Regulatory T Cells Are Depleted in Low-grade Lymphoma by the Combination of Local low-dose Radiation Followed by Intratumoral CpG-ODN

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INTRODUCTION

BACKGROUND: Non-Hodgkin B cell lymphomas are often infiltrated by immune effector cells including T, NK and dendritic cells. But despite their presence within the tumor they fail to control tumor growth. Some T cells can even play a supportive role to maintain the tumor and prevent the function of anti-tumor immune cells.

Regulatory T cells (TRegs) function normally to dampen immune responses and prevent auto-immunity by direct cell contact or by secreting suppressive cytokines i.e. TGF-b and Interleukin-10. Follicular Lymphoma tumor cells can induce the conversion of CD4 T cells into TRegs, thereby evading anti-tumor immune responses.

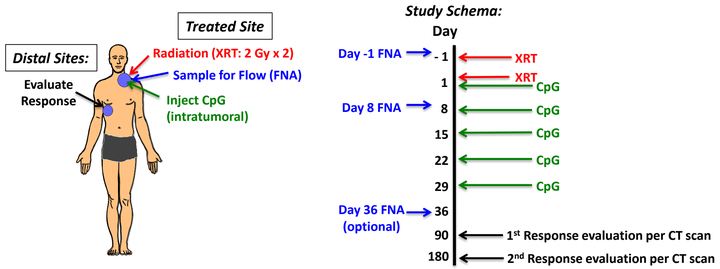
Follicular helper T cells (Tfh), another class of regulatory T cells, are found within normal follicles of lymphoid organs. Migrating into the germinal center, they promote the survival and differentiation of follicular B cells. In lymphoma, Tfh cells can send a survival signal to tumor B cells through CD40L/CD40 interactions.

In an ongoing multi-center phase I clinical trial (NCT02266147), patients with previously untreated low-grade lymphoma receive low dose (2Gyx2) radiotherapy to a single, tumor site, followed by intratumoral injection into the same site of a CpG-ODN (SD-101, Dynavax Technologies), an immune-modulatory molecule that targets Toll-like Receptor 9 (TLR-9). Doses ranged from 1 mg to 8 mg per injection in successive cohorts.

To date, 13 patients have been entered into the dose escalation phase of this study: 9 follicular lymphoma (FL), 1 chronic lymphocytic lymphoma (CLL), 2 small lymphocytic lymphoma (SLL) and 1 marginal zone lymphoma (MZL). Therapy has been well tolerated. Local injection site reactions and fever, the most common side effects, resolved by 48 hours. There have been no related SAEs.

Ten of 13 patients had FNAs that yielded enough viable cells for evaluation pre and 1 week post treatment.

CLINICAL STUDY



MATERIALS & METHODS

- FNA samples were sent to a central site by overnight delivery in media with 5% fetal calf serum and processed into a single-cell suspension within 24 hours.
- Cells were stained for surface markers, fixed and permeabilized and stained for intracellular targets then run on an LSRII Flow Cytometer (BD). Data was analyzed using cloud-based software within Cytobank.org.
- Cells were stained with 1 – 4 Panels with 13 antibodies each to delineate T, B, NK, dendritic, myeloid cells, and their subsets. Antibodies against activation antigens, as well as T cell exhaustion, inhibition and function were also used to characterize these cells.
- Each panel required a minimum of 1 x 10^5 viable cells. Most samples were > 90% viable.

COI: Craig Berman, Robert Coffman and Robert Janssen, employees Dynavax Technologies Corp. No other relevant conflict of interest to disclose.

RESULTS

Treated Tumors Respond to low-dose Radiation and Intratumoral CpG by Day 90

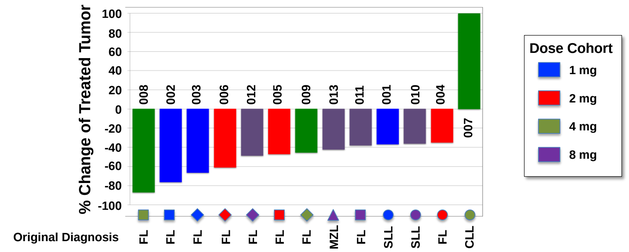


Figure 1: 12 of 13 patients showed decrease in tumor size (cm^2) at Day 90 evaluation as compared to pretreatment baseline. By Day 180, patient **007** showed tumor regression of the treated site to -15.4% below baseline. Note: Patient **012** received only 1 dose of CpG. Each patient is represented by same symbol and color throughout plots. Patient ID #'s given above.

Most of the Cells Analyzed are Lymphocytes

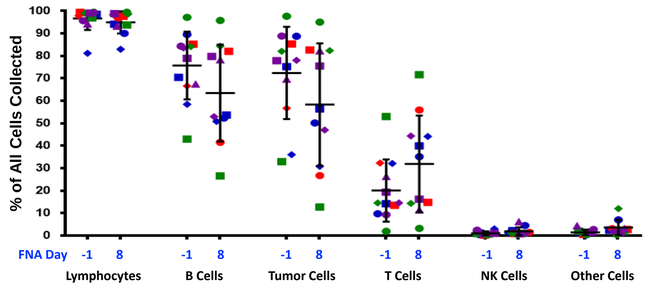


Figure 2: Data represents all evaluable FNA samples of treated sites received at Day -1 or Day 8. Gating on all viable cells, 95.8 ± 5.0% are lymphocytes per FSC vs SSC, 69.8 ± 19.1% are B cells and 25.7 ± 18.5% are T cells. 94.0 ± 13.3% of B cells are tumor per CD19 and light chain restriction at Day -1 decreasing to 87.3 ± 20.0% at Day 8.

T Cells increase at the Treated Site by Day 8

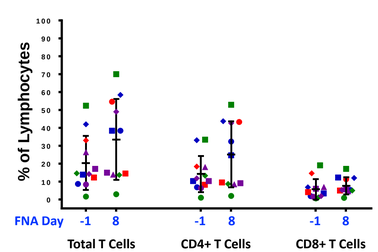


Figure 3: Total T cells increased in 7 of 10 patients with evaluable FNAs from Day -1 to Day 8, ranging from > 300% to 18% . Both CD4 and CD8 T cells increased in 5 of 7 patients. CD4 T cells in Patient **008** increased from 33.4% to 52.9% with little to no change in CD8 T cells. CD8 T cells increased in Patient **009** from 1.2 to 5.0% with a decrease in CD4 T cells, 13.3% to 8.7%.

RESULTS

Regulatory T Cells (FoxP3+ T Regs and Follicular Helper T Cells) are Depleted at the Treated Site by Day 8

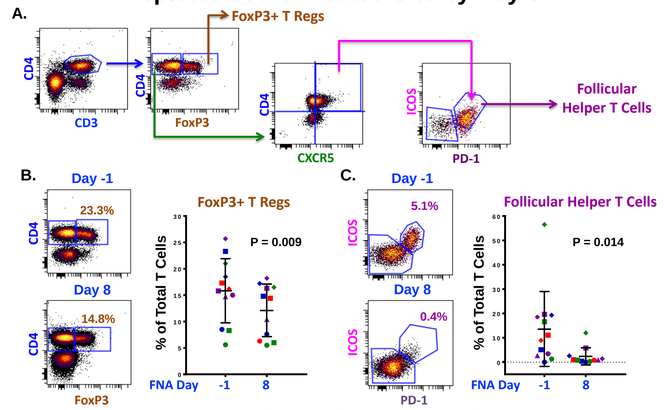


Figure 4: A.) Gating schema for T Regulatory cells and Follicular Helper T cells. **B.)** T Regs were reduced in 8 of 10 patients at Day 8, average 22.3 ± 9.5%. No change in T Regs observed in Patient **007** and a minimal increase in Patient **011**, 15.8% to 16.3%. **C.)** All 9 patients with measurable Tfh cells at baseline showed significant reduction in Tfh cells by Day 8, average 83.3 ± 9.9%. Patient **001** had no measurable Tfh cells at Day -1 or Day 8. **B + C.)** FACS plots from patient **002** at Day -1 and Day 8 for T Regs and Follicular Helper T cells.

Abscopal Effects are Observed at Day 90

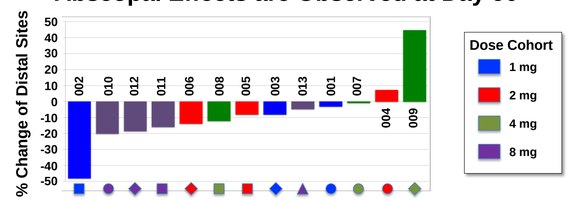


Figure 5: Tumor regressions were noted in 11 of 13 patients at Day 90. Values represent the % decrease in the sum of the areas (cm^2) of all distal lesions measured. Patient **002** reached a partial response (PR) per Cheson criteria at Day 180. Note: Patient **012** received only 1 dose of CpG.

CONCLUSIONS

- The combination of CpG and low-dose irradiation resulted in tumor regression at 90 days in both treated tumors (12/13 patients) and untreated tumors (11/13 patients).
- Immune modulating therapies can be delivered directly into a tumor to minimize systemic toxicity and induce changes in the tumor microenvironment, potentially inducing a global anti-tumor immune response.
- The combination of low-dose radiation and CpG reduce the proportion of TRegs and Tfh cells thereby modulating their inhibitory effects and tumor growth promoting effects, respectively.
- Monitoring the cell populations at the treated site by repetitive sampling and flow cytometry reveals the pharmocodynamic effects of anti-cancer therapies, especially those intended to trigger anti-tumor immune responses.
- The expansion phase of the study is presently open to accrual but has been modified to allow for sampling of a distant untreated site to better understand the abscopal effects of therapy.