ABSTRACT

The E-selectin ligand HCELL (hematopoietic cell E-selectin ligand) is expressed by normal hematopoietic stem cells (Merarban et al 2011) as a functional glycoform of CD44. We observed high level CD44 expression (99% ± 1.4%) by blasts from 55 consecutive patients with acute myeloid leukemia (AML) and by putative CD34+CD38- leukemia stem cells (LSCs) (99.8% ± 0.4%). The mean fluorescence intensity (MFI) for CD44 expression by AML blasts is one to two logs higher than the MFI for 16 other adhesion receptors. We find that the majority of blasts from patients also express an E-selectin ligand by flow cytometry: >75% of 22 primary patient blasts exhibit >10% binding of E-selectin ligand with mean expression 12.2% ± 0.7% (29% ± 6.2%). We also observed that HCELL is expressed by normal peripheral blood mononuclear cells and by normal human umbilical vein endothelial cells (HUVEC).

RESULTS

1. Flow Cytometry Analysis for E-selectin (IgG chimera binding by AML blasts and leukemia stem cells)

2. Flow Cytometry Analysis with HEC42 Antibody Staining

3. Binding of AML blasts to E selectin and blocking by GMI 1271

4. In vitro chemotaxis cytotoxicity of daunorubicin and cytarabine on AML blasts on E selectin with or without GMI 1271

5. In vivo cytotoxicity with combination of chemotherapy and GMI 1271 in NODscid IL2Rgc-/- mice engrafted with primary human AML.

Method

Flow cytometry analysis of HEC42 and E-selectin

AML blasts were stained for 30 minutes at 4°C with CD25-APC-Cy7 to gate on the leukemia blasts, and CD44-APC and E-selectin (CD62E) PE were used to gate on the AML stem cells. The conjugated anti-CLA-FITC (CLA: Cutaneous lymphocyte antigen, HECA-452 (clone) antibody and Human E-selectin/CD62E Fc Chimera-PE were used to assess E-selectin ligand expression. The samples were washed and then fixed with 1% paraformaldehyde. Cells were measured by FACScan (BD biosciences) and the results were analysed using Flowjo software.

SUMMARY

A Novel Small Molecule E-Selectin Inhibitor GMI-1271 Blocks Adhesion of AML Blasts to E-Selectin and Mobilizes Blood Cells in NODscid IL2Rgc-/- Mice Engrafted with Human AML

Sylvia Chien, Xin Zhao, MD, Margaret Brown, Akanksha Saxena, John T. Patton, PhD, John L. Magnani, PhD and Pamela S Becker, MD, PhD

Division of Hematology and Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA and GlycoMimetics Inc., Gaithersburg, MD

INTRODUCTION

CD44 plays a pivotal role in the bone marrow homing of leukemia cells. For example, anti-CD44 antibodies prevent engraftment of U937 cells in immunodeficient mice (Okumura et al 2005; 11:167-174). Bcr-abl positive leukemia stem cells are dependent on CD44 for homing to the marrow (Krause et al 2006; 115: 1175-1180). Nkml-All cell line home to E-selectin/CD44+ regions of the bone marrow microvasculature (Sipkins Di et al, Nature 2001, 405: 969-973), dependent on both E-selectin and CD44.

Moreover, level of CD44 expression confers prognostic significance, in that high level expression of CD44 correlates with leukemic relapse (Quere et al 2010), and high soluble CD44 in hematopoietic stem cell grafts from patients with AML also correlates with increased rate of relapse (Krause et al 2011, ArchPatholLabMed 2011;134:1033-1038).


Lastly, selectins appear to play a role in the interaction of certain cancer cells with their microenvironment. For example, a pan-selectin inhibitor disrupts myeloma cell interaction with the bone marrow and sensitized myeloma cells to bortezomib (Abas A Blood 2012; 119:1468-78). Lastly, adhesion of colon carcinoma cells to selectin led to activation of survival pathways (Pourquet et al 2011).

Therefore, we hypothesized that disruption might also impair these survival pathways and enhance the efficiency of chemotherapy.

GMI-1271 is a potent new rationally designed small molecule E-selectin antagonist.