

## **AN UPDATE ON RADIO-IMMUNOTHERAPY**

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In the United States alone, approximately 56,000 people will be diagnosed with Non-Hodgkin's Lymphoma (NHL) in 2005 and a significant proportion of these patients will fall into the low-grade or follicular B-cell subgroup of NHL. Patients with low-grade NHL may remain in remission for extended periods of time, but eventually most will have relapses that occur more frequently over the course of the disease. Historically, standard treatment consisted of multiagent chemotherapy, in some cases followed by radiation therapy. In 1997, a new era in the treatment of NHL began with FDA approval of Rituximab, the first monoclonal antibody approved for the treatment of patients with NHL. Rituximab is a chimeric anti-CD20 antibody that is indicated for relapsed or refractory low-grade or follicular, CD20+, B-cell Non-Hodgkin's Lymphoma. Rituximab's target, the CD20 surface molecule, is a B-lymphocyte specific non-glycosylated, integral phosphoprotein implicated in the regulation of trans-membrane calcium conductance, cell cycle progression, and B-lymphocyte proliferation. CD20 belongs to the MS4A (membrane spanning 4A) gene family, which is expressed in hematopoietic cells, and consists of at least 25 members clustered at human chromosome locus 11q12-13. Binding of antibodies to the CD20 protein is reported to increase calcium conductivity across the plasma membrane, arrest cell cycle at G1 phase and inhibit *in vitro* B-cell differentiation and mitogen induced immunoglobulin secretion. Cross-linking of antibodies is also observed to induce activation of protein kinases and

CD20 phosphorylation. The initiation of anti-CD20 mediated phosphorylation and intracellular signaling depends on type of antibody used. The binding of the mouse/human chimeric antibody rituximab (Rtx) induces lipid raft formation, signal transduction, and initiation of apoptosis. Binding of the antibody to the CD20 molecule on the outer surface of the B-lymphocyte may result in death of the target cell through several different mechanisms including apoptosis, antibody dependent cell mediated cytotoxicity, and complement dependent cytolysis. These effects may be somewhat variable when applied during other cytotoxic stimuli such as chemotherapy or radiation. The desire to use other concurrent cytotoxic effectors became clear when clinical data with Rituximab showed that though major responses occurred in approximately 50% of CD20+ indolent lymphoma patients, these responses were often of limited duration.

Radioimmunotherapy involves linking a radioactive isotope to this sort of cell-targeted antibody. In the case of NHL, this concept makes intuitive sense for a number of reasons: NHL cells are intrinsically sensitive to the cytotoxic effect of radiation, targeting the CD20 molecule allows delivery of the radioisotopes to the tumor and minimizes the normal tissue effects, and beta particles emitted from the isotopes can also kill neighboring CD20 negative cells (the “cross-fire” effect).

The United States Food and Drug Administration (FDA) has approved two radio-immunotherapeutic compounds for use in NHL. Both drugs couple a radioactive isotope to a murine monoclonal anti CD20 antibody. FDA approval for yttrium-90 ibritumomab tiuxetan (Zevalin<sup>®</sup>) came in February of 2002, and similar clearance was provided in June

of 2003 for iodine-131 tositumomab (Bexxar®). The approved medical indication for the use of these agents is in the treatment of patients with relapsed or refractory low-grade, follicular, or transformed B-cell Non-Hodgkin's Lymphoma. Although quite similar in design, there are several important differences between these FDA-approved biologically targeted radiopharmaceuticals.

For Y-90 Zevalin, a chelator side arm chain (tiuxetan) binds the metallic radioisotope, Yttrium-90, to the heavy chain of the murine anti-CD20 antibody. The characteristics of Yttrium-90 and its high-energy beta particle emission spectrum are critical elements of the Zevalin regimen. Treatment of an NHL patient with Zevalin involves several coordinated steps, the first of which provides an evaluation of the biodistribution of the antibody and isotope. Initially, non-radioactive Rituximab is infused at a dose of 250 mg/m<sup>2</sup> followed by a smaller "tracer" dose of <sup>111</sup>In ibritumomab tiuxetan. Indium-111 is used as a surrogate in this first step since Yttrium-90, which is used in the therapeutic portion, has a similar chemistry but cannot be readily visualized on a gamma camera. Multiple scans are obtained to assess an acceptable biodistribution and lack of dangerous pooling in normal tissues such as the lung. If the distribution is acceptable, the therapeutic infusion is given 7-9 days after the beginning of the process. A large IV dose of Rituximab is given 4 hours before the Zevalin dose, which is infused at a weight-adjusted level of 0.3-0.4 mCi/kg up to a max dose of 32 mCi. The preliminary dose of Rituximab saturates the CD20 receptors on normal tissues and allows deeper, more homogenous distribution of the Zevalin compound. After infusion, the patient is released without major radiation precautions, again due to the nature of the pure beta emission of

the Yttrium-90 radioisotope. The most common toxicity associated with radioimmunotherapy is a transient decrease in blood counts, with a typical nadir around 4-8 weeks after infusion.

Treatment of NHL patients with I-131 Bexxar involves some distinctive issues when compared to Zevalin, mostly related to the differing properties of the radioisotopes. Iodine-131 has an emission spectrum involving both beta and gamma ray emissions. This dual emission spectrum permits scans without the need for a surrogate isotope such as the Indium-111. While pre-treatment administration of “cold” antibody is used for both compounds, the pre-treatment imaging studies performed in Bexxar administration are used to tailor the dose to each specific patient, based on individualized bio-clearance characteristics for that patient. The clearance characteristics obtained from the scans are used to calculate the I-131 millicurie dose necessary to produce a total body equivalent dose of 65-75 cGy. The natural physiologic avidity of I-131 for the thyroid requires protection by oral iodine supplements, which serve to block I-131 uptake by the gland. Finally, while most patients qualify for immediate release, considerable precautions are needed after radioimmunotherapy with Bexxar, again due to radiation safety issues inherent in the penetrating gamma ray emissions. Specific instructions to patients include sleeping in a separate bed from others, refraining from long trips, maintaining >6 feet from others, and avoiding contact with young children & pregnant women. Both Zevalin and Bexxar have shown considerable clinical efficacy even against heavily pretreated NHL patients. In multiple trials, overall response rates of 60-80 percent are reported with approximately 30% of patients achieving a complete response.

There is increasing interest in the expanded use of these biologically targeted radiopharmaceuticals. Although they are currently FDA approved only for treatment of indolent B cell NHL, the Southwest Oncology Group (SWOG) is currently evaluating the role of anti-CD20 radioimmunotherapy in the initial management of patients with aggressive NHL. In this trial, patients first receive standard dose CHOP followed by involved field radiation. After an interval of 3-6 weeks, Zevalin is administered and response is assessed. At the University of Michigan, Bexxar is being evaluated in patients with previously untreated, advanced-stage, low-grade NHL. The results, originally presented at the 2002 American Society of Hematology Annual Meeting, show a 95% overall response rate with a notable 74% of patients achieving a complete response. Similarly, several investigators are evaluating the effects of Zevalin along with rituximab maintenance in patients with untreated low-grade follicular lymphoma and are achieving long-lasting remissions. There is also interest in using radioimmunotherapy as re-treatment in selected cases of NHL. The data suggests that re-treatment can again produce complete response rates of around 25%. At the most recent meeting of the American Society for Clinical Oncology, data were also presented using Zevalin along with chemotherapy as a conditioning regimen for elderly patients with aggressive NHL and also as a kind of “liquid TBI” for stem cell transplant. In both cases, early data appear promising. The use of conformal external beam treatment of bulky disease sites prior to RIT is also under investigation at the Cleveland Clinic and elsewhere.

The identification of specific cell surface molecules on cancer cells has recently led to several key advances in the treatment of malignant disease. This progress is evident in the treatment of B cell NHL, where several different antibody-based therapeutics directed against the CD20 surface protein are now in wide clinical use. Two anti-CD20 radiolabeled compounds, Y-90 Zevalin and I-131 Bexxar, have already been approved by the FDA for treatment of indolent NHL and other approved indications are likely in the near future. In an era of unprecedented medical advances, these compounds represent the first of what is likely to be a whole family of new biologically targeted radiotherapy agents useful in the treatment of cancer and related conditions.