Blocking Vascular Niche E-Selectin Dampens AML Stem Cell Regeneration/Survival Potential In Vivo By Inhibiting MAPK/ERK and PI3K/AKT Signalling Pathways

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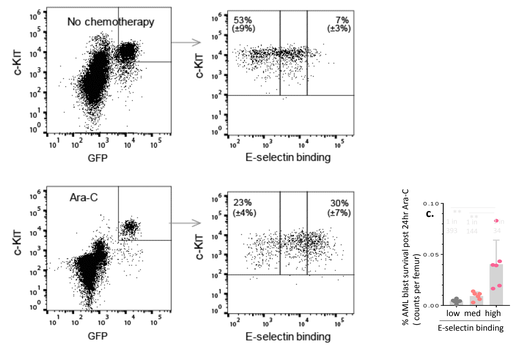
Rationale

We have previously shown vascular E (endothelial)-selectin to play a key role in niche-mediated chemo-resistance in Acute Myeloid Leukaemia (AML).
Now we report that the cell surface glycosylation of AML blasts –and thus their E-selectin-binding potential- alters during therapy and queried whether these variations influence treatment outcome.

1a.. AML blasts with highest E-selectin binding survive chemotherapy



1b.. FACs sorted AML blasts show E-selectin blocade dampens AML resgenearaion potentail

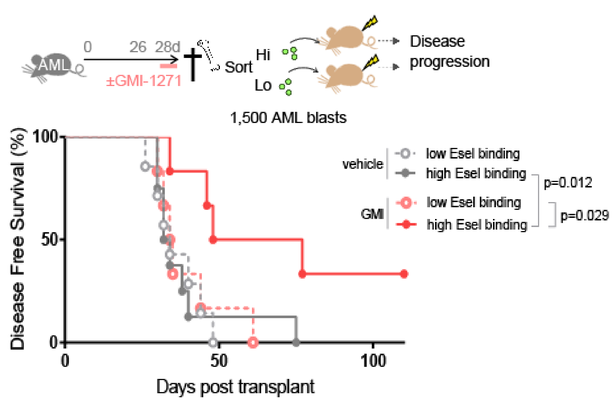


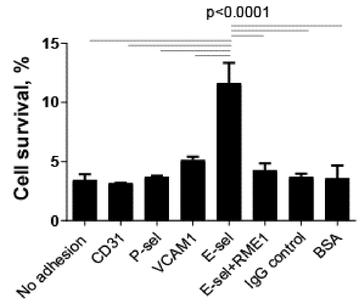
Figure 1. High E-selectin-binding potential characterizes the AML blasts more likely to survive chemotherapy. Mice with MLL-AF9 leukemia (n=6 / group) were administered 24hr high-dose cytarabine (Ara-C) chemotherapy, or vehicle control, prior to harvest of bone marrow cells for flow cytometry analyses. **(a).** Dot plots show gating strategy and altered distribution of E-selectin binding among GFP⁺ Kit⁺ AML blasts in bone marrow before (*top panel*) and after chemotherapy (*bottom panel*). Shown is representative dot plot from one mouse per group. **(b)** *Experimental outline*. Bone marrow cells from (n=6) mice were separated by FACS and 1,500 sorted AML blasts (GFP⁺ Kit⁺) with highest or lowest E-selectin-binding (using the gating strategy shown in top panel above) were transplanted into 2.5Gy conditioned recipient mice (n=8 / group). In one cohort of donors GMI-1271 was administered 200mg/kg BiD for last 48hr prior to harvest and sort. *Bottom panel*. Shown is Kaplan Meier disease-free survival of recipient mice transplanted with 1,500 sorted AML blasts. Solid lines represent survival of recipients of high E-selectin-binding whereas dashed lines are recipients of low E-selectin-binding AML blasts. Grey curves indicate recipients of vehicle treated donors, while red/orange indicates recipients of sorted AML blasts from GMI-1271-treated donors. Statistics, Log-Rank (Mandel-Cox) survival curve comparison. No significant difference in leukemia regeneration (relapsing potential) was observed in recipients of vehicle treated donors. However in high E-selectin binding blasts, GMI-1271 pre-treatment of donors significantly reduced regeneration potential of sorted AML blasts, resulting in extended duration of disease-free survival (median survival of 62 days, compared to matching vehicle 34 days, p=0.012).

Introduction

The vascular adhesion molecule E-selectin has been shown to be a key component of the Bone Marrow (BM) Haematopoietic Stem Cell (HSC) niche with a role in facilitating HSC activation at the expense of self-renewal (Winkler et al., Nat Med, 2012).

How do AML cells respond to E-selectin at the vascular niche?

2a. . In vitro adhesion to E-selectin directly mediates cytoarabine resistance in AML



2.b . Therapeutic blockade of E-selectin at the same time as chemotherapy doubles treatment efficacy.

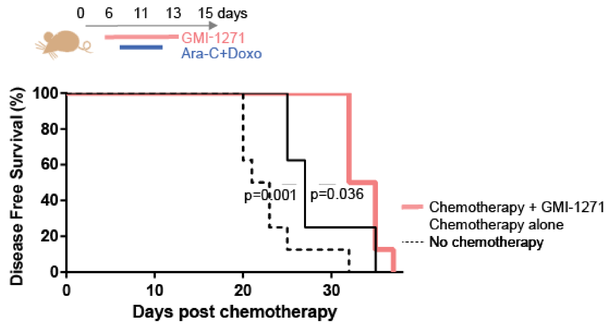


Figure 2. Absence or therapeutic blockade of E-selectin sensitizes AML to chemotherapy and extends duration of disease-free survival in mice. (a) MLL-AF9 AML cells from mouse bone marrow were cultured for 3 days in the presence of 25 ng ml^{-1} cytarabine (AraC) in wells pre-coated with either recombinant mouse PECAM-1 (CD31), P-selectin (P-sel), VCAM-1 (VCAM1), or E-selectin (E-sel) human IgG1 fusion protein as indicated. "No adhesion" control wells were coated with bovine serum albumin (BSA). In some wells 10μg/mL E-selectin blocking antibody RME-1 or isotype control rat anti-mouse IgG were added as indicated. Plotted are percentages of cell survival compared to matching non-chemotherapy-treated wells by quantitative enumeration of viable GFP⁺ cells by flow cytometry. Mean ± SD of pooled data from three replicate experiments with n=3, 5 and 5 replicate wells/condition. P values calculated by one-way ANOVA with Bonferroni correction for multiple comparisons. (**b**) Wildtype C57BL/6 mice with MLL-AF9 AML were administered GMI-1271 40mg/kg BiD or saline before and during a 5-day induction chemotherapy regimen (doxorubicin 1mg/kg 3d and cytarabine 100mg/kg 5d) as indicated and monitored for duration of disease-free survival. Shown is Kaplan Meier curve (n=8 mice / group). Statistics; Log Rank (Mandel-Cox) survival curve comparison.

. Immunoblotting of bone marrow lysates form leukemic mice demonstrate E-selectin blockade (GMI-1271 administration) dampens activation of components of AKT, NFkkB, mTOR and MAPK pathways important to malignant cell survival.

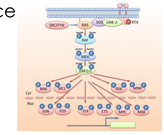


Image from Lui et al., Acta Pharmaceutica Sincia 2018

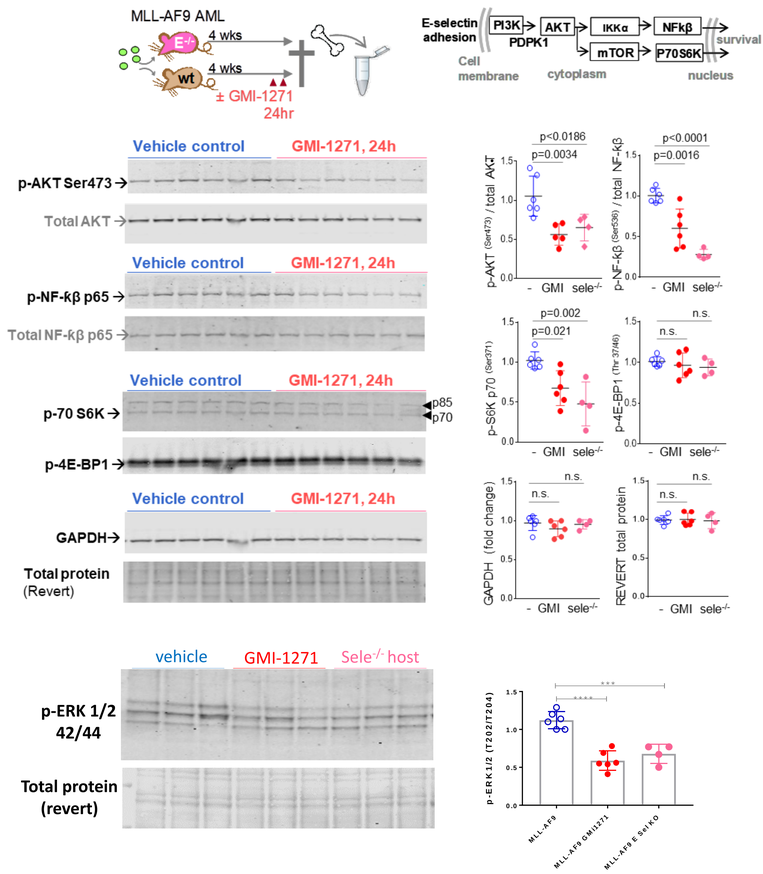


Figure 3. E-selectin blockade *in vivo* dampens PI3K/AKT/NF-κB signaling. (a) Outline of experimental plan and intracellular signaling pathways investigated. **(b).** Quantitative immunoblotting of bone marrow lysates with indicated antibodies using linear Li-COR detection. Lysates were from wildtype leukemia mice administered GMI-1271 (24h 200mg kg^{-1}, BiD) or vehicle control. Each lane contains 15 μg bone marrow lysate protein from an individual mouse (n=6 / group). (**c**). Quantitative band intensity analyses by Li-COR. Includes additional data from analyses of same AML transplanted into (n=4) *Sele*$^{-/-}$ hosts from additional blots. Each dot represents data from a single mouse expressed as fold change over saline vehicle injected control.

Reagents and Methods

To understand mechanisms of chemo-sensitisation, leukemic mice were administered the small molecule antagonist of E-selectin GMI-1271 (from GlycoMimetics), or saline vehicle control for ± cytarabine chemotherapy then analysed for AML survival.
Murine Leukaemia models used include murine mono-myelocytic leukaemia induced by MLL-AF9 or MLL-ENL (11q23 translocation) translocation.

Conclusion and significance

Here we show that (1) vascular niche E-selectin blockade by GMI-1271 dampens malignant AML reconstitution/survival potential *in vivo* when administered as sole agent alone,
(2) E-selectin blockade mediates these effects via dampening a range of intracellular survival/regeneration signaling pathways in the malignant cell, and finally
(3) these data suggest E-selectin blockade may synergise with other specific pathway inhibitors to improve treatment outcomes - but only for malignant cells that are appropriately glycosylated to interact with E-selectin. A Phase III Clinical trial to study efficacy of GMI-1271 in combination with chemotherapy in AML patients (NCT03616470) is currently in progress.

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