Vascular E-selectin Protects Leukemia Cells from Chemotherapy by Directly Activating Pro-survival NF-kB Signalling

Therapeutic E-selectin Blockade with GMI-1271 Inhibits NF-kB Activation in AML.

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Significance

Median survival for adults diagnosed with Acute Myeloid Leukaemia (AML) remains only ... years despite best practise. New strategies are needed to improve treatment.

Humanised mouse models show the leukaemia repopulating cells that are in contact with blood vessels in the bone marrow are most likely to survive chemotherapy. (Ninomiya et al., Leukaemia 2007)

We hypothesise that adhesion to vascular niche E-(endothelial)-selectin alone can promote leukemia stem cell (LSC) survival in vivo.

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).

Only ~3% of BM endothelial cells normally express E-selectin. However we find E-selectin to be greatly upregulated ~10-fold in mice engrafted with AML). This raises the question whether E-selectin-mediated signalling between HSC and AML LIC differs.

Reagents & Methods

To understand mechanisms of chemo-sensitisation, leukemic mice were administered the small molecule glycomimetic antagonist of E-selectin GMI-1271 (from GlycoMimetics), or saline vehicle control for 4 days ± cytarabine chemotherapy then analysed for AML survival. In some experiments NF-kB commercial small molecule inhibitor BMS-345541 was also used.

Leukaemia models used include murine mono-myelocytic leukaemia induced by MLL-AF9 (11q23 translocation) and granulocytic leukaemia induced by AML1-ETO9a (t(8;21) translocation). Results also verified using human CD34+ leukaemia cell line KG1a in vitro.

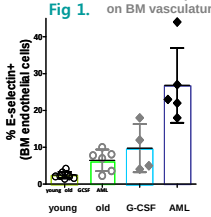
OBSERVATIONS

Fig 1. Vascular E-selectin becomes highly expressed on bone marrow endothelial cells during AML.

Figure 1. % of BM endothelial cells expressing E-selectin increases with age, G-CSF and during AML.

Bone Marrow (BM) cells from ♂ young (10wk), old (100wk), 3 day GCSF-treated and in mice with MLL-AF9-induced AML. Femoral endosteal cells were surface stained for E-selectin expression on gated endothelial cell fraction (Lin⁻ CD45⁻ CD31⁺)

Fig 1. E-selectin expression on BM vasculature



Figs 2,3. Adhesion to E-selectin activates pro-survival signalling in AML cells.

Pro-survival signalling is reversed by addition of small molecule E-selectin antagonists such as GMI-1271

Figure 2. In vitro chemo-sensitivity assay. Bone marrow leukemic blasts were cultured 4 days in wells pre-coated with immobilised recombinant adhesion molecules (E-selectin, VCAM-1) and controls (BSA, IgG) in presence of ± Cytarabine (Ara-C).

Shown are % surviving leukemic blasts.

Figure 3a. Leukemic blasts that survive cytarabine chemotherapy in vivo are those with highest E-selectin binding potential.

Figure 3b. These data are consistent with previous publications (Ninomiya et al., 2007) showing the few human CD34+ AML cells that survive chemotherapy in immunocompromised mice are adjacent to bone marrow endosteal vasculature (where we find E-selectin is expressed.

Fig 2. Adhesion to E-selectin protects from chemotherapy in vitro.

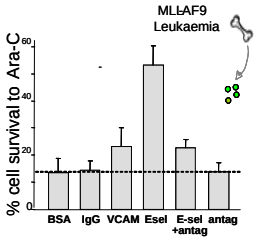


Fig 3a..

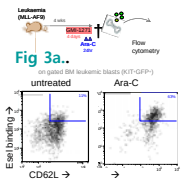
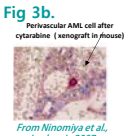


Fig 3b.



MECHANISM

RNA sequence analysis — NF-kB intracellular signalling pathway is dampened by E-selectin blockade with GMI-1271 in vivo

FACS purified BM leukaemic blasts from mice administered ± GMI-1271 (4 days) were processed for RNA sequencing .

NF-kB activation is frequent in AML. NF-kB activation is well known to promote survival to chemotherapy. (Review. Godwin et al., Frontiers in Oncology 2013)

Figure 4. E-selectin mediated adhesion upregulates NF-kB – GFP reporter in murine leukaemia cell line. Readout by flow cytometry for GFP after 2hr adhesion to immobilised recombinant E-selectin compared to other vascular adhesion molecules in wells.

Fig 4. NF-kB – GFP reporter Leukaemia cells

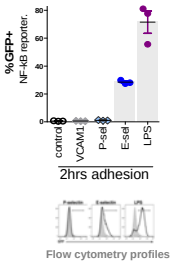


Fig 5.

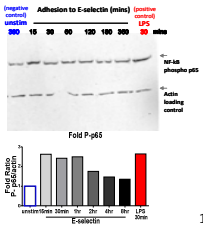


Figure 5. Adhesion to E-selectin leads to phosphorylation activation of NF-kB p65. Murine leukemia cells were cultured in contact with E-selectin for times (mins) specified, then cell lysates immunoblotted for NF-kB phospho p65 and actin loading control.

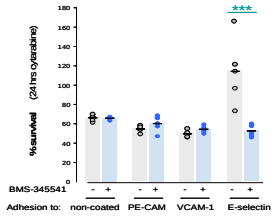
Bottom is intensity scan of blot showing fold increase of phospho NF-kB p65 relative to actin control.

15 min adhesion to E-selectin achieves same phosphorylation of NF-kB p65 as LPS (100ng/mL).

Fig 6. Blocking NF-kB signalling in AML cells in vitro reverses E-selectin-mediated chemoresistance.

Figure 6. Human CD34+ AML cell line KG1a cultured 24hrs in contact with vascular adhesion molecules (PECAM-1/CD31, VCAM-1, E-selectin) in presence of cytarabine chemotherapy ± NF-kB inhibitor BMS-345541 (10uM).

Shown is % cell survival after 24hrs cytarabine.



THERAPEUTIC APPLICATION

Fig 7. In mice E-selectin blockade *(GMI-1271)* is as efficacious as NF-kB blockade at chemo-sensitisation of bone marrow AML cells in vivo.

Figure 7. Quantification of NF-kB or E-selectin antagonists on LSC (colony) survival in mice.

Leukemic mice were administered GMI-1271 (3 days 40mg/kg BID, E-selectin antagonist) or BMS-345541 (24hrs, 75mg/kg BID) together with 24hrs high dose cytarabine chemotherapy. 1% of femoral bone marrow was plated in colony assays to enumerate surviving functional LSC colony initiating cells.

Both GMI-1271 and BMS-345541 synergise with cytarabine in vivo to sensitise LIC.

Fig 4. In vivo blockade of E-selectin and NF-kB similarly chemo-sensitises AML LSC (CFC).

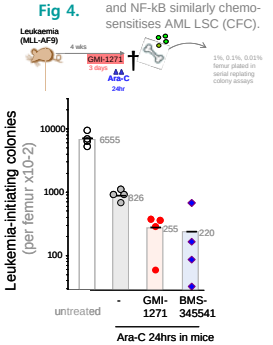


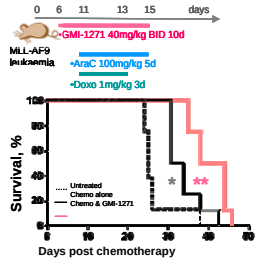
Fig 8. GMI-1271 administered together with chemotherapy significantly extends overall survival in mice.

Figure 8. Co-administration of GMI-1271 together with 5 day standard mixed Cytarabine/Doxorubicin chemotherapy regime prolongs survival of mice with AML.

Methoc 24 mice transplanted with MLL-AF9 leukaer ia and administered either saline, cytarabine + doxorubicin alone (chemo) or chemo + GMI-1271

(n=8/gp) *P<0.05, ** P<0.01 Log-Rank (Mantel-Cox) test.

Chemotherapy alone extended survival by 23% which was doubled with GMI-1271 co-administration



Conclusions

Upstream blockade of E-selectin by GMI-1271 not only inhibits NF-kB activation but also mobilizes LSC out of the protective BM niche & prevents re-entry thereby breaking the chemo resistance observed with these cells.

A Phase I/II Clinical trial to study efficacy of GMI-1271 in combination with chemotherapy in AML patients (NCT02306291) is currently in progress.