Abstract # 1757

MOBILIZATION OF TUMOR-PRIMED, MARROW INFILTRATING LYMPHOCYTES INTO PERIPHERAL BLOOD WITH INHIBITORS OF E-SELECTIN OR E-SELECTIN AND CXCR4

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Abstract

Marrow infiltrating lymphocytes (MILs) primed to tumor antigens have been described in patients with hematologic malignancies and in metastatic disease arising from carcinomas. The presence of tumor-reactive MILs in these patients has suggested the possibility of their utilization in T-cell immunotherapeutic approaches. Inherent in this approach are considerations that mediate MIL interactions with the microenvironment and how these may be governed for adoptive or active immunotherapy. Both E-selectin and CXCR4 are known to regulate the homing and retention of T cells to the bone marrow. GMI-1271 and GMI-1359 are potent, small molecule glycomimetic antagonists of E-selectin and E-selectin/CXCR-4, respectively. GMI-1359 is a potent small molecule glycomimetic dual antagonist targeting E-selectin and CXCR-4. In the present studies tumor-specific MILs were established in BALB/c mice that had been induced to reject the syngeneic CT26 colon carcinoma via treatment with anti-CTLA-4 T cell checkpoint antibody, and the subsequent effects of antagonizing E-selectin and/or CXCR4 with GMI-1271 or GMI-1359 on the mobilization and distribution of these bone-marrow derived tumor-specific CD8+ T cells, were determined. CT26-immune mice were treated for three days with saline, GMI-1271 (40 mg/kg), or GMI-1359 (40 mg/kg) and 12 hours following the last injection, the phenotype and functional activity of CD8+ T cells were determined in bone marrow and peripheral blood. Additional controls included CT26-immune mice treated with G-CSF (0.125 mg/kg) and tumor naïve mice treated with saline. Treatment of mice with GMI-1271 and to a greater extent with GMI-1359 lead to an approximate 3-4 fold increase in CD8+CD62L+CD44- naïve and CD8+CD62L+CD44+ central memory T cells in peripheral blood. This was not observed following treatment of tumor-immune mice with G-CSF. Treatment of mice with GMI-1271 or GMI-1359 did not affect distribution of CD8+CD62L-CD44+ effector memory T cells in peripheral blood. The increase in percentages of CD8+ naïve and central memory T cells in peripheral blood following treatment with GMI-1271 or GMI-1359 functionally correlated with increased production of IFN-γ *ex vivo* in response to irradiated CT26 tumor cells or the immunodominant CT26 peptide, AH-1. Collectively these results demonstrate the mobilization or redistribution of marrow infiltrative tumor specific CD8+ T cells into peripheral blood as a consequence of E-selectin and/or E-selectin and CXCR4 antagonism. Once in the periphery, these MILs could (1) be collected for adoptive immunotherapy approaches or (2) serve as a systemic augmentation of T cells for combination with immune stimulants as a foundation to boost active immunotherapy.

Background

- In the bone marrow (BM), infiltrating lymphocytes (MIL) primed to tumor antigens have been described in hematological malignancies and in metastatic disease arising from carcinomas.
- Both E-selectin and CXCR4 are known to regulate the homing and retention of T cells to the BM.
- Strategies to block E-selectin and/or CXCR4 may decrease retention/homing of T cells in the BM and increase their distribution into peripheral blood thereby creating a more favorable compartment for either adoptive or active immunotherapy approaches.

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Results

Table 1. Binding of GMI-1271 and GMI-1359 against E-selectin or CXCR4

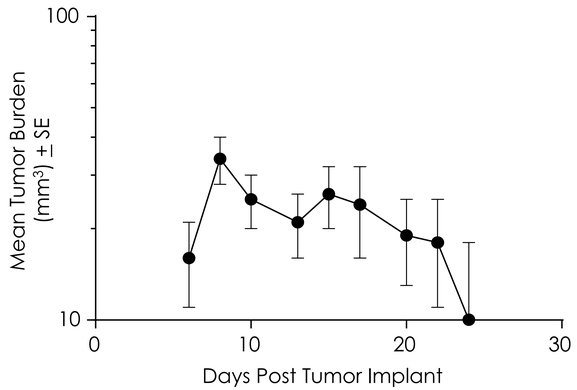
GMI-1359 (dual E-selectin/CXCR4 antagonist) and an E-selectin specific antagonist (ANT) were assessed for inhibition of (1) sialyl LeX binding to immobilized E-selectin and (2) CXCR4 binding to Raji cells. IC50's (μM) were determined and summarized in Table 1.

Compound	E-selectin	CXCR4
GMI-1359	1.0	0.5
GMI-1271	2.4	>10000

Summary (Table 1). The small molecule glycomimetic, GMI-1359, inhibits ligand binding to both E-selectin and CXCR4. As previously reported, GMI-1271 is a selective inhibitor of ligand binding to E-selectin. GMI-1359 and GMI-1271 were subsequently evaluated for redistribution (mobilization) of bone marrow infiltrative, tumor specific CD8+ T cells.

Figure 1. Generation of Tumor Primed Mice with anti-CTLA4 in the CT26 Colon Carcinoma Model

Female Balb/c mice (7 wks) received subcutaneous injections with CT26 colon carcinoma cells (5 x 10^5/mouse). Beginning 3 days post injection, mice were treated with 10 mg/kg anti-CTLA-4 (clone 9D9) on days 3, 6, 10, 13 and 17. Tumor volumes were measured twice/week from day 0 to day 24 and the mean tumor burden (±SE) was calculated to assess response.



Summary (Figure 1).
- Treatment of Balb/c mice with anti-CTLA4 regresses small CT-26 tumors and tumor-free mice are resistant to tumor re-challenge.
- This model was chosen to study tumor-reactive MILs and their mobilization by GMI-1271 and GMI-1359.

Results

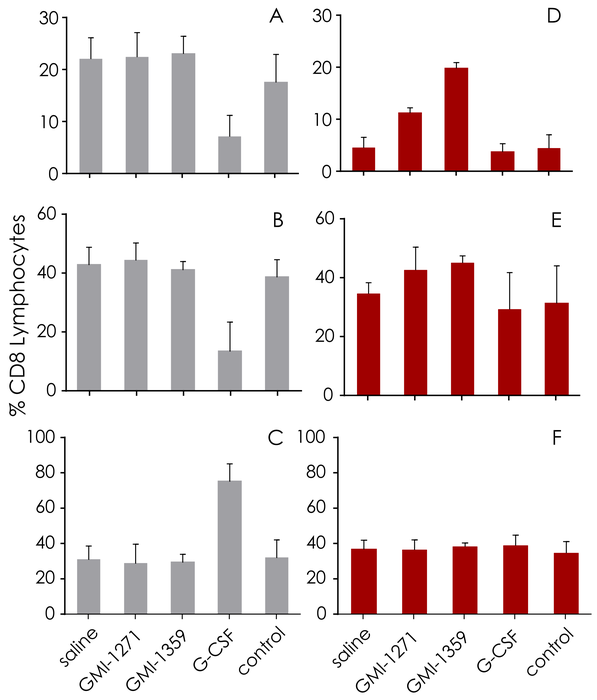
Table 2. Study Protocol to Assess Mobilization of Tumor-primed, Marrow Infiltrating Lymphocytes with GMI-1271, GMI-1359 or G-CSF

On day 25, mice rendered tumor free with anti-CTLA-4 treatment, were distributed into 4 groups (n=5 mice/group) and treated as described. G-CSF treatment was included for comparison.

Group	Treatment Regimen	Parameters (12 hrs post final dose)
saline	10 mL/kg IP bid x 3d	• PB & BM CD8 phenotype • Tumor specific PB & BM in vitro responses: defined as CD8+/IFNγ+ following tumor pulse
GMI-1271	40 mg/kg IP bid x 3d	
GMI-1359	40 mg/kg IP bid x 3d	
G-CSF	0.125 mg/kg SC bid x 3d	

Figure 2. Distribution of CD8 T Cells in CT26-Immune Balb/c Mice following Administration of GMI-1271, GMI-1359 or G-CSF

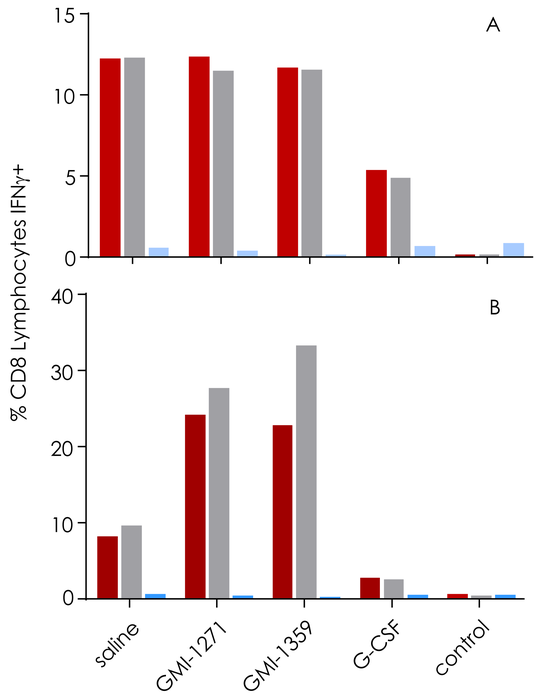
Twelve hrs post final dose, bone marrow (■) and peripheral blood (■) from individual mice were processed for flow cytometry. Control, tumor naïve mice were included. The percentage of CD62L+, CD44- (A, D), CD62L+, CD44+ (B,E) and CD62L-, CD44+ (C,F) were determined in the total CD8+ T cell gate and summarized below.



Results

Figure 3. Mobilization of Tumor-primed Marrow Infiltrating Lymphocytes into Peripheral Blood with GMI-1271 and GMI-1359

Bone marrow (A) and peripheral blood (B) from each treatment group were pooled and the percentage of IFNγ+ CD8 lymphocytes was determined by flow cytometry following stimulation with irradiated CT26 cells (■), AH-1 peptide (■) or media alone (■).



Summary (Figure 2 and 3).
- Compared to saline treatment, GMI-1271 and GMI-1359 mobilizes CD62L+ MILs into peripheral blood by 2- and 4-fold, respectively.
- These mobilized MILs are primed to respond to tumor antigens and produce IFNγ.

Conclusion

- In this preclinical model, glycomimetic inhibitors of E-selectin (GMI-1271) or E-selectin and CXCR4 (GMI-1359), was shown to mobilize and distribute tumor-primed MILs into peripheral blood.
- Mobilized MILs could (1) be collected for engineering of T cells or (2) serve as a systemic augmentation of T cells for combination with immune stimulants and the development of new strategies for immunotherapy