Ingrid Winkler

Title. Mobilisation of reconstituting HSC is boosted by E-selectin antagonist GMI-1271

Blood
Bone marrow

Active
Dormant

myeloid
lymphoid

Active
Dormant

myeloid
lymphoid

Active
Dormant

myeloid
lymphoid

Active
Dormant

myeloid
lymphoid

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HSC niche chapter, Stem Cell Biology, Humana press
HSC

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Vascular niche E-selectin regulates hematopoietic stem cell dormancy, self renewal and chemoresistance

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Vascular niche E-selectin regulates hematopoietic stem cell dormancy, self renewal and chemoresistance

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HSC proliferation

8-fold less HSC turnover in E⁻/⁻
Vascular E-selectin - low levels in steady-state

Endothelial cells in: control mice

3% positive in steady-state
Vascular E-selectin - increases following stress

Endothelial cells in:
- control mice
- Post-irradiation

E-selectin
G-CSF increases E-selectin expression at HSC vascular niche

% E-selectin +, BM endothelial cells

G-CSF alone

G-CSF 4d
250ug/kg/day

Australia
G-CSF increases E-selectin expression at HSC vascular niche

- **G-CSF alone**
  - % E-selectin +, BM endothelial cells:
    - 0% at 0 days
    - 4% at 4 days
    - 21% at 6 days
    - 14% at 10 days

- **CYP + G-CSF**
  - % E-selectin +, BM endothelial cells:
    - 0% at 0 days
    - 46% at 5 days
    - 14% at 6 days
    - 4% at 8 days

**Summary:**
- G-CSF alone increases E-selectin expression over time in BM endothelial cells.
- CYP + G-CSF shows a marked increase in E-selectin expression, particularly at 5 days.
G-CSF increases E-selectin expression at HSC Vascular niche
Absence of E-selectin improves mobilisation of reconstituting cells
Absence of E-selectin improves mobilisation of reconstituting cells

- CFC per mL blood
  - WT
  - Esel\(^{-/-}\)
  - Control
  - G-CSF

- % donor CD45.2\(^+\), in blood
  - WT
  - Esel\(^{-/-}\)
  - G-CSF

- Reconstitution Units, mL blood
  - WT
  - Esel\(^{-/-}\)
  - G-CSF

**Absence of E-selectin improves mobilisation of reconstituting cells**

- Absence of E-selectin improves mobilisation of reconstituting cells.

**Methods:**
- G-CSF 125mg/kg BID
- 25μL blood + 2x10^5 congenic WBM
- WT vs. Esel\(^{-/-}\)

**Results:**
- CFC per mL blood:
  - Control: WT > Esel\(^{-/-}\)
  - G-CSF: WT < Esel\(^{-/-}\)

- % donor CD45.2\(^+\), in blood:
  - WT: 39%
  - Esel\(^{-/-}\): 76%

- Reconstitution Units, mL blood:
  - WT: 66
  - Esel\(^{-/-}\): 634

**Conclusion:**
- Absence of E-selectin improves the mobilisation of reconstituting cells in the blood.

**Australia**
Therapeutic blockade of E-selectin boosts reconstitution potential

GMI-1271 – synthetic E-selectin GlycoMimetic antagonist. Specifically blocks E-selectin binding site

G-CSF 250μg/kg/d ± GMI-1271 20mg/kg BID

Australia
Therapeutic blockade of E-selectin boosts reconstitution potential

GMI-1271 – synthetic E-selectin GlycoMimetic antagonist.

G-CSF 250ug/kg/d ± GMI-1271 20mg/kg BID

25μL blood + 2x10⁵ congenic WBM in long-term competitive transplant assay

% donor CD45.2+ in recipient blood

Reconstitution units, per mL donor blood

G-CSF 250ug/kg/d ± GMI-1271 20mg/kg BID

In long-term competitive transplant assay

GMI-1271 – synthetic E-selectin GlycoMimetic antagonist.

Australia
Limiting dilution transplant

% donor CD45.2+ reconstitution (recipient blood, 16 weeks)

- 25 uL blood
- 5 uL blood
- 1 uL blood

G-CSF
G-CSF + GMI-1271

< 0.5% negative threshold

+ 2x10^5 congenic BM cells in Long-term Reconstitution Assay
Limiting dilution transplant

25 uL blood

G-CSF

G-CSF + GMI-1271

% donor CD45.2+ reconstitution (recipient blood, 16 weeks)

36%

0.1

100%

G-CSF

G-CSF + GMI-1271

5 uL blood

17%

1%

10%

100%

G-CSF

G-CSF + GMI-1271

1 uL blood

0.3%

1%

10%

100%

G-CSF

G-CSF + GMI-1271

Reconstitution Units per mL mobilised blood (Poisson Distribution)

462

21

G-CSF

G-CSF + GMI-1271

+ 2x10^5 congenic BM cells in Long-term Reconstitution Assay

Limiting dilution transplant

G-CSF 250ug/kg/day

± GMI-1271 20mg/kg BID

25uL

5uL

1uL

Australia
Hypothesis.

E-selectin as a ‘gate-keeper’ dampening potential of migratory HSC

....compromises reconstituting potential of 95% of harvested peripheral blood HSC
Transwell assays – in vitro recapitulation

- Count & phenotype,
- Transplant for reconstitution potential

BM KIT+ HSPC, 10^6
(GCSF 3d & GMI-1271 4d treated CD45.2+ donor)

- SDF-1

Control IgG Fc-coated

E-selectin-Fc coated

** Mannwhitney Australia
Transwells – recapitulate in vitro

BM KIT^+ HSPC, 10^6 (GCSF 3d & GMI-1271 4d treated CD45.2 donor)

Control IgG Fc-coated

E-selectin-Fc coated

LKS^+ , lower well

IgG-Fc  Esel-coating

SDF-1
Transwells – recapitulate in vitro

BM KIT+ HSPC, 10^6
(GCSF 3d & GMI-1271 4d treated CD45.2 donor)

** mannwhitney

Long-term competitive repopulation assay. ½ lower well transplanted in competition with 2x10^5 congenic BM cells.
SUMMARY

Key Findings

• G-CSF ↑ E-selectin expression

• Interaction with E-selectin during transmigration compromises ~95% of HSC

......unexpected disadvantage with current mobilisation regimes.....

Remedy

• administer E-selectin antagonist together with G-CSF
**SUMMARY**

**Key Findings**

- G-CSF ↑ E-selectin expression
- Interaction with E-selectin during transmigration compromises ~95% of HSC

......unexpected disadvantage with current mobilisation regimes.....

**Remedy**

- administer E-selectin antagonist together with G-CSF

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**E-selectin.** A vascular gate-keeper dampening potential of migratory HSC
Thank you for listening…

Collaborators
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HSC biology team
Stem Cells & Cancer team