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NCI RADIATION RESEARCH PROGRAM MEETING REPORT

TRANSLATIONAL RESEARCH IN RADIATION ONCOLOGY

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INTRODUCTION

A 1.5 day workshop was convened at the request of the Radiation Research Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute (RRP/DCTDNCI) for the purpose of defining the current state of the science in tumor biology as it may be applied to treatment with ionizing radiation and to discuss potential clinical applications. The first morning session included overview presentations of seven research areas that were felt to be potential areas of new investigation: Opening Remarks (C. N. Coleman, R. Cumberlin); Presentations: Normal Tissue (P. Okunieff), Signal Transduction (R. Schmidt-Ullrich, A. Dritschilo), Angiogenesis (R. Weichselbaum), Apoptosis (Z. Fuks), Microenvironmental Effects (J. M. Brown), Chemotherapy–Radiotherapy Interactions (T. Lawrence, D. Brizel, J. Mitchell), and Radiation Induced Gene Expression, (R. Weichselbaum). Appendix I includes the list of attendees.

The remainder of the Workshop was organized into breakout sessions pertaining to four general research areas that were scheduled for the afternoon with the preliminary conclusions discussed by the entire group on the final morning. Written reports were prepared by the breakout chairs with this document assembled by Drs. Cumberlin and Coleman. The four topics were consolidated from the seven topics on the agenda and were: (*1*) Angiogenesis and gene expression; (*2*) Signal transduction and apoptosis; (*3*) Radiation-chemotherapy interactions combined with tumor microenvironment; and (*4*) Normal tissue tolerance.

ANGIOGENESIS AND GENE EXPRESSION (CHAIR–JAMES R. OLESON, M.D., PH.D.)

There are clear rationales for combining ionizing radiation (RT) with antiangiogenic (AA) and antivascular (AV) agents and for combining nonionizing radiation with agents that result in antivascular effects in the treatment of cancer. It is known that several of the growth factors and cytokines involved in angiogenesis are also involved in the response to radiation. Examples are VEGF, TGF-β, and PDGF. Such treatment approaches require the preclinical study of mechanisms of action using models that test the sequencing of radiation with the chemical or biologic agent of interest and include appropriate pharmacokinetic analysis. Means of optimizing such approaches must be determined preclinically, and means of validating the effects that can be used clinically must be developed.

Some of the questions to be answered include:

1. Will inhibition of VEGF signalling enhance the radiation response of most tumors, or will it be necessary to inhibit multiple pathways?
2. Will it be necessary to inhibit different factors or pathways at different stages of tumor development?
3. How significant is the problem of redundancy during persistent hypoxic stimulation of tumor angiogenesis?
4. What are the most appropriate and reliable indicators of efficacy for use in preclinical and clinical studies?

Major areas of research to answer these questions include the following:

- Characterize the effects of antiangiogenic and antivascular agents with and without radiotherapy *in vitro*, and assess the heterogeneity of effects in a variety of assay systems.
- Extend the *in vitro* studies to preclinical *in vivo* models. Predict what tumor types respond to AA/AV agents and/or RT and assess heterogeneity among tumor lines and within tumors.
- Develop invasive and noninvasive assays of AA/AV/RT effects that can be validated in preclinical models and extended to clinical studies.
- Develop preclinical models to optimize the use of various AA/AV agents with fractionated radiotherapy in a variety of tumor types including subcutaneous and orthotopic xenografts and spontaneous tumors. Study drug or bio-

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logic agent delivery systems in general and site-specific delivery systems. Include systems based on gene expression approaches.
- Identify the relevant cytokines involved in effects of various AA/AV agents, which may vary with tumor type, growth stage, normal tissue bed, and other phenotypic and genotypic variants of a particular tumor histology. Assess mechanisms of action in a variety of preclinical models and extend the studies to include radiation.
- Determine and validate acute and late normal tissue effects of AA/AV/RT approaches in preclinical models and extend the models into clinical trials.
- Evaluate the effectiveness of photodynamic therapy using novel drugs and fiberoptic light delivery systems in preclinical and clinical trials for superficial malignant and premalignant diseases.

SIGNAL TRANSDUCTION AND APOPTOSIS (CHAIR—ANATOLY DRITSCHILO, M.D.)

The breakout group considered an arbitrary classification of proteins involved in cellular signaling for purposes of discussion. These several targets are amenable to combined modality and gene therapy approaches. Tests for proof of principle have been completed in cell systems and in animal systems. These include:

- Upstream signaling (membrane/cytoplasmic), such as receptor tyrosine kinases (ERBB) and RAS/RAF/MAP-K
- Nuclear signaling such as p53, NF-κB, PARP, and Ku/DNA-PK
- Apoptosis induction or protection such as bcl2/bax and sphingomyelin

The mechanisms for disruption of these pathways include the use of:

- Antisense (AS) oligonucleotides
- Dominant negative vectors
- Growth factors (KGF, FGF)
- Cytokines (TNF, IFN)
- Chemical inhibitors (Farnesyl transferase inhibitors)

Specific targets for future therapeutic strategies may include ATM, BRCA1, BRCA2, and NBS1. Initial efforts should explore tumor specific characteristics of these large DNA repair proteins. Chips/array patterns of responses of tumors to X-rays may provide insight into these biochemical and molecular pathways. NCI support for development of gene therapy vectors would be helpful in testing these strategies.

The group felt that a suitable mechanism to gain access to new therapeutic molecules under development was needed. A working group for drug identification was suggested as well as limited screening of new drugs, and drug identification resources should be developed.

There was thought to be a gap in the NCI funding mechanisms for translational research. Traditionally, the individual investigator will pilot a new therapy with the RO1 or PO1 mechanism and the cooperative groups will follow this up with large scale clinical trials through the U10 mechanism. Small consortia (3–5 members) of UO1 grantees or multi-institutional PO1s were suggested for rapid testing of new concepts. Such funding would fill the gap between RO1 and cooperative group mechanisms.

Collaborative agreements for technologically complex treatment strategies require logistically difficult, expensive protocols. Such therapeutic interventions as gene therapy for radiation sensitization are difficult to perform under current funding mechanisms because of the substantial infrastructure requirements. Collaborative arrangements with the NCI for utilization of NCI resources should be considered.

RADIATION–CHEMOTHERAPY INTERACTIONS AND THE TUMOR MICROENVIRONMENT (CHAIR—J. MARTIN BROWN, PH.D.)

This breakout panel divided this topic into four broad areas. The report and recommendations for each area are given separately below:

Radiation–chemotherapy interactions

Specific questions needing to be addressed:

1. Can we optimize the interaction between radiation and currently used anticancer drugs to maximize the therapeutic ratio?

Though this was felt to be an area many do not consider suitable for funding through traditional hypothesis-driven research, it is, nevertheless, extremely important because of the widespread clinical use and success of combined chemotherapy and radiotherapy. Prior attempts to develop optimum schedules without regard to mechanisms have largely failed. Despite this, mechanism-based studies with animal tumor and normal tissue models could yield benefits. Such mechanisms could develop from studies outlined below.

2. Can we develop methods of imaging (confirmed by biopsy) the results of radiation–chemotherapy interactions?

Although laboratory studies can suggest the mechanism of drug–radiation interactions, data from patients will be required to understand how these interactions occur during actual therapy. Such studies would ideally be conducted in patients with imaging used to determine pharmacokinetics and pharmacodynamics, as this would permit multiple, real-time measurement. PET and magnetic resonance spectroscopy (MRS) appear to be most promising, as they can image physiologic processes relevant to drug–radiation interactions. Biopsies will need to be obtained to validate new imaging technologies and to determine microdistribution of drugs. Substantial animal and *in vitro* studies will need to be carried out to develop these new imaging techniques.

3. Can we develop new radiation modifiers?

There are several ways of doing this that should be explored. First, a radiation assay could be included in the NCI screen of roughly 10,000 new compounds per year against 3 or 4 cell lines. Second, rational drug design aimed at specific targets known to affect radiation sensitivity, for example, the molecular components of DNA-PK, ATM, and the Nijmegen Breakage Syndrome (NBS) genes could be explored. This could employ combinatorial chemistry with high-throughput screening for these targets. Third, gene expression arrays for radiation could be used to identify patens correlating with sensitivity and resistance. This could help identify new, potential targets for sensitizing tumor cells to radiation.

4. What is the mechanism of radiation protection of normal tissues by various agents such as amifostine?
5. How can we optimize the combination of hormones and radiation for hormone-dependent tumors such as prostate and breast?
6. Do factors other than genetic ones influence the response of tumors to radiation/chemotherapy interactions?

Tumor microenvironment

The microenvironment of solid tumors differs from that of normal tissues in a number of important aspects, the majority of which stem from differences between the two vasculature systems. Compared to the regular, ordered vasculature of normal tissues with a hierarchy from arteries to arterioles, capillaries, venules, and veins, blood vessels in tumors are often highly abnormal, distended capillaries with leaky walls and sluggish blood flow. Tumor growth also requires continuous new vessel growth, or angiogenesis. These differences lead to difficulties in cancer treatment. For example, hypoxia that develops in solid tumors leads to resistance to radiotherapy and to anticancer drugs as well as to increasing tumor aggressiveness. However, these differences can also be exploited for selective cancer treatment. Some success in this field has already been achieved with the development of the hypoxia-activated drug tirapazamine developed under a contract from the Radiation Research Program at NCI. However, there is still a major opportunity for improving cancer therapy using this important difference between normal and malignant tissues. Specific questions needing to be addressed:

1. Can we develop new, microenvironmentally activated cytotoxic or cytostatic drugs?

Such activation could be via hypoxia, low pH, nutrient deprivation, or any combination of the three. Development of such compounds could be based on a rational drug approach, for example, using the known hypoxia-activating moiety of a di-N-oxide or nitro group, or could be by addition of a hypoxic culture to the NCI screening program for new chemotherapeutic drugs. Another possibility would be to use gene expression arrays using chip technology based on the hypothesis that genes induced under hypoxia/low pH would be targets for anticancer drug therapy.

2. Are the human focused model systems emphasized by the NCI in its drug-screening program optimal for studies of tumor radiobiology and the tumor microenvironment?

Do the advantages of the use of human cells outweigh the added costs and biosafety problems inherent in the use of such systems? Do the problems raised by the immune deficits (and sometimes repair deficits, for example, in SCID mice) of the hosts and by the tumor host incompatibilities inherent in xenograft systems outweigh the advantages offered by the use of human tumor cells? Is implantation site (*e.g.*, subcutaneous vs. orthotopic transplantation) important for these studies? Are there advantages to the use of spontaneous rodent (or large animal) tumors and early transplanted generation tumors or well characterized, murine tumor cell lines in syngeneic rodents for some radiation biology studies?

3. Can we develop and validate more user-friendly methods for determining tumor oxygenation?

Tumor hypoxia may exert a powerful, negative influence on the treatment of many human malignancies. The identification of patients with hypoxic tumors for treatment strategies designed to ameliorate or exploit hypoxia represents a potentially important benefit of such pretreatment measurements of tumor oxygenation. Current electrode measurement techniques are cumbersome and not readily available to most investigators. Development of more user-friendly methods of measuring oxygen would facilitate larger, more rapid accrual into studies evaluating new treatment strategies. Also, techniques that lead to a better understanding of the physiological/metabolic mechanisms of hypoxia (oxygen supply vs. consumption) will facilitate the rational development of these strategies.

4. Does hypoxia predict for resistance to chemotherapy in the clinic?

There is compelling evidence from preclinical studies that such cells are resistant to conventional chemotherapeutic drugs both because of the issue of drug concentration and because hypoxic cells also tend to be nonproliferative. Although this question is not specifically addressed to radiotherapy, it is important in considering chemotherapy–radiotherapy interactions.

5. Does tumor hypoxia cause increased tumor aggression and metastasis and if so, how?

A number of clinical studies have shown association between tumor hypoxia and the development of distant metastases. A component of the decreased local control in hypoxic tumors to radiotherapy could also be the result of increased tumor aggressiveness—for example, by increasing the fraction of clonogenic or proliferating cells. It is

extremely important to know which, if any, of the aspects of the tumor microenvironment are responsible for this.

Infrastructure support needed that is common to both areas

1. Development of a website

This would include information on ongoing translational trials, of methods of discussing possible ideas for trials and for explaining funding opportunities.

2. Organization of tissue (urine and plasma) acquisition
3. Workshop for education in clinical trials

This would include information on such aspects as trial design, biopsy procedures, and methods to store material.

4. Establishment of a Laboratory Network

This should include seed money to permit an investigator at one institution to ask another investigator elsewhere to run a lab test or drug assay.

NCI support for the scientific side of collaboration between the radiation research community and the pharmaceutical industry

Many pharmaceutical companies do not appear to be interested in supporting scientific studies related to their compounds that are not directed at obtaining an FDA approved indication for the use of the compound. However, it is often the case that the drug could be used more effectively if its mechanism were better understood. Although sometimes this type of work can be funded through an RO1 mechanism, often the task is not sufficiently basic science driven to do well in a traditional study section. A funding mechanism that could support this effort (perhaps requiring some matching funds from the company) could be useful to permit this type of research.

NORMAL TISSUE EFFECTS (CHAIR—PAUL G. OKUNIEFF, M.D.)

Normal tissue tolerances to irradiation and to combined-modality therapy, are critical determinants of eventual success of all newly developed therapies. The α/β formulation has been the best estimator of late effects, and has driven the development of several successfully completed studies on time dose and fractionation. This approach, however, does not allow a physician to identify, pretreatment, individual patients at high risk for acute or chronic side effects and provides no targets for prevention or alleviation of toxicity. Further, it does not explain the highly variable expression of toxicity between patients. More recently, several studies in preclinical models have shown that late effects are a balance between cell killing and inflammatory processes. The former predominating in acute reactions and the later possibly dominating in late effects. The theme of translational studies over the next few years therefore must unravel the causes of radiation toxicity other than fractionation and dose.

The goal of late effects prevention differ from most therapeutic studies of new treatment for cancer. For example, it is rare that a Phase I study would be recommended to prevent toxicity. Feasibility studies are possible in combination with therapeutic trials, however, it is not recommended that a dangerous clinical trial be designed to test a protective agent. Similarly, it is unlikely the chronically manifest toxicity will be easily or completely reversible, thus, prevention strategies are expected to be more successful that treatment strategies. The low risk of severe acute toxicity in current therapy, and the weak tools available to consistently measure toxicity less than Grade IV, make it necessary to identify high-risk subsets of patients on whom to focus translational studies.

The broad translational goals of therapy therefore must be one of the following:

- Develop clinically relevant scoring systems that can be used consistently by physicians and that provide the detail needed to unravel organ specific mechanism. Clinical specimens will be needed, and protocols must be designed with a goal to evaluate scoring systems.
- Measure the extent to which molecular determinants of cell killing and tissue inflammation modify the tolerance to radiation. Identify subgroups of patients at high risk for toxicity, with the eventual goal of selecting those patients for protocols with preventive therapies.
- Test strategies for prevention and treatment of radiation toxicity using drugs that have preclinical evidence of success such as ACE, anti-TNF, FGFs, COX2, and sulfhydryl drugs.

Radiation toxicity scales for scoring of acute and late toxicity were first developed over 50 years ago and have been in international use since 1980. The NCI has recently established an effort to develop acute toxicity scales that are broadly appropriate for combined-modality therapy and that have recently gone into clinical testing. Internationally used late toxicity scoring systems were developed by the RTOG/EORTC in 1980, and have been in the process of revision. New scales that are clinically useful and provide sufficient information for correlative studies with molecular mechanisms have been developed and are in clinical testing by the RTOG and ECOG. Ultimately, late-toxicity scoring systems will be needed for chemotherapy and surgery as well.

A normal irradiated-tissue bank collected from prospectively treated patients, which could be dispensed to investigators for research purposes, would be useful. Tissue should be encoded with a common toxicity criteria and include both plasma and DNA. Patients treated to different anatomic sites must be collected in sufficient numbers to detect genetic and protein level differences. Because late toxicity is observed primarily in cancer survivors, data repositories could be accumulated rapidly.

Genetics of radiation repair and cellular killing require greater information than currently available. There are some well-defined genetic markers of radiation response and experimental evidence from SF2Gy analyses that markers will

be found that predict for individual variation in intrinsic sensitivity to radiation toxicity. Current targets include, EGFR, ATM, RB, Ku70/80, and others. Ultimately gene chip arrays should be useful in identifying haplotypes, polymorphisms, and mutations predisposing patients to toxicity.

Inflammatory and fibrovascular response contributes greatly to the development of late and acute radiation toxicity. Its impact has recently been studied with observations that promise to identify powerful methods of prevention. The mechanisms of inflammation are, however, not fully understood.

Modification of toxicity in animal models, and in patients, is being seen with anticytokine therapies including counteracting cytokine injection, blocking antibodies, and drug inhibition. The impact of cellular components of inflammation has not been studied and the role of apoptosis is not fully understood; only a few individual organs have been studied. Emphasis should be placed on organs most limiting radiation delivery including the lung, liver, brain, and small bowel.

Toxicity from combined-modality therapy will become increasingly important as new agents are taken into Phase I and II trials and then used as standard combined modality therapy. Many cytotoxic drugs, such as doxorubicin and bleomycin, are known to have synergistic toxicity with radiation. Care must be taken to study the effects of combined therapies on normal tissues to assure new agents offer selective sensitization of tumor.

The RTOG has had great success in the study of late toxicity for patients with cancers at certain anatomic locations, and has successfully run several studies aimed at toxicity prevention. There are many new drugs that deserve study including COX2 inhibitors, antibody therapy against cytokine targets, cytokine suppressing drugs, and FGF7. These should be tested for both demonstrable toxicity and for toxicity prevention.

The NCI can play the following role in assisting the performance of the above mentioned research:

- Assist in convening the LENT III conference with the EORTC for next spring or summer to review data collected on the LENT II scales and to update the LENT II scales.
- Create a consortium of institutions to measure toxicity in a consistent manner and create a database on serum and DNA for future gene array and protein array analyses
- Provide financial support for national groups to collect and centrally store and analyse specimens and toxicity data from protocol treated patients
- Make development of molecular chips that are particularly relevant for detecting markers of inflammation and DNA repair a high priority for funding
- Facilitate discovery of new compounds based on preclinically identified targets and their ligands. Drug manufacturers currently have little interest in developing radiation protection drugs. Drug development could be augmented though the NCI drug development program, or though influence of the NCI on the priorities of drug companies.

CONCLUSIONS FROM THIS WORKSHOP

The application of molecular mechanisms will play an increasing role in the treatment of cancer with ionizing radiation. These will be involved not only in enhancing tumor response but also in reducing normal tissue injury. A great many questions remain unanswered, however, that are best addressed through more canonical research methodology. These include development of microenvironmentally activated cytotoxic/cytostatic drugs or radiation modifiers and the discovery of promising new sensitizing/protective compounds through conventional screening. The study of normal tissue effects will assume increasing importance as combined chemotherapy–radiotherapy becomes more effective but also more toxic.

The Workshop summary was prepared from the reports Working Group Chairs by Drs. Cumberlin and Coleman.

ADDENDUM TO TRANSLATIONAL RESEARCH IN RADIATION ONCOLOGY WORKSHOP REPORT (UPDATE: JUNE 20, 2000)

Since this Workshop was held, the RRP has taken the following preliminary steps toward implementing some of the recommendations. Updates will be made in the RRS and ASTRO newsletters and on the RRP website:

1. A proposal is being considered by DCTD to develop a Radiation Modifier Evaluation Module (RAMEM) at the FCRDC at Ft. Detrick. This would include some screening of new compounds and *in vitro* and *in vivo* evaluation of radiation–drug interaction.
2. We have a new RRP website (http://www.nci.nih.gov/rrp/) that will include Workshop reports and other information. Among the projects for the Website will be the inclusion of information for the radiation research community as to new agents under development.
3. The RRP is holding discussions with a number of academic departments that are considering a multi-institutional PO 1 grant for translational research.
4. Future RRP workshop will address hypoxia imaging and detection, and late effects, among others.

APPENDIX I. WORKSHOP PARTICIPANTS

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