E-Selectin Ligand Expression By Leukemic Blasts Is Associated with Prognosis in Patients with AML

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INTRODUCTION

The E-selectin ligand is a carbohydrate structure (sialyl Lewis x) expressed on myeloid cells and plays a role in cell adhesion and activation by binding specifically to E-selectin on endothelial cells. Acute myeloid leukemia (AML) blasts express the E-selectin ligand on glycoforms of CD44, CD62L and CD65. Interaction of AML blasts in the marrow microenvironment is believed to involve a number of receptors, including CXCR4, VLA4, and CD44. E-selectin in the vascular niche regulates dormancy for hematopoietic stem cells (Winkler IG et al. Nat Med. 2012;18:1651-7), and chemoresistance and survival in AML. E-selectin inhibitors are now being studied in clinical trials in combination with chemotherapy for hematologic malignancies. Here, we evaluate the clinical significance of the expression of the E-selectin ligand using a panel of AML patient blood and bone marrow samples.

METHODS

We studied 89 serially acquired AML patient samples obtained with informed consent on an IRB approved protocol. Mononuclear cells were isolated from blood and bone marrow specimens after density depletion on Lymphocyte Separation Medium (Mediatech, Inc. Manassas, VA).

We used multicolor flow cytometry to examine binding of an E-selectin-Fc chimeric protein or staining by HECA452 antibody that recognizes sialyl Lewis a/x carbohydrate epitopes. The blast population was gated by forward scatter vs. side scatter, then CD45 vs. side scatter. Leukemia stem cells (LSCs) were defined as CD34+CD38-CD123+. All antibodies were obtained from Becton Dickinson Biosciences. After labeling, the cells were washed with PBS and fixed with 2% paraformaldehyde. Flow cytometry analysis was performed using FACScanto with FACSDiva (BD Biosciences. San Jose, CA). Data analysis was performed with FlowJo software (Tree Star, Inc.).

Correlations were conducted for the following clinical parameters:
Gender
Age
Antecedent Hematological Disorder
New diagnosis vs Relapsed/Refractory
Complete Remission
Duration of first Complete remission
Cytogenic risk
FLT3 ITD mutation
NPM1 mutation
White blood count at presentation
Overall Survival

Statistical analysis was performed using the following:
Welch Two Sample t-test
Wilcoxon rank sum test with continuity correction
Pearson's product-moment correlation

RESULTS

Patient Characteristics

Feature			
Gender	Male 49		Female 38
Age	Median 60		Range 26-85
Status	New diagnosis 40		Relapsed, refractory 49
Cytogenetics	Favorable 7	Intermediate 35	Unfavorable 48
Antecedent Hematologic Disorder	Yes 24		No 65
Response	CR/Cri 30		Refractory 54
Mutation Status	NPM1 17 mutated, 47 neg		FLT3 ITD 29 mutated 45 neg

RESULTS

E-selectin Ligand Expression by AML Blasts and LSCs

Fig. 1. E Selectin Ligand % Expression by AML Blasts

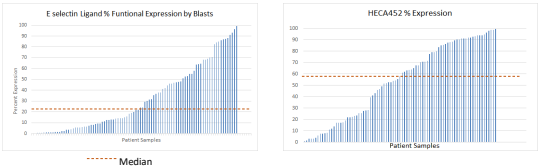

Figure 1. Left. Percent E selectin ligand functional expression (binding of Esel/Fc chimera) ranged from 0.4 to 99.4%, median 20.9%, mean 32.1%.
Right. Percent staining by HECA452 antibody ranged from 0.9 to 99.6%, median 58%, mean 54.3% These data suggest that the epitope recognized by the HECA452 antibody may be present, but not always functionally active.
Correlation E-sel/Fc binding to HECA (Pearson's): p-value = 1.543e-06

Fig. 2. E Selectin Ligand Expression by Blasts-MFI

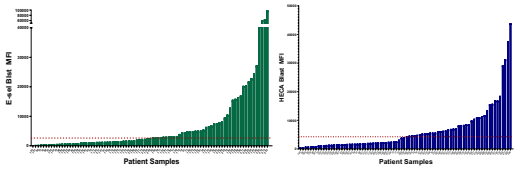

Figure 2. Left. Mean Fluorescence Intensity (MFI) for binding of Esel/Fc chimera ranged from 242 to 99,340, Median 2600, Mean 7702.
Right. MFI for staining by HECA452 antibody ranged from 601 to 43,951, Median 4242, Mean 6853. Correlation Esel/Fc binding to HECA: 5.815e-08

Fig.3. E-selectin Ligand % Expression By LSCs

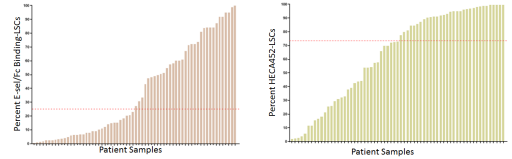

Figure 3. Left. Percent E selectin ligand functional expression (binding of Esel/Fc chimera) by LSCs ranged from 0.6 to 100%, median 25%, mean 37.9%.
Right. Percent staining of LSCs by HECA452 antibody ranged from 1.9 to 99.8%, median 73%, mean 64%

Fig.4. E-selectin Ligand Expression By LSCs-MFI

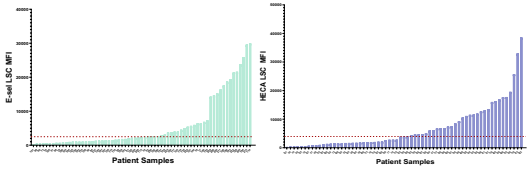

Figure 4. Left. MFI for binding of Esel/Fc chimera by LSCs-ranged from 338 to 29,980, Median 2340, Mean 5973.
Right. MFI for staining by HECA452 antibody ranged from 321to 38,565, Median 3931, Mean 6860.

Figure 5

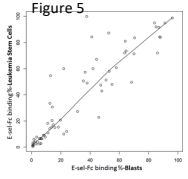

Fig.5. Correlation E-selectin Ligand Expression Blasts vs LSCs

Figure 5. E-selectin ligand expression by blasts was highly correlated to expression by leukemia stem cells from the same patient, p< 2.2e-16 (Pearson's product-moment correlation) and correlation coefficient 0.90.

RESULTS

Correlations of E-selectin Ligand Expression with Clinical Parameters

Fig. 6. E Selectin Ligand Expression with AML Status: New Diagnosis vs Relapse

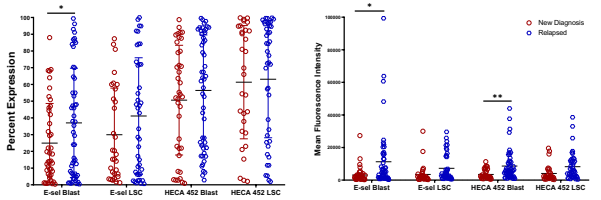

Figure 6. Left. Percent expression for new diagnosis vs. relapse. For percent binding of E-sel/Fc chimera (E-sel blast) new diagnosis (ND) mean 25% vs. relapse (R) 38% (*p=0.037). For percent HECA (Blast), ND 51% vs R 57% (p=0.40)
Right. The MFI was lower for E-sel/Fc chimera binding (Blast) and staining by HECA452 (Blast) for new diagnosis (ND) compared to relapse (R). MFI ND-2,884 vs R-11,764 for E-sel/Fc (*p=0.00074 Wilcoxon, p=0.0026 t-test), and MFI ND-3387 vs R-8994 by HECA staining (**p=0.0013 Wilcoxon, p=0.00038 t-test).

Fig. 7. E Selectin Ligand Expression with Favorable/Intermediate vs Unfavorable Cytogenetics

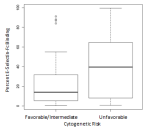

Figure 7. There was a significant difference for E-selectin ligand % expression by E-sel/Fc binding for favorable/intermediate (mean 24%) vs. unfavorable (mean 39%) cytogenetics. (p=0.019 Welch, p=0.033 Wilcoxon)

Fig. 8. E Selectin Ligand Expression with FLT3ITD or NPM1 Mutation Status

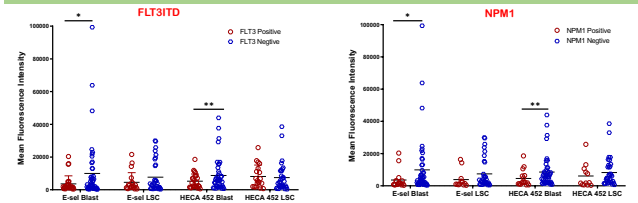

Figure 8. Left. There was a significant difference for E-selectin ligand Blast MFI for FLT3ITD mutated (M) vs. wild type (WT), for E-sel/Fc binding, mean M 3858 vs WT 9919,* p=0.042, and for HECA MFI, M 5100 vs WT 8733, **p=0.055. Right. For NPM1 mutated, E sel/Fc binding (Blast) MFI 3597 M vs. 9839 WT, *p=0.038 and for HECA (Blast) MFI, M 4562 and WT 8537, **p=0.049.

Summary

- E-selectin ligands are variably expressed by AML blasts and LSCs.
- Mean fluorescence intensity of E-selectin-Fc binding is 4-fold higher for relapsed/refractory patients than for newly diagnosed patients (p=0.0026), suggesting that sequestration in the vascular niche of the marrow is associated with chemotherapy resistance.
- Percent E-selectin-Fc binding is higher in patients with unfavorable than favorable/intermediate risk (p=0.019), correlating with worse prognosis.
- Moreover, expression of E-selectin ligands by LSCs is correlated with blasts from the same patients, suggesting a role for E-selectin in leukemia survival and propagation.

Acknowledgement/COI

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