Intratumoral Injection of G100 (TLR4 Agonist Glycopyranosyl Lipid A) Modulates Tumor Microenvironment and Induces CD8 T Cell-dependent, Systemic Anti-tumor Immunity

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ABSTRACT

The tumor microenvironment (TME) plays a critical role in controlling the balance between tumor progression and immune surveillance. Infiltration of T cells, especially CD8 T cells has been associated with a good prognosis. We hypothesized that G100, a novel synthetic TLR4 agonist formulated in a stable emulsion (SE), can modulate TME when directly injected into the tumor and trigger both a local and systemic effective immune response.

Balb/C mice with implanted syngeneic A20 lymphoma received intratumoral (IT) injection of G100 (100 µg GLA/SE) or control PBS three times a week. This treatment significantly inhibited tumor growth and resulted in complete tumor regression in approximately 60% of treated mice. To investigate the effects of G100 on TME, tumors were collected and analyzed using flow cytometry for gene expression by Nanostring and immune phenotyping studies by FACS. Out of the 770 immune response genes included in the mouse PanCancer Immune Profiling panel, 20 genes were significantly upregulated in PBS-treated tumors. The upregulated genes include DC function-related genes (C101, CD81, CDS1, and ly6g) and T cell and NK cell function genes and multiple chemokines and cytokines (Il1b, Il2A, Il3, Il6, Ifng, Tcfs7, Cks2, Gm609, Ccl5, Ccl2, Ccl12, Cxcl11, Cxcl9, Cxcl10, and Cxcl9). T cell chemokine markers (Il12a and Jaks) and Cxcl10 (Ifn11) were also induced. G100-induced inflammation is also reflected at the cellular level as shown by increased T cells and NK cells in tumor via FACS analysis. To determine the immune cells that mediated tumor rejection, mice were selectively depleted of CD4 or CD8 cells during G100 treatment. Results showed that the anti-tumor effect of G100 is dependent on CD8 T cells. G100-induced tumor protection was durable as mice surviving the first tumor challenge rejected a secondary tumor challenge without additional G100 treatment. Moreover, our results suggest that G100 induces a proinflammatory cytokine and chemokine milieu that changes a "cold" tumor to a "hot" tumor, which facilitates the development of a CD8 T cell-dependent potent and durable anti-tumor effect. The induction of PD-1L-1 also suggests the potential synergy between G100 and checkpoint blockade therapy. These preclinical data support an on-going clinical trial of IT G100 in patients with follicular non-Hodgkin’s lymphoma (NCT01210147).

BACKGROUND

- Glycopyranosyl Lipid A (GLA), the central component of Immune Design’s GLA® platform, is a clinical-stage synthetic TLR4 agonist that activates DC and enhances Th1 immune responses.
- GLA has been safely administered to more than 1,000 humans as a vaccine adjutant and intratumoral to cancer patients.
- G100 (intratumoral injection of GLA in a stable emulsion) is currently evaluated in cancer patients in multiple clinical trials

- NCT01395427: G100 in Merkel Cell Carcinoma
- NCT01415756: GLA-activated w orldwide in follicular lymphoma
- NCT01701845: GLA and radiation in soft tissue cancer

RESULTS

1. G100 leads to complete regression of A20 tumors

2. G100 modulates the gene expression in tumor microenvironment (TME)

3. The anti-tumor effect of G100 is dependent on CD8 T cells

SUMMARY

- Intratumoral G100 has potent local and abscopal anti-tumor effect in murine A20 lymphoma model
- G100 modulates TME by inducing proinflammatory cytokine/chemokines and increasing infiltration of T cells and NK cells
- G100 enhances antigen-specific CD8 T cell response and the anti-tumor effect is dependent on CD8 T cells

Figure 1. A20 tumors were collected 3 days after i.t. injection of G100. Tumor RNA was used for gene expression analysis using the mouse panCancer Immune Profiling kit from Nanostring, Seattle. (b) heatmaps showing the expression of different subsets of genes that are upregulated in G100 group (green) as compared to control group (purple). (c) heatmap plots showing the top 20-upregulated genes in G100-treated tumors. (d) cell education markers are induced by G100.

Figure 2. (A) TILs from mice treated with G100 were analyzed by flow cytometry. (B) Percent survival for PBS or G100 treated groups.

Figure 3. (A) Representative FACS plots showing the levels of total T cells, CD4 or CD8 T cells, and Foxp3+ Tregs in splenocytes in TIL from mice treated with control PBS, G100, or SE. (B) Summary graphs of T cells and NK cells in TIL from each treatment group.

Figure 4. Balb/c mice with bilateral A20 tumors received i.t. G100 (10 µg, 3x/wk, for three weeks) into one tumor and tumor growth on both sides was monitored. (A) Kaplan-Meier showing the growth of treated tumor. (B) G100 inhibits the growth of untreated tumors. (C) G100 prolongs the overall survival (Kaplan-Meier for treated and untreated tumor combined).

Figure 5. CD8 T cells mediate the anti-tumor effects of G100. (A) Splenocytes from G100-treated mice reconstitute A20 tumor cells in vitro and secrete IFNγ. (B) Depletion of CD8 T cell but not CD4 T cells ablates the anti-tumor effects of G100. (C) Co-inoculation of CD8 T cells from GLA-treated tumor rejection mice with A20 tumor cells confer tumor protection.