COMBINATION OF A GLYCOMIMETIC ANTAGONIST TO E-SELECTIN AND CXCR4, GMI-1359, WITH AN ANTI-PD-L1 ANTIBODY ATTENUATES REGULATORY T CELL INFILTRATION AND ACCELERATES TIME TO COMPLETE RESPONSE IN THE MURINE CT26 TUMOR MODEL

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Abstract

Regulatory T cells (T_{reg}) modulate anti-tumor immune responses by suppressing T cell activation. T_{reg} are induced and maintained by immunoregulatory receptors, such as PD-L1, and respond to homing signals within the inflamed tumor microenvironment that include the endothelial cell surface protein, E- selectin, and the CXCR4 ligand, SDF-1. GMI-1359 is a small molecule glycomimetic compound beginning clinical evaluation with dual inhibitory activity against E-selectin and CXCR4. The aim of the current study was to determine if GMI-1359 either alone or in combination with anti-mPD-L1 antibody affected the in vivo growth of CT26 colon carcinoma and to assess percentages of infiltrative intratumoral cells expressing immune markers.

Female Balb/c mice were implanted subcutaneously with 5x10^5 CT26.WT tumor cells. Beginning 3 days post tumor injection, mice (n=15/group) were treated with saline, GMI-1359 (40 mg/kg for 12 consecutive days), isotype control antibody (anti-KLH) or anti-mPD-L1 antibody (10F.9G2, 10 mg/kg on days 3, 6, 10, 13, and 17), or the combination of GMI-1359 and anti-mPD-L1 antibody or anti-KLH. On day 15, tumors and spleens (n=5/group) were excised and the impact of treatment on T cells (total CD4+ and CD8+, and CCR7+/CD62L+ subsets of each), regulatory T cells (T_{reg}; CD4/CD25/FoxP3), and myeloid derived suppressor cells (MDSC; CD11b+/Gr1+) was determined by flow cytometry. The remaining mice were followed for tumor response.

Treatments were well tolerated. Mice in control groups and single agent GMI-1359 were all identified with progressive disease. In contrast, treatment with anti-mPD-L1 alone or in combination with GMI-1359 produced a 40% complete response (CR) rate. The median time to CR was shorter when anti-mPD-L1 was combined with GMI-1359 compared to anti-mPD-L1 alone (14 vs. 23 days, respectively, p<0.0471). Evaluation of tumor infiltrating cells showed that combination therapy with GMI-1359 and anti-mPD-L1 antibody reduced the percentage of T_{reg} compared to treatment with saline, GMI-1359 or the anti-mPD-L1 antibody as single treatments (0.9% vs. 3.3%, 2.9% and 1.9%, respectively). No other T cell subsets were affected. In spleens, the median percentage of T_{reg} were unaffected by any of the treatments and suggest that the reduction in intratumoral T_{reg} by combined treatment with anti-PD-L1 and GMI-1359 was an attenuated response to maintenance and homing signals in the tumor microenvironment.

In conclusion, these studies demonstrate that the dual E-selectin/CXCR4 antagonist, GMI-1359, in combination with anti-mPD-L1 antibody attenuates the induction and distribution of intratumoral T_{regs} and this reduction in T_{regs} is associated with a more rapid immunotherapeutic anti-tumor response.

Introduction

GMI-1359 is a rationally-designed parenteral small molecule glycomimetic (MW = 1115) with dual antagonistic activity to ligands for E-selectin and CXCR4. The compound currently is undergoing an initial safety and pharmacokinetic evaluation in normal human volunteers.

Table 1. Competitive Binding Activity (IC50) of GMI-1359 Against E-selectin and CXCR4

GMI-1359 was assessed for inhibition of sialyl LeX binding to immobilized E-selectin and α-CXCR4 antibody binding to Raji cells. IC50's (μM) were determined.

Compound	E-selectin	CXCR4
GMI-1359	1.0	0.5

Summary. The small molecule glycomimetic, GMI-1359, inhibits ligand binding to both E-selectin and CXCR4 with approximate equal potencies.

Figure 1. Protocol Schematic for the Combination of anti-PD-L1 and GMI-1359

Female Balb/c mice were implanted subcutaneously with 5x10^5 CT26.WT tumor cells. Beginning 3 days post tumor injection, mice (n=15/group) were treated with saline, GMI-1359 (40 mg/kg for 12 consecutive days), isotype control antibody (anti-KLH) or anti-mPD-L1 antibody (10F.9G2, 10 mg/kg on days 3, 6, 10, 13, and 17), or the combination of GMI-1359 and anti-mPD-L1 antibody or anti-KLH. On day 15, tumors and spleens (n=5/group) were excised and the impact of treatment on T cells, T_{reg} and MDSC was determined by flow cytometry. The remaining mice were followed for tumor response.

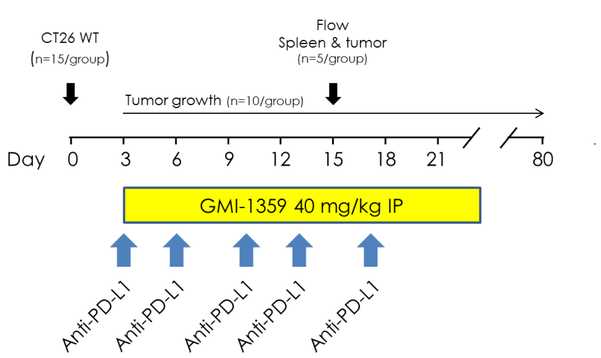


Figure 2. Percentages of CD4+, CD8+ and Regulatory T Cells in Spleen and Tumor on Study Day 15

Twenty-four hours following the final dose of GMI-1359, five mice from each treatment group were euthanized and spleens and tumors were processed for flow cytometry. Tumors were dissociated according to Miltenyi Dissociation Protocol for soft tumors. Single cell suspensions from spleen were obtained by maceration. The following cell determinants were assessed: CD4, CD8a, CD11b, CD25, FoxP3, GR1, CD62L, CTLA-4, PD-1, and PD-L1. Shown below are the results for percentage of total CD4+ and CD8+ lymphocytes and regulatory T cells, and the ratio of CD8 T cells to regulatory T cells.

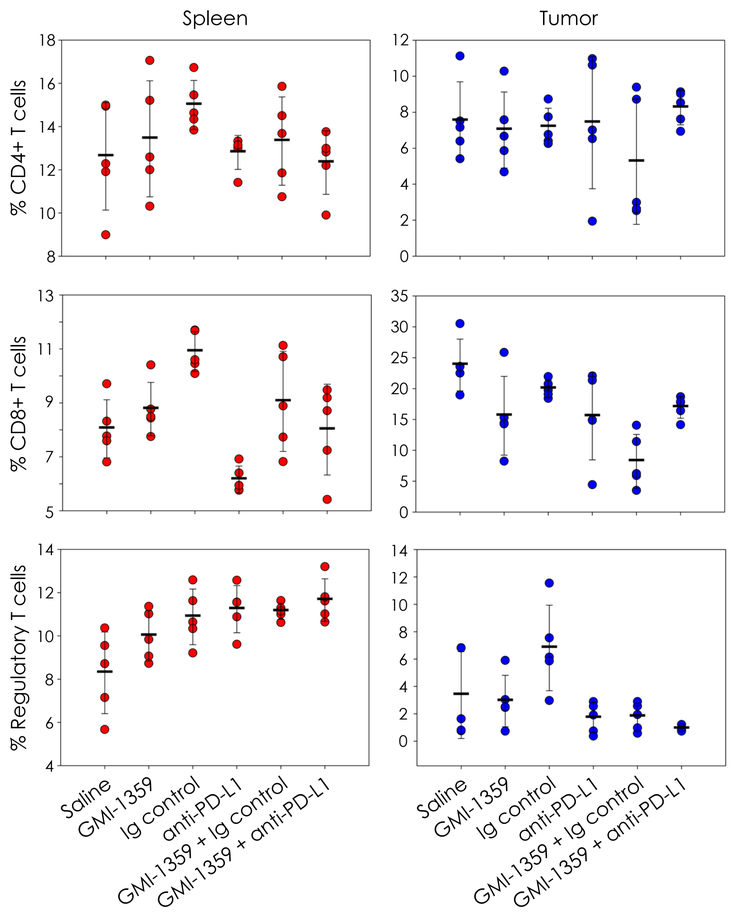


Table 2. Ratio of CD8/Regulatory T cells in Spleen and Tumor on Study Day 15

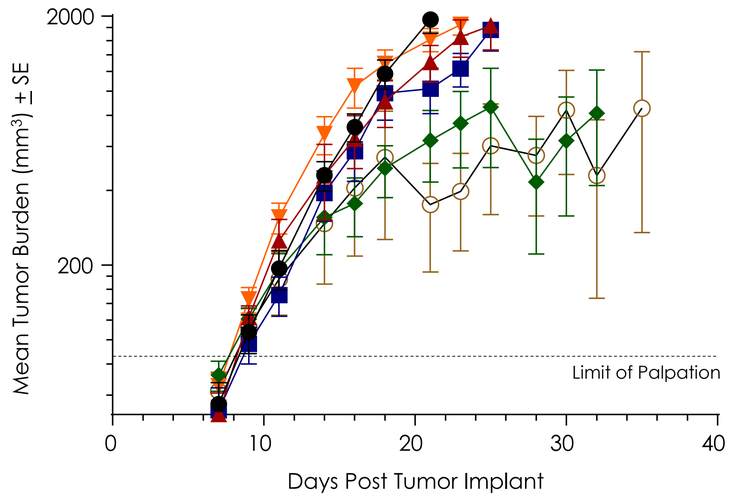
Treatment Group	Spleen	Tumor
Saline	0.97	7.07
GMI-1359	0.88	5.33
Ig control	1.00	2.93
Anti-PD-L1	0.55	9.15
GMI-1359 + Ig control	0.81	4.60
GMI-1359 + anti-PD-L1	0.69	18.71

Summary. Combination therapy with GMI-1359 and anti-PD-L1 antibody reduced the percentage of intratumoral T_{reg} compared to treatment with saline, GMI-1359 or the anti-mPD-L1 antibody as single treatments (0.9% vs. 3.3%, 2.9% and 1.9%, respectively). No other T cell subsets were affected. The reduction in intratumoral T_{reg} cells resulted in a more favorable increase in the ratio of total CD8 T cells to T_{reg}. In spleens, the median percentage of T_{reg} were unaffected by any of the treatments and suggest that the reduction in intratumoral T_{reg} by combined treatment with anti-PD-L1 and GMI-1359 was an attenuated response to maintenance and homing signals in the tumor microenvironment.

Figure 3. Mean Tumor Burden Group Comparison and Response Summary

Tumor measurements were recorded three times weekly. Tumor burden (mm^3) was estimated from caliper measurements by the formula for the volume of a prolate ellipsoid. Animals with tumors in excess of 2000 mm^3 were euthanized. The primary endpoints used to evaluate efficacy were: tumor growth delay, the number of tumor-free survivors at the end of the study, and the incidences of progressive disease, stable disease and regressing disease.

Group	Tumor Growth Delay (days)	% Progressive Disease	% Regressing Disease	% Tumor-free survivors
Saline	NA	100	0	0
GMI-1359	2.5	100	0	0
Ig control	3.2	100	0	0
Anti-PD-L1	11.7	60	40	40
GMI-1359 + Ig control	-0.1	100	0	0
GMI-1359 + anti-PD-L1	13.5	60	40	40



Summary. All treatments of tumor-bearing mice were well tolerated, resulting in no treatment-related mortality. Treatment with GMI-1359 in combination with anti-PD-L1 or anti-PD-L1 alone was associated with a tumor-growth delay of 13.5 and 11.7 days, respectively, and a 40% complete response rate.

Table 3. Time to Complete Response Rate in Tumor-bearing Mice Treated with anti-PD-L1 or anti-PD-L1 in Combination with GMI-1359

Treatment Group	Complete response rate	Days post Rx initiation to complete response (median)[a]
α-PD-L1	40	14, 21, 25, 30 (23)
GMI-1359 + anti-PD-L1	40	7, 14, 14, 16 (14)[b]

[a]Treatment completed day 20 post tumor injection
[b]p=0.0471 vs. anti-PD-L1 alone

Summary. The median time to complete response of tumor-bearing mice treated with GMI-1359 in combination with anti-PD-L1 occurred earlier than with anti-PD-L1 alone (14 vs. 23 days, respectively). All complete responders rejected a subsequent challenge with CT-26 WT tumor cells.

Conclusions

These studies demonstrate that in comparison to anti-PD-L1 treatment alone, the dual E-selectin/CXCR4 antagonist, GMI-1359, in combination with anti-PD-L1 antibody:

- attenuated the specific induction and distribution of T_{regs} into tumor
- increased the ratio of intratumoral CD8 T cells to T_{regs}
- generated a more robust immunotherapeutic anti-tumor response

GlycoMimetics, Inc.