save time, resources and animal exposure. To be useful, such systems must also be validated. The expectation is that the sponsoring company must validate the system and probably submit data from the new system along with the traditional data, so called "traditional plus additional" approach. Clearly, this discourages the development and deployment of novel technologies for the drug development process.

Several other examples will be discussed during the presentation, and a specific proposal will be made to encourage regulatory agencies to validate some exemplary systems so that the novel technologies benefit not only other regulatory agencies but also the U.S. Pharmaceutical Industry in general.

Regulatory Perspectives/Principles for Evaluating New Methodologies-II

X3-017 A LEGAL PERSPECTIVE ON REGULATORY REQUIREMENTS AFFECTING THE DISCOVERY AND DEVELOPMENT OF THERAPEUTIC COMPOUNDS, Peter Barton Hutt, Covington & Burling, Washington, D.C.

Congress established a system of premarket approval for biological drugs in 1902. All other drugs were made subject to government policing in 1906, to premarket notification in 1938, and to our present system of premarket approval in 1962. In none of those statutes, however, has Congress sought to limit the discretion of FDA in determining appropriate standards for drug approval. Faced with constant criticism from Congress and in the public media resulting from drug tragedies, FDA has in fact adopted an extremely conservative and risk adverse to drug approval. This means that patients who need these drugs must wait longer, and indeed that some drugs will not be developed at all. The only exception has been AIDS drugs. Several reports have urged reform in the new drug approval system, but there is no indication that FDA has changed its attitude and approach to this matter or is likely to do so in the future.

Late Abstracts

THE SCID-hu MOUSE AS A TOOL FOR PRE-CLINICAL EVALUATION OF ANTIVIRALS

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The SCID-hu mouse model was developed, in part, to serve as a preclinical *in vivo* model for discrimination of antiviral compounds against HIV which are shown to have *in vitro* activity. Constructed by transplantation of interactive human lymphoid organs into the immunodeficient C. B17 *scid/scid* stock, the SCID-hu mouse may be infected by HIV in a time and dose-dependent manner. Standardized, low passage number viral stocks have been prepared from culture of a number of HIV isolates in PHA-activated human T cell blasts. Prior to use in animals the viral inocula are characterized by p24 content and $TCID_{50}$ assay for biological titer. Intrathymic inoculation of the molecularly-cloned HIV isolates JR-CSF, NL-4 and Xho as well as a number of clinical isolates was found to result in infection of the human thymus engrafted into the SCID-hu mouse. Infection can be detected by p24 ELISA, DNA PCR, viral isolation, and cellular RNA PCR at early times post-inoculation and later by deletion of CD4+ thymocytes. Administration of 3'-azido-3'deoxy-thymidine (AZT) or dideoxyinosine (ddI) prior to intravenous infection of HIV into SCID-hu mice with transplanted human thymus resulted in suppresssion of infection. Continued studies in HIV-infected SCID-hu mice may facilitate both the discovery of effective antiviral compounds, the value of combinations of drugs relevant endpoints for evaluation of efficacy in humans.

SURROGATE ENDPOINTS IN TRIALS OF ANTIHYPERTENSION, Henry R. Black, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612

Though often not appreciated, the reason we treat hypertension is not merely to lower the numbers but rather to reduce the mortality and morbidity attri- butable to elevated levels of blood pressure. Thus, we should interpret the success or failure of antihypertensive therapy, not by whether the drug reduces blood pressure (a surrogate end point) but rather by whether treatment reduces clinical end points such as mortality from any cause, cardiovascular mortality and cardiovascular morbidity (coronary artery disease, cardiovascular disease, renal disease, congestive heart failure and other related conditions.)

Recent analysis of long term epidemiologic data and the results of clinical trials have suggested that the reduction in blood pressure achieved with treatment are not necessarily accompanied by the expected reduction in clinical end points. For cerebrovascular disease, for example, the 5-6 mm Hg reduction in diastolic blood pressure (the average level obtained 14 large non-industry sponsored clinical trials), was predicted to have reduced the stroke rate by 39% and 42% reduction occurred. But for coronary artery disease the reduction in events was only 14%, not the 24% predicted.

The possible reasons for this "Coronary Artery Disease Paradox" will be dis-cussed and newer data, which is more consistent with the epidemiologic pre- diction, will be presented. Finally, I will comment of the possible uses and abuses of surrogate end points in hypertension.

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Preclinical in Vitro

X3-100 HUMAN TUMOR CELL LINES FOR DRUG DISCOVERY: DETOXIFICATION ABILITY AND TOXICITY OF MODEL QUINONES, Zora Djuric, Frederick A. Valeriote, Thomas H. Corbett and Laurence H. Baker. Dept. Internal Medicine, Wayne State University, Detroit, MI 48201

The primary testing of potential antitumor drugs in our laboratory involves use of human tumor cell lines. We have examined possible biochemical mechanisms that may contribute to the differential sensitivity of the cell lines towards various compounds using menadione and 1,4-dimethoxynapthoquinone (DNQ) as model cytotoxic quinone-type compounds. Both DT diaphorase and GSH transferase, which are important for the detoxification of quinones, were measured in cell lines from continuous passage in culture. Human CEM leukemia cells exhibited increased GSH transferase and DT diaphorase activity relative to human solid tumor cell lines CX1, HCT8, H116 and H125. The toxicity of menadione and DNQ was measured using the disc diffusion assay. The cells were plated in soft agar, a paper disc containing the compounds was placed on the plate and the area of colony inhibition around the disc was measured as an index of toxicity. The area of colony inhibition was dose-dependent for both quinones. The toxicity of menadione was greater in CEM cells than in the solid tumor cell lines. DNQ, which cannot be detoxified by glutathione conjugation, exhibited lesser differences in toxicity between cell lines. Our preliminary data indicate that there are similar enzymatic differences between mouse leukemia and solid tumor lines. These data indicate that the detoxification ability of cells may be an important determinant of the differential toxicity of quinones to human cells in vitro. Supported by grant 1PO1 CA46560.

X3-101 EFFECT OF SUCCINATE ESTERS OF α-TOCOPHEROL AND CHOLESTEROL ON ADRIAMYCIN-INDUCED CYTOTOXICITY TO LEUKEMIA AND NORMAL BONE MARROW CELLS, Michael B. Fortuna, Zora Djuric, Carleen K. Everett, David F. Trent[a] and Marc W. Fariss[a], Dept. Internal Medicine, Wayne State Univ., Detroit, MI 48201 and [a]Dept. Pathology, Medical College of Virginia, Richmond, VA 23298

A fundamental concern in anticancer drug therapy is the lack of specificity, with large numbers of both normal and tumor cells being killed. Fariss and co-workers have reported that tocopherol hemisuccinate (TS) administration protects normal cells from the toxic effects of a wide array of insults including adriamycin (ADR). In contrast to the protective effects of TS on normal cells, tumor cell viability is reportedly diminished after exposure to TS. In the present study, we have examined the effect of TS on ADR toxicity in mouse leukemia cell lines, L1210 and C1498, and in mouse normal bone marrow in vitro. We also compared the effects of TS with cholesterol hemisuccinate (CS). At low concentrations (< 50 μM), both succinate esters elicited slightly mitogenic effects on all three cell types. At higher concentrations (100-200 μM) the esters were toxic to leukemia cells but toxicity was not apparent in the normal bone marrow, CFU-GM cells. Using the disc diffusion assay, the effect of the esters on adriamycin toxicity were examined. The cells were plated in soft agar, a paper disc containing adriamycin was placed on the plate and the area of colony inhibition measured as an index of adriamycin toxicity. In some instances, the cells were pretreated with the succinate esters. Using mitogenic concentrations of the esters, very little effect on ADR toxicity was observed. Using higher doses, however, both TS and CS pretreatment resulted in increased ADR toxicity to the leukemia cells without changing toxicity to the normal cells. These data suggest that TS and CS may be useful in selectively potentiating tumor cell kill during cancer chemotherapy with ADR. The results also indicate that the observed activity of TS does not result from the action of α-tocopherol since a similar effect was observed with CS. Supported by grant 05452 from NIEHS and the Wayne State University Ben Kasle Trust Fund for Cancer Research.

X3-102 Novel *In Vitro* Assays For Elucidating Molecular Mechanisms Of Toxicity, Paul D. Ponath, Spencer B. Farr, Xenometrix, Inc., 812 Huntington Ave., Boston, MA 02115

Because of the vast number of new chemical entities synthesized each year as well as the tremendous expense and time required for testing these compounds there is a need among pharmaceutical, consumer and cosmetic, and chemical manufacturers for simple, inexpensive methods for obtaining information about the potential toxicity, mutagenicity or carcinogenicity of these compounds. None of the short-term *in vitro* assays currently available provide information about the underlying molecular mechanisms for bioactivity and toxicity of a compound and, therefore, are not useful predictors of animal toxicity. For example, currently employed mutagenicity assays detect alteration at the DNA level only. They can neither detect direct damage to protein or lipid membranes, nor inhibition of DNA synthesis. None yield information about the cellular mechanism by which a toxin exerts its effects.

We have developed a set of novel *in vitro* assays which allow investigators to quantify the toxicity, mutagenicity or carcinogenicity of a compound more accurately and reliably than currently available tests. Furthermore, these assays provide information about the specific molecular mechanism underlying the toxicity of particular compounds. These assays are simple, rapid, and inexpensive and should prove valuable for predicting the toxicity of a compound prior to performing expensive, time-consuming animal tests. These assays will reduce the number of compounds a company takes through expensive *in vivo* assays and thus dramatically reduce the cost of testing as well as the number of animals subjected to testing. The mechanistic information provided by these assays is especially useful in the discovery and rational design of new therapeutic agents.

The yeast "DEL" assay and its mammalian equivalents predict carcinogenicity more accurately than the conventional assays by detecting both inter- and intra-chromosomal homologous and non-homologous recombination. The bacterial and mammalian "Farr-Tox" assays detect non-mutagenic carcinogens as well as numerous types of cell damage and cell stress by measuring the response of a specific set of genes to particular categories of stress.

Preclinical in Vitro and in Vivo

X3-103 DEVELOPMENT OF A NEW THERAPEUTIC STRATEGY TO IMPROVE TACRINES' USE IN THE TREATMENT OF ALZHEIMER'S DISEASE, Marc W. Fariss and Jeyananthan Chelliah, Environmental and Molecular Toxicology, Department of Pathology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298

The most promising drug for the treatment of Alzheimer's disease is the anti-cholinesterase inhibitor, Tacrine (THA). The widespread clinical use of THA, however, has been prevented by THA's hepatotoxic potential and concerns about Tacrines' efficacy. Using in vitro and in vivo techniques to assess hepatotoxicity and cholinergic efficacy, we have discovered that the administration of tocopheryl hemisuccinate (TS) and cholesteryl hemisuccinate (CS) can prevent THA-induced hepatotoxicity as well as potentiate the cholinergic and anti-cholinesterase activity of THA. Succinate ester-mediated prevention of THA-induced hepatotoxicity was observed in freshly isolated rat hepatocyte suspensions (2 to 5 mM THA) and in fasted male Sprague-Dawley rats (15, 25 and 30 mg/kg THA, oral gavage). The THA-mediated hepatotoxicity observed in rats was similar to the clinical manifestations of THA liver injury; 3 to 8 fold increase in liver specific serum enzyme levels, reversible upon discontinuation of THA dosing and little or no alteration in liver histopathology. During the hepatotoxicity studies we also observed that TS and CS pretreatment significantly potentiated THA-induced cholinergic behavioral effects such as salivation and exophthalmos. The inhibitory effect of TS and CS on cholinesterase activity was confirmed in vitro using purified commercial preparations of acetylcholinesterase (ACHE) and pseudocholinesterase (PCHE). These data indicate that TS and CS are weak inhibitors of PCHE (IC50 of 100 and 168 uM respectively) but potent inhibitors of ACHE (IC50 of 3.0 and 1.0 uM respectively). In fact the presence of TS and CS dramatically potentiated the anti-ACHE action of THA. Thus by eliminating the hepatotoxic effect of THA while potentiating THA's cholinergic efficacy, TS and CS administration in combination with THA represent a promising new therapeutic strategy for the treatment of Alzheimer's disease. Funded by NIEHS grant 05452.

X3-104 PROTEIN THERAPEUTICS TARGETING HUMAN MALIGNANCIES CHARACTERIZED BY ALTERED EXPRESSION OF THE RETINOBLASTOMA (RB) TUMOR SUPPRESSOR GENE, Duane Johnson, H. Michael Shepard, Canji Inc., 3030 Science Park Road, #302, San Diego, CA 92121

The retinoblastoma and p53 tumor suppressor genes appear to play a major role in the development and aggressiveness of several human cancers. Altered expression of the retinoblastoma protein, $p110^{RB}$, has been reported to predict decreased survival time for patients with bladder cancer, acute myelogenous leukemia, and perhaps non-small cell lung cancer. One method for therapy of malignancies associated with altered RB might be replacing the absent or altered retinoblastoma protein in tumor cells that lack it. We have engineered two different proteins with this purpose in mind. Preliminary data suggest that such constructs are active in *in vitro* assays, including T antigen binding and inhibition of growth of tumor cells which are deficient in expression of $p110^{RB}$. *In vivo* studies with the appropriate tumor models are in progress. Such proteins may represent a unique and effective therapeutic approach for human malignancies characterized by altered expression of tumor suppressor genes.

X3-105 THE THERAPEUTIC POTENTIAL OF CELL-IMPERMEABLE INHIBITORS OF PHOSPHOLIPASE A_2. Saul Yedgar, Department of Biochemistry, Hebrew University - Hadassah Medical School, Jerusalem 91010, Israel.

Phospholipase A_2 (PLA_2), which hydrolyzes cell-membrane phospholipids, is the key enyzme in the production of arachidonic acid, the precursor for the eicosanoids (prostaglandins, leukotrienes, thromboxanes and HETES). The eicosanoids play a central role in the development of various diseases, such as allergy, inflammation, thrombosis and cancer. Therefore, PLA_2 inhibitors have been proposed for the treatment of these diseases. However, inhibitors which penetrate the cell impair the cell viability. Thus, inhibitors of PLA_2 activity at the membrane which do not enter the cell were desirable, but not available.

Cell-impermeable PLA_2 inhibitors (PLI) which fulfill this requirement have been designed and synthesized for the first time in our laboratory. This was done by the binding of PLA_2 inhibitors to macromolecular (polymeric) carriers, in a way which preserves the capacity of the inhibiting molecule to interact with the cell membrane and inhibit the PLA_2 activity at the cell membrane, but its internalization is prevented by the carrier.

These PLIs were found effective in inhibiting the development of a number of related pathological conditions. Among them are: human platelet aggregation; arthritis in rats; basophil activation; paralysis induced in rats by experimental autoimmune encephalomyelitis (a model for multiple sclerosis); growth of murine and human leukemia cells.

The inhibitory capacity of these preparations depends on the molar ratio of the inhibiting molecule to the carrier, and can be modified accordingly. The preparation's size can also be modified, by selecting the carrier, to obtain the proper size for targeting to the desired organ.

The rapidly growing research into eicosanoids has demonstrated the pertinence of PLA_2 action at the cell-membrane to the various physiological conditions.

The pattern of our cell-impermeable PLA_2 inhibitors provides a basis for a variety of preparations which can be employed for diverse treatments of PLA_2-related diseases.

Preclinical in Vivo

X3-106 **TAb 250-GELONIN: AN IMMUNOTOXIN EFFECTIVE AT INHIBITING GROWTH OF TUMOR CELLS THAT OVEREXPRESS THE c-erbB-2 PROTEIN.** Beatrice C. Langton[1], Jody R. Brink[1], Wendy L. Schraufnagel[1], Laura K. Shawver[1], Lawrence Cheung[2] and Michael G. Rosenblum[2]. [1]Department of Oncology, Berlex Biosciences, Alameda, CA, [2]MD Anderson, Houston, TX.

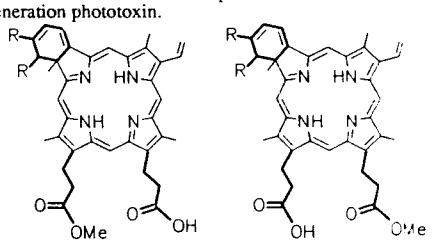
The c-erbB-2 protein (gp185) is overexpressed in 20-30% of breast, lung and ovarian cancers and is associated with disease advancement, clinically manifested by an early relapse and a shortened survival time. Antibodies targeted to the c-erbB-2 protein can inhibit, *in vitro* and *in vivo*, the growth of mammary, ovarian and lung tumor cell lines that overexpress gp185. These effects are often cytostatic unless immune effector functions are recruited or the antibodies are combined with cytotoxic agents such as chemotherapeutic agents or toxins. We describe here a toxin-antibody conjugate directed against gp185. The toxin, gelonin, is a ribosome inactivating protein which lacks a cellular binding region and is thus relatively non-toxic. However, once internalized, gelonin maintains excellent potency similar to that of other previously described plant toxins such as ricin. The antibody, TAb 250, is a murine monoclonal antibody that binds an extracellular epitope of gp185 with excellent specificity, failing to react with most normal tissues. We have previously shown that TAb 250-gelonin specifically binds c-erbB-2, is internalized by c-erbB-2-expressing cells, inhibits the growth of mammary and ovarian tumor cell lines that overexpress c-erbB-2, and that the cytotoxicity correlates with the number of cell surface receptors (Proc. Amer. Assoc. Canc. Res. 33:3340). We describe here the biodistribution, pharmacokinetics and *in vivo* efficacy (growth and survival models) of this immunoconjugate. TAb 250-gelonin distributes specifically by 24-48 hours to tumors that overexpress c-erbB-2 and inhibits the *in vivo* growth (subcutaneous tumors) of the c-erbB-2 expressing ovarian cell line, SKOV-3. Additionally, the survival of animals bearing intraperitoneal tumors treated with TAb 250-gelonin is increased up to 50% compared to animals treated with TAb 250, gelonin or a combination of the unconjugated proteins. This data suggests that such an immunoconjugate could be used clinically against cancers that overexpress c-erbB-2 to reduce tumor burden and perhaps enhance survival time.

X3-107 DEVELOPMENT OF BPDMA AS A PHOTODYNAMIC THERAPEUTIC AGENT, Ethan D. Sternberg, David Dolphin, Julia Levy and Elizabeth Waterfield, Quadra Logic Technologies and University of British Columbia, Vancouver, British Columbia, Canada.

Photodynamic Therapy (PDT) has clinical applications in fields ranging from cancer therapy to viral inactivation. PHOTOFRIN^R, an oligomeric mixture of hematoporphyrin, has had extensive clinical success in the treatment of a number of cancers including esophagael, lung and bladder. Quadra Logic Technologies and Lederle Labora tories have submitted an NDA in a number of countries for this drug.

The mechanism of PDT is based on a photosensitizer. This is a molecule whereupon irradiation, singlet oxygen is produced. This highly reactive form of oxygen has the ability to react with biomolecules ranging from proteins to nucleic acids. The result of which is a range of disruption of intracellular enzyme mechanisms.

We at QLT and Lederle have begun clinical trials on a second generation compound, BPDMA. It addresses and eliminates the problems associated with the first generation compound. The development of this second generation compound has included investigating: first, the *in vitro* phototoxicity of it and several analogues: second, the photophysical properties of these compounds; third, the ability to effect a cure *in vivo* in a mouse cancer model; fourth, the formulation of the hydrophobic molecule in a liposome delivery system: and fifth, the clinical trial set up at Massachusetts General and in Vancouver. This poster will address some of the special concerns in developing a second generation phototoxin.



R = CO_2Me

A.M. Richter et al., Photosensitizing Efficiency of Two Regioisomers of Benzoporphyrin Derivative Monoacid Ring A (BPDMA). *Biochemical Pharmacology*, **43**, 2349-2358.

X3-108 ENHANCEMENT OF MYELOID CELL FORMATION BY PHOSPHOROTHIOATE ANTISENSE OLIGONUCLEOTIDES TO CHOLINESTERASES, Haim Zakut[1], D. Patinkin[1], G. Ehrlich[1], D. Ginzberg[1], F. Eckstein[1] & H. Soreq[1] [1]Dept. of Obstetrics & Gynecology, The Edith Wolfson Medical Center, Sackler Faculty of Medicine, Tel-Aviv University Holon (58100), Israel, [1]Dept. of Biological Chemistry, The Life Sciences Institute, The Hebrew University of Jerusalem, Israel, [1]Max-Planck Institute for Experimental Medicine, Gottingen, Germany

Defective myeloid cell formation is a major problem associated with various clinical and toxicity syndromes, including chemotherapy and immuno-suppressive disorders. We have used short, 15-mer (4500 m.w.) antisense" phosphorothioate oligodeoxynucleotides suppressing cholinesterases (CHE) mRNAs (Patinkin et al., Mol. Cell. Biol. 10, 6046-6050, 1990) and the mRNA for CHED, a cdc, homolog (Lapidot-Lifson et al., Proc. Natl. Acad. Sci., 89, 579-583, 1992) to enhance myeloid cell formation in primary murine bone marrow cultures and by intraperitoneal injection of balb-c mice (5µg oligonucleotide per gram weight). Absolute enhancement of about 3-fold in myeloid cells composition was observed in culture up to 10 days. In vivo, a parallel relative increment was detected up to 60 days post-treatment. RNA-PCR analyses displayed suppressed levels of the target mRNAs, suggesting that the effect was due to induction of RNase-mediated nucleolytic degradation. Thus, antisense technology may lead to the development of novel therapeutic preparations for the targetted modulation of bone marrow composition.

X3-109 PHARMACOLOGIC AND TOXICOLOGIC EVALUATIONS OF CD5 PLUS™. Ada H.C. Kung, Dianne M. Fishwild, Karen Lin, Nneka Ottah and Fred R. Kohn, Department of Pharmacology and Toxicology, XOMA Corporation, Berkeley, CA 94710

CD5 Plus™ is an immunoconjugate constructed with a murine anti-CD5 monoclonal antibody and ricin A chain (RTA) for use in the treatment of acute graft-versus-host disease following bone marrow transplantation. In the process of evaluation of both the pharmacologic and toxicologic properties of XomaZyme-CD5 Plus, we have conducted the following animal studies: 1) Pharmacokinetic studies in both the monkey and the rat, 2) Toxicology studies in both the monkey and the rat, and 3) Efficacy studies in the SCID/PBMC mouse.

The elimination half-lives determined for both the rat and the monkey were within the range observed in man. The toxicities observed after repeated intravenous administration of CD5 Plus™ were largely related to general inflammatory or stress related responses due to the RTA component of the immunoconjugate. The administration of CD5 Plus™ significantly depleted human PBMC from various organs of SCID mice. The results from these studies have been integral in designing clinical studies with CD5 Plus™ and have provided information on its mode of action *in vivo*.

X3-110 PRECLINICAL EVALUATION OF RADIOLABELED IMMUNOCONJUGATES FOR CANCER TREATMENT Syed M. Quadri, Jing Lai, Abrar Siddiqui, Hamid Pour, and Huib M. Vriesendorp, Department of Radiotherapy, The University of Texas, M. D. Anderson Cancer Center, Houston, Texas 77030

Progress in clinical Radioimmunoglobulin Therapy (RIT) is hindered by the complex preclinical analysis of RIT reagents and its poor correlation with clinical outcome. The initial screen for antibody reactivity with human tissues can be performed on tissue sections with immunoperoxidase techniques. Optimal antibody class and size have to be determined in vivo in nude mice with a s/c growing human malignancy. Chemically stabilized $F(ab')_2$ fragments appear to be most promising. The selection of the therapeutic isotope depends on the size of the tumor to be treated. Larger tumors require isotopes high energy beta emissions. Currently, Yttrium-90 is the most appropriate isotope for the average human tumor (> 1cm in diameter). Radiometals such as Yttrium-90 require a chelator conjugated to the immunoglobulin for a successful radioimmunoconjugate. A new chelate, a carbon back bone modified DTPA derivative has shown superior complexation with Yttrium. Radioimmunoconjugates are tested for in vitro stability in serum under physiological conditions. Stable conjugates are further evaluated for pharmacokinetics and tumor targeting in nude mice with a s/c xenograft of human tumor. Subsequently, normal tissue toxicity is best evaluated in a large animal model such as the beagle dog as the nude mice information is of insufficient predictive value for human patients (bone marrow damage, liver damage). High uptake by normal liver (dog) and low uptake by tumor (mouse) remain obstacles to further improvement. Hydrolysable ("labile") linkers and chemically stabilized $F(ab)_2$ fragments and fractionation of RIT appear to offer improvements. However, further optimization is needed. Quality control prior to clinical application includes: testing for sterility, pyrogenicity, immunoreactivity and radio chemical purity.

The application of the described flowsheet for preclinical analysis provides a better characterization of RIT reagents and allows for a rational selection of reagents and treatment parameters for further clinical study. Hopefully, these improved selection procedures will accelerate progress in clinic RIT.

X3-111 CLINICAL EVALUATION OF RADIOLABELED IMMUNOGLOBULIN THERAPY, HM Vriesendorp, SM Quadri, Department of Radiotherapy, The University of Texas, M. D. Anderson Cancer Center, Houston, Texas 77030

Radiolabeled Immunoglobulins can provide systemic cancer treatment with a better therapeutic ratio than cancer chemotherapy. The specificity of the antibody and the localized energy deposition of the isotope used can deliver high radiation doses to tumor and spare normal tissues. The preclinical evaluation of radioimmunoglobulins is addressed in a companion abstract. Here new guidelines for clinical analysis are proposed on the basis of early clinical results. Patients had Hodgkin's disease, ovarian cancer, colon cancer or pancreas cancer. Antibodies were reactive to ferritin or adenocarcinoma antigens. Isotopes were Iodine-131, Rhenium 186, Indium-111 or Yttrium 90.

The radioactivity is best prescribed per Kg body weight, not body surface area. Patients receive a diagnostic low activity injection first and are analyzed for 1) in vivo stability of radioimmunoconjugate 2) tumor targeting and 3) tumor/normal tissue dosimetry. A few days thereafter a therapeutic, high activity, injection is given only if the diagnostic study shows that the reagent is stable, targets the tumor and delivers sufficient radiation to the appropriate volumes. Activity escalations can precede more rapidly for reagents with in vivo distributions that are similar to previously analyzed reagents. Dose limiting tissues are bone marrow first and liver or kidney second. The sequential phases of clinical studies as developed for cancer chemotherapy do not apply to radiolabeled immunoglobulin. The in vivo accountability of the radiolabeled proteins allows for a more cautious clinical development with lower risks to patients. We propose the following phases 0) Preclinical analysis 1) In vitro stability and tumor targeting 2a) Optimizing therapeutic ratio 2b) Activity escalation 3) Randomized trial.

Application of our proposal requires single agent studies with radiolabeled antibodies in patients with measurable and radiosensitive disease. It limits the number of patients that will receive radiolabeled antibodies without therapeutic potential (unstable radioimmunoconjugates or antibodies that do not target the tumor). It prevents premature negative evaluations of radiolabeled antibodies and should accelerate the safe clinical application of this promising new group of systemic cancer agents.

X3-112 CARDIOVASCULAR EFFECTS OF A NOVEL CHIMERIC MOLECULE FOR TREATING HEART FAILUE (CHF).
J.E. Shaffer, M.K. Grizzle and D.K. Anderson, Department of Pharmacology, Glaxo Research Institute, RTP, NC 27709.
The hemodynamic and beta adrenergic blocking effects of GI104313 (6-{4-[N-[2-[3-(2-cyanophenoxy)-2-hydroxypropylamino]-2-methylpropyl] carbamoylmethoxy-3-chlorophenyl]}-4,5-dihydro-3(2H)-pyridazinone), a chimeric molecule containing a PDE inhibiting pyradazinone and a beta blocking phenoxypropanolamine, were examined in anesthetized dogs. The results of these studies were compared to those of indolidan, a known PDE inhibitor, and xamoterol, a partial beta-adrenoceptor agonist. The compounds were infused at 6 increasing dose rates, in 10 min intervals. Isoproterenol (ISO, 0.5 μg/kg) was administered prior to each dose increment to determine beta-adrenoceptor responsiveness; these studies were performed in either the presence or absence of atenolol to abolish beta-adrenoceptor dependent effects. GI104313 elicited dose-dependent increases in heart rate, contractility (+dP/dt) and cardiac output and decreases in arterial blood pressure, left ventricular end diastolic pressure and systemic vascular resistance in un-pretreated and atenolol pretreated dogs. However, GI104313 was less potent, hemodynamically, in atenolol pretreated animals. This was evidenced by a 4-fold dextral shift in the dose response relation for several hemodynamic variables. In un-pretreated dogs, GI104313 elicited potent dose-dependent blockade of the heart rate, diastolic blood pressure and +dP/dt responses to ISO. Greater than 95% inhibition of the ISO response was attained at 1 μmol/kg GI104313 for all observed variables. Indolidan increased contractility and heart rate and decreased diastolic blood pressure in a dose related fashion, but did not affect ISO responses. Both indolidan and xamoterol produced responses hemodynamically similar to GI104313 except that xamoterol's effects were dextrally shifted >100-fold by atenolol, whereas indolidan's effects were shifted only 2-fold. GI104313 is a unique chimeric molecule which exhibits beta adrenoceptor blockade and cardiac inotropy via a non-adrenergic mechanism, most likely due to PDE inhibition.

X3-113 **Transiently transgenic Xenopus embryos expressing human acetylcholinesterase: an in vivo model for testing the efficacy of cholinergic drugs and poisons.**
Hermona Soreq, Shlomo Seidman and Revital Ben Aziz-Aloya. Department of Biological Chemistry, The Hebrew University of Jerusalem, Israel 91904.

Acetylcholinesterase (ACHE) controls the termination of intercellular communication in muscle, brain and multiple embryonic tissues, and functions also as a natural scavenger for a variety of cholinergic drugs and organophosphorous (OP) insecticides (Soreq et al., TIBS 17: 353-358, 1992). The cloned cDNA encoding human ACHE (Soreq et al., PNAS 87: 9688-9692, 1990) linked to the potent cytomegalovirus (CMV) promoter-enhancer, was microinjected into oocytes and early cleavage Xenopus embryos, where it was efficiently expressed to yield authentic, catalytically active human ACHE (Ben Aziz-Aloya et al., PNAS, in press) with its distinct substrate and inhibitor specificities, as determined by a microtiter plate assay enabling numerous kinetic measurements simultaneously (Neville et al., EMBO: 11, 1641-1649, 1992). Cytochemical staining and electron microscopy further revealed x10-fold accumulation of overexpressed ACHE in neuromuscular junctions of apparently normal, hatched 2 day old embryos injected with the CMVACHE construct. When exposed to the OP insecticide Paraoxon, these embryos improved survival under exposure to otherwise lethal amounts of this poison. Transiently transgenic Xenopus embryos thus offer a versatile, convenient possibility for directly testing, in an in vivo situation, the inhibitory potency of cholinergic drugs and poisons on human ACHE, so that the therapeutic index and poisoning capacity of such drugs can be predicted at microscale.

Economic Models

X3-114 INTERNATIONAL PHARMACEUTICAL R&D: A GLOBAL PERSPECTIVE. **A.L. Drasdo, C.E. Lumley, R.G. Halliday & S.R. Walker**, Centre for Medicines Research, Woodmansterne Rd, Carshalton, Surrey, SM5 4DS, UK.
Pharmaceutical research and development is a vital factor in the industry's performance, and is characterised by high investment. The Centre for Medicines Research has carried out surveys amongst the top pharmaceutical companies worldwide to obtain information on international pharmaceutical R&D expenditure and strategies. It is estimated that the companies surveyed were responsible for over 70% of the world's pharmaceutical revenue R&D expenditure in 1989. Considering the companies according to their home base, some findings of these surveys appeared to show national traits. Some of these differences might also be related to the size of R&D budgets, whilst others might relate to language or cultural factors leading to varying interpretations of questions. The respondent Japanese companies were smaller than the European or American, in terms of R&D budget and staff numbers per company. They hold more of their staff in their home base and synthesise fewer compounds for each NCE marketed. The European respondents included the highest proportion of companies with over 50% of projects identified as high risk, had longer key planning terms and the lowest proportion of R&D staff in their home regions. The respondent US companies were the largest, and synthesised more compounds for each NCE marketed. The top European companies as a group invested a larger proportion of their sales in R&D than the American or Japanese companies.
There are what may be early indications of a sustained increase in geographical dispersion of R&D expenditure by Japanese companies in particular, possibly a manifestation of a determined strategy to internationalise. Interestingly the UK was a favoured site for R&D, second only to the USA according to the respondents of these surveys; the CMR has also conducted studies of the detailed breakdown of R&D breakdown in the UK.
World wide pharmaceutical R&D expenditure (excluding capital) is estimated to have been $20bn in 1990, based on the results of these surveys, and has grown by an average of 16% annually. The ratio of R&D/sales has also increased, and the average for all companies rose from 10.1% in 1981 to nearly 15% in 1990. The growth in investment emphasises the large and increasing commitment of the industry to the development of new medicines, a factor crucial to therapeutic innovation.

X3-115 DISCOVERY AND DEVELOPMENT OF THERAPEUTIC COMPOUNDS: THE AUSTRALIAN EXPERIENCE, Dr Ross A Macdonald, AMRAD Corporation Limited, Melbourne, Victoria, Australia

Australian research scientists are well noted for their contributions to the advancement of biomedical science, with names such as Florey, Metcalf and Burnet being synonymous with discoveries of great medical significance. Despite representation in Australia by nearly every major pharmaceutical company, development of local discoveries to marketable products has tended not to occur within Australia. Rather, much of the pre-clinical testing, toxicology and early phase human testing of Australian discoveries has taken place in Europe or the United States.

The initiation of a program to develop Australian biomedical discoveries in Australia and the establishment of necessary infrastructure to ensure global commercial success, will be described. The development pathway of several therapeutic compounds discovered in Australia will be used to illustrate the emergence of a fully integrated local pharmaceutical industry.

Late Abstract

THE ANTITUMOR ACTIVITY OF THE POLYAMINE ANALOG, N'-N"-BIS(ETHYL) NORSPERMINE (BENSPM) IS ASSOCIATED WITH INCREASED TUMOR CELL SPERMIDINE/SPERMINE N'-ACETYLTRANSFERASE (SSAT). Bernacki, R.J., Porter, C.W., Oberman, E. and Pera, P., Roswell Park Cancer Institute, Buffalo, NY 14263 and Bergeron, R.J., Univ. Florida, Gainesville, FL 32610.

Appropriately designed spermine analogs have been synthesized which selectively modulate polyamine homeostasis resulting in tumor growth inhibition. Human melanoma, ovarian and lung carcinoma cell lines displayed high growth sensitivity to these spermine analogs. This activity correlated with the induction of the polyamine catabolizing enzyme, spermidine/spermine N^1-acetyltransferase (SSAT). The analog with greatest ability to induce SSAT, BENSPM, also displayed the most impressive antitumor activity. Against three human melanoma xenografts transplanted s.c. into nude athymic mice, minimally toxic doses of BENSPM, produced tumor regressions, sustained suppression of tumor growth for up to 2 months, and some cures. Similar responses were obtained with A121 human ovarian carcinoma and A549 lung adenocarcinoma xenografts. Mechanistic studies revealed that the initial antitumor responses correlated with near-total polyamine depletion due to massive increases in SSAT activity which occurred in tumor but not normal tissues. Relative to traditional and other experimental anticancer agents, BENSPM is structurally and functionally representative of a new class of compounds which illustrate a novel chemotherapeutic strategy. Based on the promising antitumor activity in challenging human tumor model systems, and an appreciation of BENSPM's mode of action, this agent is currently being developed toward clinical trial. (Supported in part by CA37606 and CA13038).