The Emerging Biology of Modern Radiation Oncology

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If you want to cure Hodgkin’s Disease, you have to think like a Reed Sternberg Cell

Henry S. Kaplan

For decades, radiation oncologists concerned themselves with increasing technical expertise. Two revolutions took place. With the advent of the modern linear accelerator, strides in cancer control with acceptable morbidity have occurred for a range of localized cancers including but not limited to those of the cervix, vagina, breast, prostate, head, and neck; for lymphoma; and for other surgically inaccessible cancers in the mediastinum. Computer-assisted tomographic/magnetic resonance imaging-based computerized treatment planning created a second revolution which has quietly increased the quality and precision of radiotherapeutic practice. Three dimensional imaging may in time further refine treatment planning. A synchronous revolution in medical oncology has presented not less but actually more responsibility for the radiation oncologist. In a multitude of patients treated to eradicate microscopic metastatic disease, curative radiotherapy must now be designed for residual bulky disease.

For alternative beams to photons, such as the use of protons for tumors millimeters from the brain stem, advances have been made. In the United States overall, however, with its lack of centralized medical care, these beams have had major trouble with funding, machine problems, and referral. As well, inherent limitations in the sparing of normal tissue and problems in collimation and subsequent treatment planning have somewhat muted early enthusiasm for a range of particle beams. Clearly, some selected neoplasms, such as slow-growing salivary gland cancers, may be well served by them, but whether the cost will justify the case load for funding many units remains to be seen.

In contrast to these technological developments, biological research has flourished and this offers to again revolutionize our field. The introduction of quantitative cell biology techniques and subsequent capability for study of mammalian X-ray survival curves in the mid-1950s ushered in an era of biological investigation into the effects of radiation and in turn an increasingly better understanding of the cellular, biochemical, and molecular mechanisms that govern the radiation response. Influenced by scientists such as Kaplan, who realized the crucial importance of understanding cellular phenomena in cancer treatment, radiation oncologists and biologists have come to realize that future advancements in radiation oncology will come as the biochemical and molecular mysteries of both the cancerous and normal cell are unraveled.

In parallel with this goal, radiation oncologists have incorporated the use of concomitant chemotherapeutic drugs and specific radiation sensitizers. Conversely, the search for radiation protectors to minimize normal tissue toxicity has continued as well, with some degree of progress. Attempting to limit late damage to normal tissues while increasing damage to tumors has led to altered fractionation schemes. Twice a day schedules in total body irradiation for transplantology, in advanced head and neck cancer, and in limited stage small cell carcinoma of the lung are rapidly becoming accepted alternatives to traditional practice. Efforts to test tumor samples for cell cycle parameters conducive to specific fractionation schedules are under way. The potential for systemic radiotherapy, through use of radiolabeled monoclonal antibodies, is being extensively explored as a means of controlling metastatic disease. From the study of the cellular response to radiation, it should be appreciated that radiobiologists have made numerous contributions toward understanding the application of chemotherapeutic drugs, especially when combined with radiation. In the study of agents that impose oxidative stress, radiobiologists have played an important role in defining the delicate interlocking enzymes responsible for detoxification of cytotoxic oxygen-related free radicals.

Modern cancer radiobiology has identified a number of innovations toward improvement of radiation treatment; however, in this brief review we will focus on only four of many areas of investigation: the complex interaction of tumor and inherent cellular radiosensitivity; chemical modifiers of radiation response; biological modifiers; and therapeutic radioimmunoconjugates. For all our efforts, the oncological effort remains unchanged from decades ago: benefitting the patient by increasing the therapeutic index.

Inherent Cellular Radiosensitivity

Survival Curves

Shortly following publication of the first mammalian X-ray survival curve, X-ray sensitivities were determined for a variety of cell types including cell lines derived from both tumor and normal tissues, mostly of rodent origin. Rodent tumor cell radiosensitivity was shown not to vary significantly among different tumor types and other normal cell types. During the 1980s more emphasis was placed on establishing human tumor cell lines from different histological types. Recent radiosensitivity analysis of human tumor cell lines has revealed a relationship between the inherent radiosensitivity, expressed as $S_{2\theta}$, and the clinical responsiveness of the respective tumor type from which the cell lines were initiated (see Fig. 1) (2-4). In general, low $S_{2\theta}$ values correlate with greater clinical responsiveness. For example, small cell lung cancer is initially quite responsive to radiotherapy. In vitro X-ray $S_{2\theta}$ values for small cell lung cancer cell lines are -0.2. In contrast, cell lines derived from glioblastoma tumors, which are very unresponsive to radiotherapy, yielded $S_{2\theta}$ values that range between 0.5 and 0.6. Similar findings have been demonstrated for rodent tumors where in vitro and in vivo sensitivities can be readily assessed (5, 6). Fig. 1 shows that there is at best a 3-fold difference between the

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2 The abbreviations used are: $S_{2\theta}$, surviving fraction at 2 Gy; BSO, buthionine sulfoximine; GSH, glutathione; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-1, interleukin 1.
EMERGING BIOLOGY OF MODERN RADIATION ONCOLOGY

The patient died of an unrelated myocardial infarction and pathological examination of the “unchanged” mass revealed no viable cancer cells, only stroma. The extent of stromal, fibrotic remnant after radiotherapy can be seen as well in Fig. 3A, in a pelvic cross-section of a patient, who eventually died of metastatic disease, treated initially with irradiation for massive pelvic sarcoma. The tumor by computer-assisted tomographic scan showed marginal response to therapy. At autopsy, this large pelvic mass, as illustrated on microscopic

most “sensitive” and “resistant” tumor cell types. While a 3-fold difference might seem small, when one considers a conventional course of radiation therapy consisting of 30 fractions over 5–6 weeks, such a difference could equate into huge differences with respect to total tumor kill. Surviving fractions of 0.2 versus 0.6 can result in differences of up to 14 logs in cell kill over the course of treatment. Such differences can be made only as estimates because implicit in such extrapolations are assumptions that the surviving fraction will remain constant for each dose delivered and that repopulation of tumor cells between doses will not be significant.

Relating inherent cellular radiosensitivity data to a clinical parameter, such “responsiveness” of a tumor all too often generates confusion over the terminology used. In the clinical arena, terms such as “responsiveness” become intermingled with “sensitive” and “resistant.” The terms “sensitive” and “resistant” ideally should be strictly used to describe inherent cellular sensitivity to particular agents as derived from in vitro cell survival curves. Unfortunately, these words imply an absolute trait and do not connote relative degrees of a trait. Using well defined survival curve parameters, cell types can thus be compared on a quantitative basis. By contrast, “responsiveness” is a crude, unquantified clinical term used to describe the extent with which a tumor responds to treatment. Therefore, the term is oftentimes used to describe gross reduction in tumor size (or the antonym “unresponsive” to describe progression or no change) using semiquantitative tumor measurements over often unspecified times. The ultimate responsiveness of a tumor to therapy is the result of not only the inherent sensitivity of the tumor cells but also a host of other important factors including size of the tumor, physiological and nutritional status of the tumor, the actual dose of a particular modality delivered to the tumor cells, a variety of little understood processes of clearance of the cells following their death, etc. Some human tumors that are “unresponsive” clinically may, in fact, consist of inherently sensitive tumor cells which can be totally destroyed by treatment even though the mass may not “shrink,” especially when there is a large matrix or stromal component (7). In Fig. 2A, a retroperitoneal sarcoma is seen prior to intensive irradiation. Three years thereafter the mass remained essentially unchanged

Fig. 1. Surviving fraction following a 2-Gy radiation dose compiled from 101 human tumor cell lines derived from tumors with varying responsiveness to radiation therapy. A good correlation was observed between the SF2 of the particular tumor cell type and the clinical radiocurability of the associated tumor. Low SF2 values correlated with greater clinical responsiveness. Note that from the radioresponsive to highly curable tumor the difference is only approximately a factor of 3. (Data adapted from Ref. 2 with permission.)

Fig. 2. A, computer-assisted tomographic scan of the abdomen at the level of the left renal artery of a patient who had a primary liposarcoma of the left leg. The leg had been amputated and the patient was treated with 8 cycles of Adriamycin and Cytoxan and 6 cycles of high dose methotrexate. Four years after chemotherapy a nonresectable right retroperitoneal mass (outlined in arrows) was found at exploration. The patient received 2 cycles of I.V. continuous infusion 5-bromodeoxyuridine, 650 mg/m²/day for 14 days, interspaced with a 2-week break. Radiation treatment was given in 37 fractions at 1.5 Gy/fraction to a total dose of 55.5 Gy over 66 days. B, computer-assisted tomographic scan of the same patient 3 years after 5-bromodeoxyuridine/X-irradiation. The size of the mass (outlined in arrows) decreased only slightly, although the patient remained healthy. The patient died of a myocardial infarct ~3 months after the scan. Detailed histopathological examination of the tumor at autopsy revealed no malignant cells.
tase, catalase, GSH and GSH-related enzymes, protein thiols, and numerous other low molecular weight thiol-containing molecules present in low concentrations in the cell. Superoxide dismutase converts superoxide to hydrogen peroxide and catalase can detoxify hydrogen peroxide to water. These two enzymes should be important in the detoxification of toxic oxygen-related species that can be produced by radiation, yet no correlations have been demonstrated linking their intracellular concentration or activity with radiation sensitivity. It is difficult to determine if high intracellular concentrations of these enzymes can afford protection against radiation. Enzymes, due to their size, do not readily traverse the cell membrane. This problem makes experiments where exogenous enzymes are added to cells (or animals) for potential radiation protection difficult to interpret. Hopefully, solutions to problems such as...
these will come using DNA transfection techniques where genes (either in the sense or antisense direction) coding for specific enzymes may be introduced into cells. Cells thus manipulated to either up- or down-regulate intracellular levels (or activities) may provide an in vitro model of the enzyme in question. As gene sequences for particular enzymes thought to be important in intracellular radiation protection become available, approaches such as this will allow for a more precise means of evaluating the contribution of a given enzyme in the radiation response.

GSH, a small tripeptide molecule, assumes a pivotal role in numerous bioreductive reactions, transport, enzyme activity, protection from harmful oxidative species, and detoxification of xenobiotics (8, 9). GSH S-transferase and GSH peroxidase are two enzymes that act in concert with GSH and provide the cell with a powerful means to detoxify both drugs and toxic oxygen-related species produced by radiation. Sulphhydryl compounds such as GSH should afford radiation protection at the cellular level by either direct donation of a hydrogen atom to oxidized substrate molecules or detoxification of organoperoxides via GSH or S-transferase or GSH peroxidase. No direct correlations with intracellular GSH levels and inherent radiation sensitivity have been reported (10–12). These findings are consistent with GSH modulation studies where GSH levels within the same cell type are altered prior to radiation treatment. These studies have been made possible by the introduction of agents which “specifically” inhibit (BSO) or stimulate (oxathizolidine) GSH synthesis or directly introduce GSH (GSH esters) intracellularly (13–15). Modulation of GSH from 5 to 300% of control values has little influence on the aerobic radiation response (16, 17). Such observations are somewhat surprising, but perhaps if higher levels of GSH could be achieved more protection would be found. GSH is important in maintaining the reduced:oxidized ratio of thiol-containing proteins and thus may be indirectly involved in any protection these thiol-containing proteins afford. Currently attention is directed to the role of cellular protein thiols and the radiation response, since these thiols are present in much higher equivalent concentrations than GSH.

Oncogenes. Another focus toward elucidation of molecular pathways responsible for inherent radiation sensitivity is on oncogenes. Cell lines derived from clinically unresponsive cancers after treatment have shown suggestive evidence of radiore sistance in some laboratories (18), and investigators have further characterized some of these cell lines as having oncogene expression, specifically c-raf (19). Unfortunately, cell lines were not derived from the patient’s tumor prior to radiotherapy for evaluation of radiation sensitivity and oncogene expression. Recent reports indicate that the introduction of various oncogenes into 3T3 cells can confer radiation resistance (20), although transfection with vector without oncogene has discordantly produced cell lines of variable radiosensitivity (21). Combined v-myc and H-ras expression in primary rat embryo cells leads to more radioreistance than if either oncogene is expressed alone in the same cells (22). Other oncogenes appear to have the reverse effect (23). In cell culture, antisense oligomers to c-raf RNA products have been shown to reverse radioreistance (24). From an epidemiological standpoint, patients with Li-Fraumeni syndrome, with predisposition to a range of cancers, show increased radioreistance in their skin fibroblasts and increased c-raf expression (25). Activation of oncogenes has been implicated in a wide variety of human malignancies. Activated oncogenes and oncogene-encoded proteins can have dramatic effects at the cellular level on multiple systems. It is not clear presently exactly how oncogenes affect inherent sensitivity to radiation. Possibilities include alterations in repair systems, intracellular detoxification systems, or chromatin structure.

Search for Specific Repair Genes. The fact that cell and tissues can repair radiation damage was known for many years prior to its quantitation at the cellular level (26). Radiation damage repair most likely is an enzymatic process. The enzyme(s) responsible for repair has not been identified. To identify “repair genes” and their products is not trivial given that repair systems probably do not differ markedly between tumor and normal cells. One approach to identify repair genes is the use of mutants that have been selected for extreme sensitivity to a given modality. Radiation-sensitive mutants have been isolated (27, 28). Transfection of such mutant cell lines with normal DNA and selection of subsequent clones with restored normal sensitivity are the first steps toward identifying repair genes. There has been some success toward this end for UV-sensitive mutants (29, 30); however, the identification and characterization of specific mammalian repair genes for X-rays have remained elusive to date.

Inherent cellular resistance to radiation could (and perhaps does) pose a formidable interference toward effective therapy. Modern DNA technology will hopefully add to our understanding in the near future as to which gene(s) is instrumental in governing the sensitivity of cells to radiation. Knowledge of the relevant gene or genes will allow for the identification of specific proteins; however, the manipulation of their discrete functions will still require a considerable amount of understanding of cellular biochemistry and tumor physiology for development of beneficial cancer treatment approaches.

Cross-Resistance between Chemotherapy and Radiation

Clinicians have often questioned whether cells that are resistant to cytotoxic drugs might also be resistant to radiation. There are clinical situations where a proportion of patients who fail chemotherapy also fail radiation therapy and vice versa. Likewise, there are patients who may fail all treatments. Many investigators attribute treatment failure to the inherent resistance of the tumor cells to the agent being used; however, as discussed above there may be many explanations for treatment failure apart from inherent cell resistance. The radiation sensitivity of in vitro cell lines that are resistant to a variety of drugs has yielded conflicting results. Cell lines resistant to Adriamycin (12, 31, 32) and cisplatin (33) and a multidrug-resistant cell line (11) exhibit radiosensitivities similar to those of parental cell line from which they were derived. The interpretation from these studies is that drug resistance does not necessarily confer radiation resistance. However, there are a few examples of cell lines resistant to cisplatin and melphalan that exhibit approximately 1.5-fold radiation resistance (based on SF2 values) compared to the parental cell from which they were derived (12). However, resistance in vitro does not always predict for resistance in vivo (34). More work in this area is needed to resolve these differences.

Conversely, a related consideration is whether radiation treatment might result in altered sensitivity to chemotherapy. There are a few in vitro reports where prior radiation exposure significantly increased the incidence of methotrexate-resistant clones (35–37). A recent report by Hill et al. (38) demonstrated that fractionated radiation treatment to Chinese hamster cells
duced drug resistance to a variety of drugs and also induced production of the p170 glycoprotein associated with multidrug resistance. Resistance induced by radiation in these studies was orders of magnitude less than that seen when the cells are induced to resistance by chronic exposure to cytotoxic drugs (38). The mechanism and implications of these observations are unclear. Certainly in the clinic this could conceivably occur and radiation could lead to changes in inherent cellular sensitivities (as a result of genomic alterations) to subsequent chemotherapy. Alternatively, radiation treatment could alter the tumor vascular supply, tumor bed, and other physiological determinants that could compromise the delivery of the drug to the clonogenic tumor cells. Much work will be required in this area to extrapolate the tumor biology of these new findings.

Chemical Modifiers

Radiation Sensitizers. Radiation biologists have labored for many years to identify agents that sensitize tumor cells to radiation. Much effort has gone into the development of agents that can selectively sensitize hypoxic cells to radiation (39). It is well documented that hypoxic cells are significantly more resistant to radiation than fully oxygenated cells (40), are present in rodent tumors (41) and most likely in human tumors (42–44), and may therefore represent a barrier to effective treatment. The best studied of this class of sensitizers are the nitroimidazoles including misonidazole which has been extensively evaluated in the clinic. Clinical trials with misonidazole in several tumor sites have not shown dramatic effects, although some positive results are available (45). Of significant concern was the dose-limiting neurological toxicity of misonidazole which compromised the obtaining of adequate tumor concentrations for maximal radiosensitization (46). Newer nitroimidazole sensitizers such as SR-2508 (47) and Ro-03-8799 (48) have been developed to lessen neurological toxicity while affording equivalent tumor radiosensitization and are presently under clinical evaluation (49–51).

Another potential avenue for clinical exploration that could lead to further enhancing radiosensitization by nitroimidazoles is the use of BSO to deplete tumor GSH levels. Both in vitro and in vivo studies have clearly shown that GSH depletion potentiates nitroimidazole radiosensitization (52, 53). BSO has recently entered Phase I clinical trials as a means of increasing melphanal tumor cytotoxicity and therefore will be available for possible use in radiation sensitizer trials.

Other significant clinical findings emerged from work with nitroimidazoles. In addition to the hypoxic radiosensitization properties of misonidazole, early studies showed that the agent was selectively toxic to hypoxic cells (54). The possibility of directly treating hypoxic tumor cells has led to a search for more efficient bioreductive drugs. New agents under evaluation are SR-4233 (55), RSU 1069 (56), and porfiromycin (57). All of these agents exhibit significantly more toxicity toward hypoxic cells than aerobic cells and have shown efficacy in solid tumors in experimental animals. The development and ultimate clinical use of these agents represent a new chemotherapeutic strategy aimed at a specific tumor cell population that has proved difficult to kill with radiation alone. Again, the radiation oncologist and medical oncologist have intertwined concerns because hypoxia can as well be a biological source for chemotherapy resistance (58, 59).

The revival of the use of halogenated pyrimidines which are selectively taken up in rapidly proliferating tissue shows promise in several tumor types commonly thought to be incurable with radiotherapy, including massive unresectable sarcomas (60–62). In order for halogenated pyrimidines to sensitize cells to radiation, they must be incorporated into cellular DNA. In the case of human tumors, which have in general rather long cell cycle times, this may require many days of continuous drug infusion to achieve adequate replacement. In vitro studies support the relationship between the extent of radiosensitization and the percentage of thymidine replacement (63). More information and research are needed regarding the percentage of thymidine replacement achievable by halogenated pyrimidines in human tumors and how it relates to treatment outcome.

Combination Chemotherapy and Radiotherapy. The combined use of chemotherapy and radiation therapy in cancer treatment would seem to be a logical and reasonable approach. Local control of the primary tumor mass has been achieved by high dose radiation therapy combined with systemic chemotherapy in place of surgery, with the hope as well of control of metastatic disease. Chemotherapeutic drugs appear to enhance the effects of radiation, making the integration of both modalities even more appealing. The experience has been in most experimental models and in some clinical trials that when drugs and radiation are combined simultaneously, normal tissue toxicity is enhanced (64–66). Research continues in this area with an emphasis on gaining a better knowledge of the optimal timing of each modality and mechanistically how each agent interacts (67).

In practice, the most widely utilized chemotherapeutic drugs from the radiation oncologist’s perspective have been agents such as Adriamycin, cisplatin, and 5-fluorouracil. Clinical trials in a range of locally advanced tumors included those of bladder, head and neck carcinomas, and extremity sarcomas. The combined effect of drugs and radiotherapy in these settings has resulted in increasing local normal tissue reaction to some extent, probably increasing local control, while having an uncertain role in adding to control of distant metastases. A major benefit of combined modality therapy has been the circumvention of major surgery in such regions as the anus and esophagus, with no obvious decrement in survival (68, 69).

Radioprotectors. Yet another means of obtaining a therapeutic advantage is the selective protection of normal tissues within the radiation treatment field. The discovery that aminothiols afforded protection against lethal whole body radiation in animals (70) prompted a massive synthesis and screening effort by Walter Reed Army Institute of Research. Emerging from this screen was the phosphorylated aminothiol, WR-2721, which showed promise of providing selective radioprotection (71). Subsequent laboratory studies have shown that a number of variables can influence the effectiveness of WR-2721 (72) and also complicate its use clinically. Administration of WR-2721 prior to each dose in fractionated radiation therapy was tolerated in a Phase I trial (73). Interestingly, WR-2721 may have application in protecting bone marrow from chemotherapy toxicity. Clinical trials evaluating WR-2721 in conjunction with cisplatin in the treatment of variety of tumors have shown encouraging results in mitigating nephrotoxicity, neurotoxicity, and hematological toxicity (74).

Another use of radioprotectors that may forego the complications associated with systemic administration is focal application. Used in this manner, protectors might be directly applied to specific organs prior to radiation treatment to the skin overlying tumor, inside various organs, and on the surfaces of mucosa. Cutaneous protection of skin using this approach has already been demonstrated (75).
Biological Modifiers

Cytokines. Advances in biological modifiers of the hematopoietic progenitor pool offer several new strategies for the radiation oncologist. The cytokines GM-CSF and erythropoietin are current examples of what can be brought to the clinic. The identification and subsequent cloning of GM-CSF offer promise in states of granulocytopenia that are commonly seen in today's multimodality-treated radiotherapy patients. GM-CSF affords radioprotection before and permits hematopoietic granulocyte reconstitution after total body irradiation in several animal models (76, 77), as well as humans (78). GM-CSF has shown promise in comparison to other cytokines when used after LD$_{100}$ exposure in animal models, including primates (79). Cytokines like GM-CSF may permit shorter treatment breaks for neutropenia, during which tumor regrowth is a fundamental problem in adequate delivery of curative radiotherapy (80–82). In addition to compromising effective radiation delivery, increasing numbers of patients are placed at risk for infection secondary to neutropenia. The hope that multi-potential CSF (II-3) would offer a means of increasing both granulocyte and platelet counts has yet to be established (83). II-1α may prove to be of clinical utility in this regard (84), although its delivery and side effects are potentially more complicated for a group we hope to maintain as outpatients. Of note, II-1 appears to enhance the radioprotective effects of WR-2721 (85).

Erythropoietin as a stimulator of RBC production and erythrocyte longevity may have utility for radiotherapists. Anemia represents a major clinical problem for the delivery of effective irradiation; it remains an independent poor prognostic variable in regression analysis of local control, and its correction with transfusion has been shown to be of benefit (86–88). The most popular model for poor radiation response with anemia suggests that low hemoglobin levels translate into lower tumor oxygenation with subsequent lessening of oxygen-related radiation sensitization (89). Anemia is common in radiation oncology patients and represents a medical radiotherapeutic concern.

Other Biologicals. The biological modification of tumor and normal tissue with other agents including tumor necrosis factor, tumor angiogenesis factor, and epidermal growth factor may offer means for beneficial manipulation of the radiation effect (90–94). The veritable explosion in the discovery of biological modifiers, and their recombinant synthesis and subsequent availability in purified form, challenges the radiation oncologist to make practical use of them; the horizon is broad and rapidly growing. The future array of possibilities is vast and may encompass biologicals that slow turnover rates of tissues susceptible to radiation damage because of rapid proliferation, and other agents that might speed rates of repair to tissues acutely damaged by radiotherapy. Such agents may increase our therapeutic index by selectively modulating normal tissue without major effects on tumor behavior or by cell type-specific modulation of localized tumor activity without markedly affecting the normal surrounding tissue. The central paradigm of a therapeutic index remains, but the tools for altering it are changing rapidly.

Radioimmunotherapy

Since Ehrlich first made the suggestion of a "magic bullet," improved specificity of the preferential killing of tumor cells by immunotherapy has been sought. The practical development of monoclonal antibodies against tumor cells, following the seminal report of Kohler and Milstein (95), permitted mass production of antibodies of predetermined specificity. The more recent preliminary production of human monoclonal antibodies from genomic libraries (96) gives further refinement to targeting specific cell membrane antigens with antibody or antibody fragments. Generally, localization of antibodies on the surface of cells will not destroy the cells, unless there is associated complement fixation, although there have been exceptions. Using radioisotopes rather than toxins or drugs to arm the antibodies has the advantage that the isotope has only to be in close proximity to neoplastic cells, whereas toxins or drugs require internalization to destroy cells.

The initial studies of Goldenberg et al. (97) with polyclonal anti-CEA antibodies were shown to image large tumors when labeled with $^{131}$I. These early imaging trials were then followed by Order et al. (98) with $^{131}$I-polyclonal antibodies directed against ferritin for the treatment of hepatoma. The early results looked promising; however, randomized studies did not show any significant contribution from antibody therapy (99). With the advent of monoclonal antibody technology, attention was directed toward lymphoma, as well as solid tumors. Initially, trials focused on reagents acquired from tumor immunologists and, therefore, concentrated on melanoma. No great clinical successes were forthcoming (100).

As other tumor antibodies became available, efforts were launched in the treatment of ovarian cancer, colon cancer, neuroblastoma, and even gliomas. Concurrently came a number of anti-lymphoma antibodies, most of which were unfortunately not truly specific for tumor but in general were capable of recognizing the nonmalignant precursor cell. The broad specificity of these antibodies is demonstrated by antibodies such as T-101, which recognizes the CD5 antigen on the surface of normal T-cells, as well as rare B-cells. Interestingly, this antigen is also found in low concentration on some low grade B-cell lymphomas and B-cell chronic lymphocytic leukemia. Most specific of the anti-lymphoma antibodies are the anti-idiotypic monoclonal antibodies, developed by Levy and Miller (101), and used with mixed success, although these antibodies were not armed with isotope, toxin, or drug. A variety of other anti-lymphoma antibodies have shown intermediate specificity (102–104).

It is possible that there may be some guidelines for future success. Clearly, two of the most important parameters in these investigations are the radiosensitivity of the specific neoplasm and favorable biodistribution of the antibodies. In animal models, where injected dose/g can reach as much as 25%, tumor therapy of heterotransplanted human tumors is possible. In humans, the best localization studies are 2 to 3 orders of magnitude less! One might therefore focus on situations when tumor is easily accessed by the antibody, most notably leukemia/lymphoma when there are circulating cells, as well as localization in specific cavities (peritoneum, pleura, and intrathecal space). Multiple injections are likely to be needed for therapeutic efficacy; this in turn creates the problem of reactive human antibodies to the therapeutic antibodies. This is likely to occur if there is no immune deficit, such as may occur in many lymphomas. To offset this difficulty, molecular biology has already shown promise in reports of decreasing immunogenicity with human chimerized antibodies (105). As an additional benefit, the biodistribution and the biological activity of antibody can be altered as the isotype is changed, or other areas of the constant portions of the molecules are altered. Both of
these potentials are likely to yield better engineered antibodies for radioimmunotherapy and other radioimmunoconjugates.

Originally, the choice of isotope was limited to $^{131}$I; however, there are now trials that utilize $^{188}$Re and $^{60}$Y. The advances in chelation chemistry (106) are likely to provide further choices of isotopes, tailoring the energy half-life, and type of emission to the specific clinical situation (Table 1). $^{131}$I is almost certainly a suboptimal choice for arming antibodies in part because of high energy $\gamma$-emission and since standard methods of iodination produce products that are easily dehalogenated over time. For the time being, chelates appear necessary to link isotope to antibody. It should be recognized that if the isotope and the antibody become disconnected, then no benefits are to be expected, only toxicity. The $\alpha$-emitting isotopes have been used in animal trials; and, although difficult to manage because of their chemistry and short half-life, they are likely to be incorporated into the clinical trials in the future (107). The appropriate choice of target will prove critical because the isotope must localize quickly. Leukemias therefore appear an optimal target.

The toxicity of these compounds is most difficult to predict. The quality of the chelate is crucial to a stable and "safe" radioimmunoconjugate (108). Once proved effective in this setting, $\alpha$-emitters with their single-hit kill capability are likely to be useful in the setting of micrometastatic disease. However, a greater understanding of the mechanisms by which the monoclonal antibodies may escape the vascular system (which is designed physiologically to keep proteins inside the vascular system in order to maintain osmotic pressure) will have to be obtained.

In some cases, the toxicity might be used to advantage, as in the case of bone marrow transplantation, where bone-localizing properties of yttrium may provide a more effective way for preparing the marrow. This could be used effectively not only in the setting of lymphomas and leukemias but also in neuroblastoma and other childhood neoplasms, with major prediction to involve bone marrow.

Biological response modifiers are also likely to play a role both in mitigating against some of the toxicity as well as by enhancing the efficacy, as demonstrated by the ability of interferon to up-regulate tumor antigen expression (109). Antibodies may not be the only way of seeking out tumor cells; other substances, such as growth factors, may also serve such functions.

Although early radioimmunotherapy trials have not yielded a success, the promise of this form of therapy for appropriate tumors continues to be attractive. The ability to humanize antibodies in order to control immunogenicity; the ability to modify the heavy chain in order to affect bioactivity, improving bifunctional chelating agents for a variety of imaging and therapeutic isotopes (110); and the availability of biological response modifiers to increase localization and potentially decrease toxicity make the concept of Ehrlich's magic bullet still attractive.

The appropriate choices of target disease and isotope should make the task more achievable, although the dosimetry of such treatment remains an enormous challenge to all investigators. The assumptions used by some investigators to assign radiation dose by "calculations" are of dubious validity. The difficulties here reflect different concepts of dose within Nuclear Medicine and Radiation Therapy. In Nuclear Medicine terminology, dose is imaged as disintegrations on film; in radiation therapy, dose must be linked to a specific target volume. Thus, the target volume must be defined first, and the basic issue is what proportion of the disintegrations actually strike that target volume, and with what kind of distribution of density. These are very different concepts and need to be resolved for effective estimates of dose to be made. The problem is compounded further by the heterogeneity within the tumor volume itself, which at this time, cannot be reliably estimated. The macrodosimetry, difficult as it is, may be outweighed by the microdosimetry especially when considerations of the relationship of dose to the localization of the clonogenic cells is considered. Several dosimetric models have been proposed to help predict rational use of differing nucleotides depending on the size of the tumor and the potential for poorly vascularized zones (111–113).

For short-lived isotopes (those with a physical half-life of 2 days or less, with even shorter biological half-life when attached to antibody) the dose rate varies markedly. As the dose rate declines, it can drop below the level at which repair of sublethal damage may exceed the effective cytotoxicity. The problem of adequate dose rates related to antibody delivery, to biological and physical radionuclide decay, and to inhomogeneity of dose distribution is only beginning to be explored thoroughly; dose rate predictions from many early studies fell below classic radiobiological levels (114, 115). These two areas microdosimetry and dose rate, figure to remain critical areas of investigation for radioimmunotherapy in the future.

Conclusions

Regardless of the modality used, a thorough understanding of the subtleties of human cancer treatment is complicated. As radiation oncology looks to biology for innovative approaches to treatment there is a call for greater interaction and cooperation between the biologist and the clinician. There is a need to foster interactions with biologists and clinicians of other cancer treatment specialties, particularly in the area of medical and surgical oncology as well as nuclear medicine. For advancements in treatment both radiation oncology and other oncological disciplines share common goals, a better understanding of the biochemical, molecular, and physiological nature of the cell and tumor. United in our efforts, we can learn much more that can ultimately be used to benefit patients. Molecular bio-

### Table 1: Rational isotopes for radioimmunotherapy

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Physical half-life</th>
<th>Type of emission and energy (MeV, max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{211}$Bi</td>
<td>1.75 h</td>
<td>$\alpha$ 6.05, 606, 8.78 + $\beta$ and $\gamma$</td>
</tr>
<tr>
<td>$^{212}$Bi</td>
<td>10.6 h</td>
<td>$\beta$ and $\gamma$ + $^{125}$I emissions</td>
</tr>
<tr>
<td>$^{213}$Bi</td>
<td>7.2 h</td>
<td>$\alpha$ 5.87 (pure)</td>
</tr>
<tr>
<td>$^{64}$Cu</td>
<td>61 days</td>
<td>$\beta$ 0.395, 0.484, 0.570; $\gamma$ 0.184, 0.093</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>8 days</td>
<td>$\beta$ 0.606; $\gamma$ 0.364</td>
</tr>
<tr>
<td>$^{90}$Y</td>
<td>64 h</td>
<td>$\beta$ 2.270 (pure)</td>
</tr>
<tr>
<td>$^{188}$Re</td>
<td>91 h</td>
<td>$\beta$ 1.07, 0.933; $\gamma$ 0.319</td>
</tr>
<tr>
<td>$^{47}$Sc</td>
<td>18 h</td>
<td>$\beta$ 2.12, 1.985; $\gamma$ 0.155</td>
</tr>
<tr>
<td>$^{89}$Y</td>
<td>83 h</td>
<td>$\beta$ 0.439, 0.600; $\gamma$ 0.160</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>60 days</td>
<td>Multiple low energy $\gamma$s, X-rays, $\beta$s</td>
</tr>
<tr>
<td>$^{125}$I</td>
<td>57 h</td>
<td>Multiple low energy $\gamma$s, X-rays, $\beta$s</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>13 h</td>
<td>Multiple low energy $\gamma$s, X-rays, $\beta$s</td>
</tr>
<tr>
<td>$^{89}$Y</td>
<td>4.4 h</td>
<td>Multiple low energy $\gamma$s, X-rays, $\beta$s</td>
</tr>
</tbody>
</table>

**Auger cascade/internal conversion**

- $^{211}$Bi
- $^{212}$Bi
- $^{64}$Cu
- $^{131}$I
- $^{90}$Y
- $^{188}$Re
- $^{47}$Sc
- $^{89}$Y
- $^{131}$I
- $^{125}$I
- $^{89}$Y
- $^{89}$Y

**Type of emission**

- $\alpha$ (+ $\beta$, $\gamma$)
- $\beta$ and $\gamma$ + $^{125}$I emissions
- $\alpha$ 5.87 (pure)
- $\beta$ 0.395, 0.484, 0.570; $\gamma$ 0.184, 0.093
- $\beta$ 0.606; $\gamma$ 0.364
- $\beta$ 2.270 (pure)
- $\beta$ 1.07, 0.933; $\gamma$ 0.319
- $\beta$ 2.12, 1.985; $\gamma$ 0.155
- $\beta$ 0.439, 0.600; $\gamma$ 0.160
- Multiple low energy $\gamma$s, X-rays, $\beta$s
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- Multiple low energy $\gamma$s, X-rays, $\beta$s

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- $^{188}$Re
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- Multiple low energy $\gamma$s, X-rays, $\beta$s
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- Multiple low energy $\gamma$s, X-rays, $\beta$s
- Multiple low energy $\gamma$s, X-rays, $\beta$s

ogy and sophisticated DNA technology holds promise for expanding knowledge on the inherent sensitivity of tumor cells to radiation and cytotoxic drugs. In the study of chemical modifiers, radiation biologists continue to uncover valuable information about how drugs affect biochemical pathways of both normal and tumor cells. In the hope for better overall tumor control, medical and radiation oncologists have converged on combined programs which appear to aid in local radiation effectiveness, although the mechanisms of sensitization remain to be fully elucidated. In the search for less hematopoietic toxicity, radiation oncologists are beginning to borrow from the lessons of modern immunology. In combined efforts with nuclear medicine and immunology, radiation oncology offers the expertise in dosimetric and oncological knowledge necessary to construct a future for optimization of radioimmunologic conjugates.

Above all else, the expertise for such trials requires collaboration between different disciplines of medicine and science who all deserve to share in the success of their combined efforts.

References


The Emerging Biology of Modern Radiation Oncology


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