

# ADVANCES IN LLM

Current Developments in the Management of Leukemia, Lymphoma, and Myeloma

Section Editor: Susan O'Brien, MD

## In Situ Vaccination as a Therapy for Low-Grade Lymphoma



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### **H&O** How can in situ vaccination improve upon standard therapies for low-grade lymphoma?

**JB** We see high response rates with standard chemotherapy, and the first time we treat a patient those responses can last for months or years. However, low-grade lymphomas are incurable with standard therapy. When we repeat those therapies, the response rates diminish with each iteration, and the durations of those responses are shorter. This is not unique to lymphoma, although it is a bit more drastic in lymphoma than in other types of cancer—it is Oncology 101 that we see diminishing returns with standard therapies.

With in situ vaccination, we have an opportunity to get augmenting returns as we repeat vaccinations. We administer more than one dose of the vaccines for measles, mumps, and rubella and for hepatitis B because a memory immune response is of greater magnitude and more rapid than a primary immune response. So, instead of the response to therapy getting worse with each iteration, there is a chance that the response will get better.

### **H&O** What was the concept behind the initial in situ vaccine?

**JB** The concept was to take advantage of the mature B-cell program. Certain Toll-like receptors (TLRs) are expressed on mature B cells and on almost all mature B-cell-derived lymphomas. By exposing lymphoma cells to a TLR agonist (such as a short synthetic stretch of DNA that has the appearance of bacterial or viral DNA), we can turn the lymphoma cells themselves into

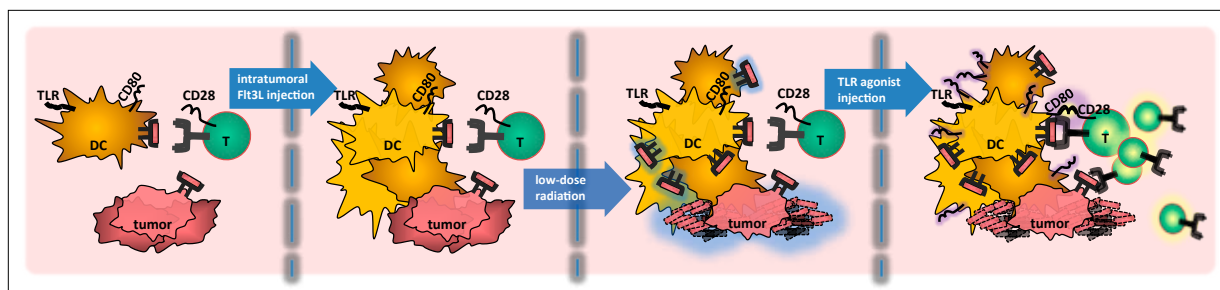
antigen-presenting cells. They will then present some of their tumor-associated antigens to antitumor T cells. Within lymphoma tumors there are also a small number of immune cells, such as dendritic cells, that could also be activated to present tumor antigens to T cells. When those antitumor T cells see the antigen presented, they express activation markers on their surface and secrete cytokines that have an antitumor effect.

### **H&O** How are in situ vaccines different than typical vaccines?

**JB** Most vaccines—for example, the measles, mumps, and rubella vaccine—are made in the factory; they are ex vivo. In situ vaccines are not made at a factory. We are literally making the vaccine right there in the patient's tumor by intratumoral administration of an immunostimulant. When we walk into the room, we have a vial of the immunostimulant, and patients ask us, "Is that the vaccine?" and we explain, "We haven't made the vaccine yet. We're going to make it right now." That is what we call in situ vaccination—making the vaccine at the tumor site.

### **H&O** How is the in situ vaccine made?

**JB** First, we treat the tumor site with a small dose of radiation, 4 Gy total, to release some tumor antigens that can be presented on immune cells or on tumor cells themselves. We then activate those immune cells with intratumoral administration of a TLR agonist. In order to assess the response, we measure disease regression far away from



**Figure.** In situ vaccination strategy using Flt3L.

Flt3L, FMS-like tyrosine kinase 3 ligand; TLR, Toll-like receptor; DC, dendritic cell; T, T cell.

that treated site. We do this because low-dose radiotherapy alone may induce regression at the treated site.

### H&O What are the clinical results of in situ vaccination?

**JB** We treated a total of 60 patients with this approach—the first cohort of which were described in our paper in the *Journal of Clinical Oncology* in 2011—and had some complete and partial remissions. Some of those remissions lasted for years. Along with disease regression, we also saw an increase in induction of antitumor T cells. This is very encouraging, but there is clearly some room for improvement.

### H&O As you mentioned previously, one problem with standard therapies is diminishing returns after many rounds. Were there any examples of improved responses after repeat in situ vaccine administration?

**JB** In these first trials, we had an opportunity to address that question. A patient who had a great response initially had a recurrence about a year and a half later that included bulky cervical and retroperitoneal adenopathy. We re-treated her at a different inguinal site, and the clinical response was even more rapid and of greater magnitude with the second vaccine.

### H&O How is current work improving upon the previous concepts of in situ vaccination?

**JB** The in situ vaccine previously used lymphoma cells as the antigen-presenting cells. We sometimes call them “amateur” antigen-presenting cells, as opposed to the dendritic cell, which is the “professional” antigen-presenting cell. Ralph M. Steinman was one of the people awarded the 2011 Nobel Prize for his work on the dendritic cell, which is a better antigen-presenting cell than any other. At the time we started this work, we did not have any way

of increasing intratumoral dendritic cells. But now we do, by using a clinical grade recombinant protein called FMS-like tyrosine kinase 3 ligand (Flt3L).

We sometimes refer to Flt3L as the erythropoietin of dendritic cells; it is the primary growth and differentiation factor of dendritic cell precursors. We can intratumorally administer Flt3L to bring many dendritic cells to the site, and then intratumorally administer a TLR agonist called poly-ICLC to activate those tumor antigen–loaded dendritic cells. Antitumor T cells, seeing this professional antigen-presenting cell, then can become potently activated. They can proliferate and induce systemic antitumor immunity (Figure).

### H&O Are there any clinical trials using the Flt3L-primed in situ vaccine?

**JB** We started a clinical trial earlier in 2014 and now have data showing immunologic and clinical responses (NCT01976585). The regimen involves 2 weeks of intratumoral Flt3L, 2 days of low-dose radiotherapy, and 2 months of weekly poly-ICLC injected into the tumor. These trials sometimes include people traveling long distances for treatment, so the weekly injections are fairly simple and practical.

### H&O What are the preliminary results of this study?

**JB** We found that there are very few dendritic cells in the tumor prior to Flt3L injection. After Flt3L injection, the number of intratumoral dendritic cells increases by several orders of magnitude. Two weeks later, we see that there are very few dendritic cells left; therefore, we temporarily recruit these dendritic cells to the tumor. Furthermore, not only do we accumulate more dendritic cells, we also activate these dendritic cells with the TLR agonist poly-ICLC. We found very few activated dendritic cells in the tumor before treatment, and 10-fold more after intratumoral TLR agonist injection. We also see T cells getting activated at the site; they are switching from what we call a central memory phenotype to an effector memory phenotype.

We also have results from the first 2 treated patients; both initially had partial responses. One patient has gone on to have a complete response, with no residual evidence of bone marrow or nodal disease. The other patient had thrombocytopenia and anemia because of bone marrow involvement in follicular lymphoma, and both of those resolved within 3 months after therapy.

**H&O** Do the results demonstrate any differences between in situ vaccines and other therapies?

**JB** Like chemotherapy, in situ vaccination kills lymphoma cells. However, unlike chemotherapy, in situ vaccination actually increases the number of T cells. Furthermore, in situ vaccination seems to distinguish between malignant and healthy B cells. In one patient, the number of malignant B cells decreased by 1 order of magnitude, and the number of healthy B cells actually increased. Somehow, the immune system seems to be specifically targeting malignant B cells by finding some antigens that are on the lymphoma cells but not on healthy B cells.

Checkpoint blockade antibodies are another active, promising area of immunotherapy research. One concern is that these can induce not only antitumor immunity, but also antiseif immunity—also known as autoimmunity. Using in situ vaccination, we hope to have a more tumor-specific immunity and minimal risk for autoimmunity. To do this, we are attempting to activate the immune system at the site of the tumor, but not systemically. Preliminary results suggest we can achieve that. We find that while the intratumoral dendritic cells are becoming potentially

activated, the systemic dendritic cells are minimally activated during the course of this therapy.

**H&O** Are there any other future plans you would like to discuss?

**JB** Yes; there is a new way for us to look at the immune systems of these patients called mass cytometry (the brand name is CyTOF), which is a newer technology than flow cytometry. Flow cytometry uses color labeling of each cell type, whereas mass cytometry uses heavy metal labeling to look at an immense cross-section of the immune cell repertoire in parallel at one time.

We can use this information to confirm our results, such as the finding of increases in the number of dendritic cells after Flt3L administration. But by looking at the entire immune system, we also can make some surprising discoveries that we would not have made otherwise. For example, patients had an increase in basophil levels and changes in other myeloid levels during Flt3L treatment. These are findings we would not have expected, and that may be relevant for how the vaccine works.

**Suggested Readings**

- Brody J, Kohrt H, Marabelle A, Levy R. Active and passive immunotherapy for lymphoma: proving principles and improving results. *J Clin Oncol.* 2011;29(14):1864-1875.
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