Vascular E-Selectin - A Gatekeeper Inducing Commitment and Loss of Self-renewal in transmigrating HSC

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Key Points
1. Bone Marrow endostal E-selectin expression peaks with peak of HSC mobilization.
2. Direct contact with vascular adhesion molecule E-selectin during transmigration compromises engraftment and self-renewal potential of >90% of mobilized HSC.
3. Therapeutic blockade of E-selectin during mobilization regimes overcomes this issue boosting HSC transplant outcomes.

Vascular E-selectin is strongly upregulated during HSC mobilization regimes - associates with number of HSC mobilized into blood.


E-selectin directly compromises HSC during transmigration. Direct contact with E-selectin during transwell migration in vitro compromises >90% of HSC reconstitution potential.

Figure 1.

Figure 2.

Figure 3.

Transmigration through E-selectin coated transwells directly compromises HSC reconstitution potential. METHOD (a, b) Cohorts of mice were administered saline or G-CSF (36 125µg/kg) 6h before GMI-1271 (100µg/kg) as indicated then donor peripheral blood collected for transplantation in Long-Term Competitive Reconstitution assay. Data shown (top) are % donor CD45.2+ reconstitution in recipient blood at 16 weeks post-transplant.

RESULTS (a) G-CSF boosts mobilization in e-selectin (-/-) but not P-selectin (slip +/-) gene-deleted mice or wildtype. (b) E-selectin blockade (administration of small-molecule antagonist GMI-1271 /Uproselan) with G-CSF boosts reconstitution potential of mobilized blood HSC. TOP. Shows % reconstitution of limiting-dilutions (25uL, 5uL and 1uL) of mobilized blood transplanted into recipient congenic mice. BOTTOM. Pseudo Panel Analysis confirming 24-fold boost with GMI-1271. To replate 33% of transplanted mice, 2uL of G-CSF/GMI-1271 mobilized blood is required vs 4x4uL of G-CSF mobilized blood. p=0.00000000 ** (sca software)

(c) Direct comparison of E-selectin blockade (GMI-1271) with high dose Plerixafor (AMD-3100) together with G-CSF. TOP. % donor blood reconstitution of 25uL mobilized blood confirming both E-selectin blockade and CXCL12 blockade have similar efficacy in boosting mobilized blood transplant outcomes. BOTTOM. Number of phenotypic HSC in mobilized blood at time of harvest shows E-selectin blockade appears to enhance “quality” of mobilized HSC while AMD-3100/Plerixafor enhances quantity.

E-selectin blockade reverses G-CSF-induced release of inflammatory Mediators in the BM that may compromise HSC.

METHODS (a) Cohorts of mice were administered saline or G-CSF 3d ± GMI-1271 for last 24hrs then euthanized and femoral flushed in 1x, 5x and 10x BM cell-free flushes were analyzed for inflammatory cytokines using LegendFLEX beads. Data shown as pg/mL of diluted femoral flush. Each dot represents data from one individual mouse.

Key reagents / Methods
• GMI-1271 (Upro) by GlycoMimetics was administered 8dally at 20-40ng/kg for time periods indicated.
• Mobilization Regimens: G-CSF (Filgrastim, Amgen) was administered to mice 125µg/kg/injection BD daily s.c. for times indicated. AMD-3100/Plerixafor (Terns) 6mg/kg i.v. was administered 2hr prior to harvest. Cyclophosphamide (CYP) single dose administered 100mg/kg i.p. as indicated.
• Mice: Wt, high E-selectin, and P-selectin gene-deleted mice were C57BL6/ background. Recipients SJL
• Blood transplantation using long-term competitive reconstitution assay (LT-CR) assay as described in Winkler et al, Nat Med 2011.

Therapeutic E-selectin blockade enhances release of inflammatory Mediators in the BM that induce HSC commitment.