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Decreasing the adverse effects of cancer therapy: Report from a NCI workshop on the preclinical development of radiation injury mitigators/protectors

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Introduction

Radiation therapy is used to treat a broad range of malignancies. The adoption of conformal treatment techniques such as intensity modulated radiation therapy (IMRT) and image guided radiation therapy (IGRT) has reduced the amount of radiation delivered to normal tissue surrounding the targeted tumor. However, the irradiated normal tissue toxicity still limits the dose that can be administered and contributes to treatment-related morbidity and decreased quality of life. Therefore, there is a need for therapeutic agents that selectively reduce radiation-induced normal tissue injury without reducing tumoricidal effectiveness, thereby increasing the therapeutic ratio of radiation therapy. Additionally, the use of these agents is expected to improve patients' quality of life through the reduction of treatment-related toxicities.

Over the past few years, the United States has increased efforts to develop countermeasures to protect people against the effects of radiological/nuclear terrorism. Agents administered following radiation exposure but before symptoms or organ toxicity occurs are called mitigators, while those given prior to radiation exposure are called protectors (1). The development of radioprotectors was largely focused around pioneering studies of Dr. Harvey Patt, who demonstrated that cysteine protected rats from lethal whole body radiation (2). Consequently, the Anti-radiation Drug Development Program of the Walter Reed Army Institute was established with the primary intent of identifying and developing radiation protectors for military use. A positive spin-off for clinical radiation oncology was the development of what is currently the only FDA approved radioprotector, amifostine (S-2[3-aminopropylamino-] ethylphosphorothioic acid).

The National Cancer Institute (NCI) supports research and development of radiation modifiers for use in cancer treatment. More recently, the National Institute of Allergy and

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Infectious Diseases (NIAID) has a research and development of program for radiation mitigators/protectors for nuclear or radiological disasters through the Centers for Medical Countermeasures against Radiation (CMCR) (3–6). The mitigators may potentially be useful in reducing the normal tissue injury from radiation therapy, which fits with the “dual-utility” philosophy of the Department of Health and Human Services of developing drugs for terrorism that also have a routine clinical use. In order to implement the use of such agents in cancer care, the agent must not protect tumors from radiation, or do so to a much lower extent than the reduction of normal tissue injury.

This NCI Workshop commentary offers preclinical guidance to help in the selection and development of radiation mitigators/protectors to be used in cancer therapy. Additionally, this commentary provides investigators with a potential blueprint for preclinical development of promising agents for cancer clinical trials (Figure 1). In order for radiation mitigators/protectors to be used in the clinical radiotherapy, the agents should: a) ameliorate toxic effects of radiotherapy; b) not decrease or inhibit the anti-tumor efficacy of radiotherapy; and c) have minimal and favorable toxicity profile (7). A complementary article discussing the key issues involved in the design of clinical investigations for such agents is in *Clinical Cancer Research* (8).

Initial Step: Selection of candidate agents

Investigators can screen for potential candidate agents using data acquired from the literature and retrospective epidemiological studies. Other sources for candidate agents include pharmaceutical companies, academic or biotechnology laboratories, the Cancer Therapy Evaluation Program (CTEP) and the Developmental Therapeutics Program (DTP) of NCI, and the CMCRs of the NIAID. From a novel drug development perspective, a candidate agent must satisfy at least one of the following Selection criteria (Figure 1) to be considered for Stage I preclinical testing and evaluation: 1) general protection/ mitigation of normal tissue from radiation damage; and/or 2) organ-specific protection/mitigation of normal tissue from radiation toxicity. Alternatively, the candidate agent should skip to the most appropriate stage (i.e., Stage II or Stage III) for continued preclinical evaluation, if the selected agent is a known drug with fully documented prior preclinical testing (i.e., known toxicity and maximum tolerated dose) and reasonable rationale for radioprotective or mitigative function.

Stage I: Assessment of toxicity and maximum tolerated dose

In Stage I, the maximum tolerated dose (MTD) and toxicity of the selected agent should be determined through a series of toxicity studies in accordance with Good Laboratory Practices/International Organization for Standardization (GLP/ISO). Candidate agents with prior FDA approval for other medical indications will not undergo toxicity studies, unless the intended route of administration is different than the FDA-approved route. Additionally, a substantial change in administration schedule may also require determination of the MTD. The MTD must be determined *in vivo* for the projected route of administration(s) using single ascending and multiple repeated dose studies, as appropriate. Correlation of MTD with pharmacokinetics will be important in determining the minimum effective dose (MED) that is predicted to be well-tolerated. Similar to standard preclinical drug testing, animals (survivors and non-survivors) should be observed (suggested at least 6 months) with documentation of any signs of toxicity. Histopathology of tissue (e.g. liver, gut, spleen, lung) and clinical chemistry analyses of bloods may aid in clarification of toxicities. Preliminary pharmacokinetics and biodistribution, such as peak concentration of candidate agent and/or metabolites in plasma and tissues, tissue-specific accumulation kinetics, and

mode of clearance from the body, should be determined. Overall, these data will aid in the determination of the dose for the proceeding stages of development.

Stage II: Determination of radiation mitigation & tumor protection activity

In Stage II, candidate agents undergo a battery of *in vitro* and *in vivo* testing to verify their mitigative or protective effect on normal tissue, not tumors, and to identify the biological mechanism of action. Experiments should be performed using the MED or a range of tolerable doses, as well as the route and timing of administration determined for the candidate agent. Proposed clinical use of the agent may influence the dose and fractionation of radiation to be employed. As needed, the agent may undergo additional *in vivo* testing incorporating chemotherapy to assess its potential use in the setting of chemoradiation therapy.

Drug effects in normal tissues

The candidate agent must demonstrate radiation mitigation in at least one organ *in vivo* in an appropriate animal model. During this stage, it will be determined whether or not the candidate agent mitigates and/or protects one specific normal tissue or multiple normal tissues from radiation toxicity. The normal tissues typically studied include gut, skin, lung, kidney, central nervous system/brain, and bone marrow. The assays used may be different for each organ system, but must be a widely-accepted and a peer-review published *in vivo* model for radiation toxicity for the specified organ. Since the focus of the drug is cancer therapy, the normal tissue experiment should focus on the organ(s) of interest and involve targeted radiation. Examination of the effectiveness against hematopoietic cell death would involve the determination of the survival benefit against whole body irradiation.

The effectiveness of the agent will be characterized by determination of the radiation dose reduction factor (DRF) for each normal tissue under consideration. The candidate agent should demonstrate a statistically significant reduction in radiation toxicity for the specified organ. The FDA may require additional assays for drug approval, such as carcinogenicity studies, if they were not already performed as part of the drug development process (9). Effective radiation mitigators/protectors might allow the radiation oncologists to increase the radiation dose with the aim of improving the probability of tumor cure. Therefore, the normal tissue complication curve for each agent should be determined with escalating doses of radiation that might be used clinically to ascertain if normal tissue protection occurs at the higher radiation doses.

Radioprotection of tumors

The radioprotective effect of the candidate agent on tumors should be determined using *in vivo* human tumor xenograft mouse models. Subcutaneous or orthotopic xenograft models using human tumor cell lines or fresh human tumor tissue are acceptable (10). *In vitro* clonogenic survival assays are recommended, but not required, for initial tumor protection screening and elucidation of the biological mechanism of novel candidate agents. Clonogenic assays should be performed on at least two (if available) relevant human cancer cell lines (e.g., MDA-MB-231 and BT-20 cell lines, if the agent will be used in patients with breast cancer). Investigators may choose to use the NCI-60 panel of human cancer cell lines; however, XTT/MTT assays are not considered a valid method to investigate tumor survival.

The rate and extent of accumulation of the candidate agent in tumors will be determined and compared to previously observed plasma and normal tissue concentrations under similar dosing/administration conditions. If the candidate agent has shown tumor protection against a certain type of tumor, as well as normal tissue protection, it should be tested against additional types of tumors using the xenograft tumor model. After additional testing, if

tumor protection is observed in only one tumor type, the candidate agent may continue for development for specific cancer diagnoses in which it did not demonstrate tumor radioprotection. If any statistically significant protection is observed *in vivo* in more than one type of tumor, further consideration of the candidate agent should be discontinued (unless the amount of normal tissue protection is substantially better). If significant tumor protection is not observed in any tumor model, development of the candidate agent may continue. It is possible, although not likely, that the candidate agent would demonstrate effectiveness as a radiosensitizer in tumors, which would potentially enhance the therapeutic effect of radiation therapy. Conceivably, an agent could both protect normal tissues and sensitize tumors. If an agent sensitizes tumors to radiation, higher radiation doses may not be necessary to improve the probability of tumor cure. The development of such an “ideal agent” as an anti-cancer therapeutic in combination with radiation therapy should continue on a parallel path.

Biological Mechanism of Action

Identification of the mechanism of action of the candidate agent for radiation mitigation/protection of normal tissue would enable classification of the agent into categories (i.e., DNA repair, free radical scavenger, mitochondrial stabilizer, cytokine blocker, stem cell regeneration promoter, etc). For some candidate agents, the biological mechanism of action may have been previously identified. If biological mechanism of action is known, additional studies may be performed to confirm that the presumed pathway is responsible for radiation mitigation and/or protection. If the mechanism of action is unknown, then appropriate *in vitro* and *in vivo* assays are encouraged to elucidate the radiation mitigative/protective function in normal tissues. In some cases, a candidate agent may proceed to the next stage of development if the biological mechanism is not definitive. For instance, if the candidate agent has minimal toxicities and experimental data supporting multiple potential mechanisms of action, the hypothesized biological mechanisms could be investigated in parallel with drug formulation and preliminary clinical trial testing. A mechanism of action and a relevant biomarker would facilitate clinical development.

Stage III: Drug evaluation, production, and formulation for clinical trials

Stage III assures that comprehensive toxicological and pharmacological testing has been performed on the candidate agent before proceeding to clinical investigation. Selected candidate agents already in use for other clinical indications may proceed directly to clinical development through Investigational New Drug approval (IND) through the FDA, although studies specific for the role of the drug as a radiation modifier may be necessary. In addition to conducting studies for FDA approval, Stage III should be used to investigate the feasibility of large-scale production (i.e., quantity desired) of a suitable dose formulation for the candidate agent.

Summary

The study of radiation mitigators/protectors for use in cancer treatment and management is complex as they must reduce normal tissue toxicity without reducing tumor cell kill. The known toxicology, pharmacokinetic, and mechanistic data for the proposed agent will direct investigators to the appropriate stage of preclinical development. Overall, we hope that this Workshop commentary facilitates the transition of radiation mitigators/protectors into clinical trials.

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Figure 1. Pathway for preclinical evaluation of novel agents

This schematic outlines the preclinical data required for novel radiation mitigators/protectors for implementation in clinical trials. A novel candidate agent should satisfy at least one of the final selection criteria to be considered for preclinical testing and evaluation.