Intratumoral G100 Rescues Radiation-Induced T Cell Depletion and Has Synergistic Anti-Tumor Effect with Local Irradiation in A20 Lymphoma

Hailing Lu1, Eric Ford2, Jeffery L. Schwartz2, Jessica Hewitt1, Frank J. Hsu3, Jan ter Meulen1, and Ramesh Rengar2

1Immune Design, Seattle, WA; 2University of Washington, Seattle, WA

ABSTRACT

Background: Radiation therapy is a standard established treatment modality for many malignancies, including lymphoma. There is growing evidence that the combination of radiation and immunotherapies can have synergistic immune and clinical effects, however, the mechanisms underlying these responses are poorly understood.

Hypothesis: We hypothesized that the potential beneficial effect of adding immunotherapy to radiation might be due to alteration of the tumor microenvironment (TME) to an inflammatory phenotype and the facilitation of T-cell infiltration.

Methods: Mice received two bilateral subcutaneous implants of 5 million A20 lymphoma cells each. When tumors were established (average tumor size ~4 mm3), mice received intratumoral injection of G100 (100 μg) as TLR agonist (GLA) in stable oil-in-water emulsion), tumor irradiation, or the combination of G100 and irradiation. X-ray radiation was delivered by using a Small Animal Radiation Research Platform (SARRP) with CT-guided imaging that can accurately target lesions. Mice were injected once daily up to one of the tumor implants. Intratumoral injection of G100 (50μg/100 μl) was administered 3 times a week for up to 3 weeks, starting on the day post radiation.

Result: The combination of G100 and radiation had synergistic antitumor effects and demonstrated a dosis effect with tumor growth delay or regression in the non-treated control tumor implant. Study of the TME in gene expression and cellular levels showed that G100 induced a larger number of pro-inflammatory cytokines and chemokines that promoted antigen processing and presentation and T-cell trafficking. By flow cytometry, G100 treated tumors showed increased infiltration of both CD8 and T-cell TILs. In contrast, tumors from animals that received only irradiation but not G100 had significantly decreased levels of 7 lymphocytes as compared to untreated tumors (P<0.05 vs. untreated tumors, p<0.001). The cytokine effect of radiation on lymphocytes. CD4 T-cell infiltrating lymphocytes (TILs) were depleted by 72% (9±2.2% vs. 24±4.4%, p=0.02) and CD8 TILs were depleted by 79% (10±3% vs. 6±0.7%, p<0.0001) or irradiated mice as compared to untreated mice. In the irradiation and G100 combination treatment group, the tumors had levels of TILs that were close to non-irradiated, G100 treated animals.

Conclusion: The anti-tumor effect of irradiation is synergistically enhanced by subsequent intratumoral treatment with G100, which also results in improved survival. The modulation of the TME by G100 rescues the T-cell depletion caused by radiation allowing for an influx of T cells, while the increase in antigen presentation overcomes a major obstacle posed by the immunosuppressive/TME. These preclinical results support the rationale for an on-going Phase I/2 clinical trial in late-stage follicular non-Hodgkin’s lymphoma (MALT) patients, who are randomized to receive G100 following local radiation with or without pembrolizumab (NCT02514473).

Methods and Results

1. Synergy between GLA and Radiation: Anti-Tumor Effects

![Figure 1. Combination therapy with G100 and focal irradiation.](image)

- **Figure 1.** Combination therapy with G100 and focal irradiation. Spleal mice were inoculated with 105 A20 tumor cells bilaterally. Mice received single dose irradiation on the right tumor on Day 10 when the average tumor size was 50±10 mm3 (Tumor area). Then, mice were inoculated intratumoral injection of G100 (50 μg, iwx/week for 1 week). (A) Treatment schema. (B) Tumor growth curves of the treated tumor. (C) Tumor growth curves of untreated tumor. (D) Overall survival of mice in different treatment groups, p<0.05 between RT and RT+G100 group. (N=9 mice).

2. Synergy between GLA and Radiation: Immune Activation

![Figure 2. Gene expression profile of tumor microenvironment.](image)

- **Figure 2.** Gene expression profile of tumor microenvironment. (A) Mice were sacrificed as a tumor reached a single dose of irradiation (10 Gy) and then 3 doses of GLA (50 μg, iwx). Tumors were collected after the last injection of G100. RNA was used for Nanopore gene expression analysis using the mouse panCancer Immune Profiling Kit. (B) Heatmap showing the unsupervised clustering of RNA samples from different treatment groups. For example, RT, RT+G100 and RT+HT+G100 are three mice in the RT group. (C) Heatmap comparing the expression of antigen processing genes in mice treated with irradiation alone (RT) or irradiation plus G100 (RT+G100). (D) Flow cytofluorimetry of Genes and Genomes (WGS) pathway showing the antigen presentation pathway with genes that are induced by G100-HT highlighted in red.

3. G100 reverses radiation-induced depletion of lymphocytes in tumor

![Figure 3. FACs analysis of TILs showing that G100 can rescue the RT-mediated depletion of lymphocytes](image)

- **Figure 3.** FACs analysis of TILs showing that G100 can rescue the RT-mediated depletion of lymphocytes. (A) Representative FACS plots showing the percentage of CD3+, CD8+ and CD4+ T-cells of total TIL from A20 tumors of mice treated with control PBS, G100, irradiation (RT), or RT+G100. (B) Summary graphs of CD4+ and CD8+ T-cells as a percentage of total TIL from each treatment group (N=15 for PBS group and N=4 for other groups).

Summary

- **G100 and radiation have additive or synergistic anti-tumor effects.**
- **G100 enhances the immunogenic effect of radiation and synergizes with RT by upregulation of genes related to antigen presentation.**
- **G100 rescues radiation-induced T cell depletion.**
- These results support the use of combined local radiation and G100 as an active immunotherapeutic approach and new treatment modality with the potential of systemic long-term effect.

BACKGROUND

- **Glycyrrhizin lipoid A (GLA) is a clinical-stage synthetic TLR agonist that activates DC and enhances TIL immune responses.**
- **GLA has been safely administered to more than 2,500 human subjects as a vaccine adjuvant or cancer therapy.**
- **G100 (intratumoral injection of GLA in a stable emulsion) is currently being evaluated in cancer patients in multiple clinical trials.**

GLA Broadly Activates In situ Immune Signaling Pathways and Induces Type 1 IFN and Multiple Pro-Inflammatory Cytokines

- **RT+GLA shows potent and sustained depletion of tumor-infiltrating lymphocytes.**

- **RT+G100 orchestrates a T cell-mediated antitumor response with TH1 cytokines.**

- **RT+G100 rescues the irradiation-induced depletion of tumor-infiltrating lymphocytes.**

- **RT+G100 induces tumor growth delay or regression in the RT-only treated control tumor implant.**

- **RT+G100 induces increased infiltration of both CD8 and T-cell TILs.**

- **RT+G100 rescues the T-cell depletion caused by radiation and allows for an influx of T cells, while the increase in antigen presentation overcomes a major obstacle posed by the immunosuppressive TME.**

Figure 1. Combination therapy with G100 and focal irradiation. Spleal mice were inoculated with 105 A20 tumor cells bilaterally. Mice received single dose irradiation on the right tumor on Day 10 when the average tumor size was 50±10 mm3 (Tumor area). Then, mice were inoculated intratumoral injection of G100 (50 μg, iwx/week for 1 week). (A) Treatment schema. (B) Tumor growth curves of the treated tumor. (C) Tumor growth curves of untreated tumor. (D) Overall survival of mice in different treatment groups, p<0.05 between RT and RT+G100 group. (N=9 mice).

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