could share, discuss, and develop animal imaging methodologies and through which results could be standardized and pooled into a widely available imaging database. Such a database could include images, standard protocols, and outcomes and could be of great utility to both academic and industry research programs.
- Consider ways to establish a database of information on methods of animal handling, equipment, standards, and ancillary equipment specifically for molecular imaging applications.

Summary Statement

Small animal imaging and associated equipment and techniques are developing rapidly as a direct result of achievements that promise to expand scientific knowledge and have direct translational benefits to a range of diseases, including cancer. At the same time, continued development depends on concerted action by researchers, academics, industry, and funding agencies to encourage agreement on and adoption of standards and harmonization in all aspects of animal imaging, including but not limited to animal handling, training, instrumentation, scanner software, image data, image analysis, methods of distribution, protocols, practice, and reporting. One key element in success will be the pooling of research experience and imaging databases to encourage gains in effectiveness and productivity in small animal imaging that will advance cancer diagnosis and treatment and extend to the many other promising areas of disease and health research.

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Chair, Instrumentation and Animal Models Session

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PRESENTATIONS

Animal Models for Human Diseases: Is There a Future Without Them?

Although this white paper focuses on rodent models of cancer, the issues about their value and limitations pertain to any intact model systems for cancer and other human diseases. The use of in vivo imaging of small animals has increased significantly in the past 5 years as the associated technologies have been rapidly developed or refined. The methods employed are increasingly useful for a number of applications. Widespread publication of the kinds of novel information obtainable through use of in vivo imaging has increased the demand for access to the instrumentation and, more important, to the knowledgeable imaging scientists and engineers who can partner with researchers who model human diseases.

Transgenic Murine Models

The original approaches for deriving transgenic tumor models—high-level overexpression of viral or cellular oncogenes in specific tissues—have for the most part been replaced by far more sophisticated techniques. Use of gene targeting in mouse embryonic stem cells can generate gain-of-function oncogene models or loss-of-function tumor suppressor models of cancer. At the present time, the targeting constructs are frequently under temporal, spatial, and dosage control (conditional approaches), so that the resulting cancer-prone mice develop diseases that are far better simulations of the natural history of the corresponding human malignancies than previous transgenics. These conditional mutants enable investigation of the roles of individual genes and their mutations in cancer etiology and the cooperation among individual mutations. Examination of the progressive stages of cancer development in these models also reveals the additional stochastic events that drive progressive of early lesions to late-stage tumors that invade and metastasize. One important feature of transgenic models is that they are immunocompetent; this affords opportunities to decipher how the immune system factors into cancer etiology, progression, and response/resistance to therapy.

Transgenic techniques are also applied to rats. Transgenic rat cancer models are an important complement to genetically engineered mouse models (GEMMS), providing species differences that illuminate cardinal features of cancer etiology and progression that are conserved from rodents to humans. The methods used to generate transgenic rats are classical DNA microinjection into oocytes, lentiviral vector–mediated DNA transfer into early embryos, and sperm-mediated transgenesis. Gene knockout models in rats are more difficult to produce, because they rely on untargeted germline mutagenesis. However, a method to screen mutants rapidly is having a major impact on the ability to generate

such models. Although gene targeting in rat embryonic stem cells is not possible (totipotent embryonic stem cells have not yet been isolated from rats), another technique, somatic cell nuclear transfer, provides the promise of eventually enabling generation of conditional knockout and gene replacement models in rats.

Because the natural history aspects of GEMMs are excellent, there is increasing application of standard-of-care therapy to examine clinical course (credentialing). Just as many of the GEMMs simulate the cancer's natural history, the clinical course in GEMMs is often highly reminiscent of patient response. The molecular features of nonresponsive and relapsing tumors are accessible through this approach. Recent experiments have defined genes and pathways required for tumor maintenance and exposed mechanisms of resistance, as well as disclosed biomarkers that indicate when a tumor is responding.

Although the natural history and clinical course of malignancies in GEMMs are vastly improved mimics of human cancer, some drawbacks pose challenges to their use in experimental therapeutics. One important element of drug response is the inbred genetic background of the model. Because of the methods used to derive mutant strains, the background may be mixed. Disease progression may be quite long and variable, as it is hypothesized to be for human cancers. Not every animal at risk for developing cancer will do so. Thus, there is a vital need for in vivo imaging to examine the natural history of disease as it presents in individual animals and to follow the course of therapy.

Xenotransplantation Murine Models

For testing of therapeutic agents, xenografts of human tumors, tumor cells, or human tumor cell lines are most often the models of choice. Xenografts are grown in immunodeficient mice, as either ectopic (subcutaneous) or orthotopic (native tumor site) grafts. Ectopic xenografts progress synchronously and are easy to observe when they reach an optimal size to begin testing. Their tumor progression is usually rapid and highly predictable.

However, the information from xenografts has limitations. If the engrafted cells are human cell lines that are maintained by many passages in vitro and adapt to culture conditions, the tumors that result from either ectopic or orthotopic xenografts do not represent the original tumor. That is because cells in culture lack the architectural and cellular complexity of the original tumor, which includes immune cells, unique tumor vasculature, and other stromal elements.

To overcome certain of these limitations, some laboratories are generating and maintaining xenograft models that are tumor tissue explants grown orthotopically. The xenografts maintain the histology and morphology of the original tumors, and the tumor genomes are stable. Just as GEMMs that are developing tumors in internal organs require imaging to follow disease progression and response to therapy, so do xenografts that are generated or maintained in internal sites.

Small Animal Cancer Models: Questions and Controversial Issues

(1) **Understanding cancer biology.** Do we have the capacity to image processes in tissues? Inflammation, immune infiltration, changes in tissue architecture, oxygen levels, pH, metabolism, signaling, blood flow, epithelial–mesenchymal transitions, cell migration, and aging changes are implicated in cancer etiology. Can we quantify such changes? Can we distinguish among various components of the immune system as potential contributors to cancer initiation and progression?

(2) **Performing experimental therapeutics.** Do we have the capacity to identify mechanisms of therapeutic response? Tumor stasis, apoptosis, senescence, dormancy, necrosis, autophagy, fibrosis, and other mechanisms are documented in vitro and are probably occurring in vivo. Will imaging enable us to quantify known or discover new parameters that are surrogate markers of response? Mouse cancer models are now being applied to serum biomarker discovery. Perhaps those biomarkers that strongly diminish upon treatment will provide clues for developing imaging surrogate response markers. Is there sufficient image resolution to discover sites of metastasis? Genetic approaches are available to time the development of tumors and their metastases to test detection limits of imaging techniques. Is there sufficient image resolution to document very early evidence of tumor recurrence after treatment?

(3) **Imaging infrastructure.** Is there value in coordinated development of imaging agents for preclinical systems and clinical trials? Is it necessary to co-locate imaging science and imaging facilities, clinical trials, cancer modelers, and animal housing? Could other mechanisms that enhance communications among the relevant communities substitute for co-location? Do we have the bioinformatics capacity to analyze and possibly integrate imaging data from preclinical testing and clinical trials? Are there ways to get around the apparent need for dedicated instruments in germ-free or specific pathogen–free facilities to enable longitudinal studies in animals? How can we have better coordination with nanotechnology and drug delivery system developers?

(4) **Future use of animal models.** Is there translational value for animal models in the realm of experimental therapeutics? Can we use imaging approaches in cell and tissue models to provide sufficiently definitive data to substitute, at least in part, for use of intact animal models for experimental therapeutics? Is there a role for in vivo imaging in animal models used for prevention studies?

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