Administration of E-selectin Antagonist GMI-1271 Improves Survival to High-Dose Chemotherapy by Alleviating Mucositis and Accelerating Neutrophil Recovery

Winkler Ingrid G[1], Barbier V1, Nutt H[1], Hasnain SZ[2], Levesque JP[1], Magnani JL[3], McGuckin MA[2].

[1]Blood and Bone Disease Program, Mater Research – University of Queensland, Brisbane, Australia, [2] Infection and immunology Program, Mater Research – University of Queensland, Brisbane, Australia, [3]GlycoMimetics, Gaithersburg, MA

ingrid.Winkler@mater.uq.edu.au

Key findings

- Therapeutic blockade of E-selectin significantly enhanced survival to repeated rounds of high-dose chemotherapy in mice.
- GMI1271 administration during chemotherapy both,
  - Accelerated neutrophil recovery
  - Reduced intestinal mucositis leading to increased mouse survival.

Background

The problem with chemotherapy

Cytotoxics target rapidly-dividing cells, including malignant cells and also normal progenitors needed to replenish:

a. the blood and immune system.
b. mucosal surfaces lining the respiratory and gastrointestinal tracts.

... resulting in neutropenia - increased susceptibility to infections- together with intestinal ulceration providing a portal of entry for bacteria.

Upto 20% of AML and high-grade lymphoma patients will die from their cancer-therapy, not their disease *(Elmaagacli, BMT, 2007).*

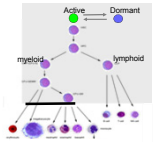
Blocking E-selectin -a possible solution

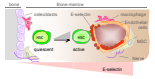
Selectins are a family of classic leukocyte adhesion molecules.
E- *endothelial* –selectin (CD62E) is expressed on endothelial cells following inflammation and is involved in recruiting and activating leukocytes.

Hematopoietic Stem Cells are regulated by their niches

Hematopoieitic Stem Cells (HSC) have two conflicting roles,
1. Remain quiescent –with heightened self-renewal potential- for long-term backup.
2. Cycle to replenish the blood and immune system.

These decisions are largely determined by the microenvironment (niche) in which the HSC reside.



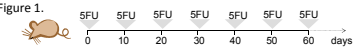


...vascular niche E-selectin awakens HSC.

We have found E-selectin on the bone marrow vasculature acts to awaken HSC. Absence or blockade of E-selectin results in an increased proportion of quiescent HSC that are 2-6-fold more able to survive chemotherapy.

Experimental Outline

Studies were performed in mice using two treatment regimes (chemotherapy or radiation). For chemotherapy, mice received repeated rounds of the anti-metabolite cytotoxic 5-fluorouracil (5-FU: 150mg/kg every 10 days). Using this regime, median survival for wildtype mice was 48 days. In contrast 80% of E-selectin gene-deleted mice (E-/-) survived >140 days ($p<0.05$). Figure 1.



Absence or therapeutic blockade of E-selectin during chemotherapy.....

E-selectin knockout mice (sele$^{-/-}$)

GMI1271 administration

... increases overall mouse survival

Fig 1.

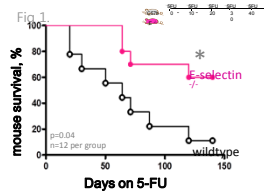
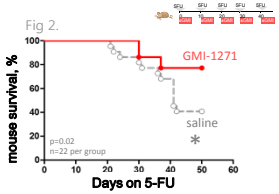


Fig 2.



Result. Absence or blockade of E-selectin –by administration of GMI-1271 for 5 days after each round of 5-FU, significantly reduced mortality in mice administered repeated rounds of chemotherapy.

Conflict of interest disclosure

- **John Magnani is an employee of GlycoMimetics.**
- **GlycoMimetics funded the parts of this work involving their compound (GMI-1271).**

.... accelerates & sustains neutrophil recovery

% mice able to recover normal neutrophil counts over repeated rounds of 5-FU.

Fig 3.

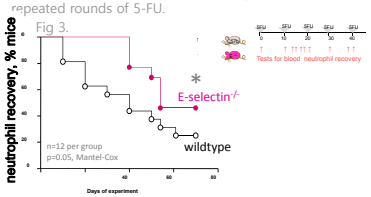
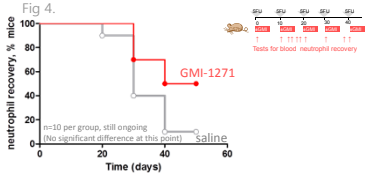


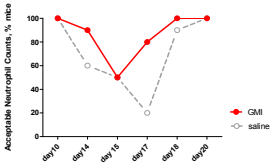
Fig 4.



Method. To determine whether acclerated neutrophil recovery could explain this survival advantage,
- Figs 3 & 4, blood was collected for neutrophil counts on the tenth day following each round of treatment -just prior to the next round of 5-FU and,
- Fig 5, the kinetics of blood neutrophil recovery during a single round of chemotherapy were determined by testbleeds at specific time-points between round 2 and round 3 of 5-FU.

Proportion of mice with neutropenia after second round of 5-FU.

Fig 5.



Result. Absence or blockade of E-selectin during chemotherapy accelerated neutrophil recoery over each round of 5-FU resulting in less mice experiencing neutropenia, and significantly increased the proportion of mice able to recover acceptable neutrophil counts over repeated rounds of therapy.

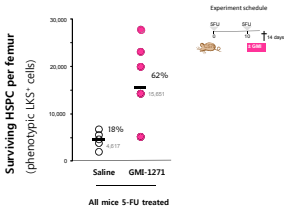
Likely mechanism

Accelerated neutrophil recovery...

GMI-1271 administration increases the proportion of HSC able to survive each round of chemotherapy, (see Fig 6).

Increased numbers of surviving HSC help accelerate subsequent bone marrow and blood recovery post-chemotherapy.

Fig 6. increased numbers of HSC survive chemotherapy when GMI-1271 is also administered.



... alleviates intestinal mucositis and therapy-related weight loss

Chemotherapy-treated mice maintain body weight if E-selectin is absent or blocked.

Fig 7.

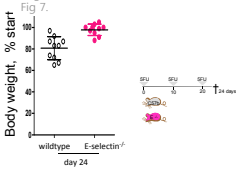
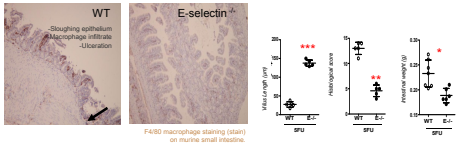


Fig 9. Chemotherapy-induced mucositis is significantly reduced in E-selectin$^{-/-}$ mice.



Method. Following indicated 5-FU regimes, small intestines were collected for histological scoring. Parallel sections were also stained with F4/80 to identify inflammatory macrophage infiltrate. We hypothesise that infiltrating inflammatory macrophages exacerbate mucosal damage.

Fig 8.

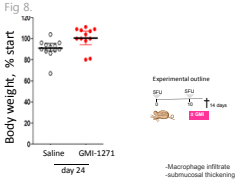
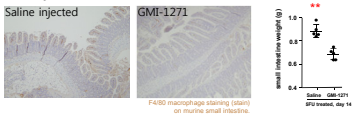


Fig 10. GMI-1271 administration during chemotherapy similarly alleviates mucositis in mice.



Result. Absence or blockade of E-selectin significantly reduced intestinal mucositis and therapy-induced weight loss.

Fig 11. Hypothesis – why blocking E-selectin may protect against mucositis

Likely mechanism

Alleviating chemotherapy-mucositis

Mucositis is now thought to be exacerbated by infiltrating inflammatory cells.

Our data show that
- E-selectin expression is upregulated in damaged intestine
- GMI-1271 administration blocks recruitment of inflammatory macrophages to damaged intestine.

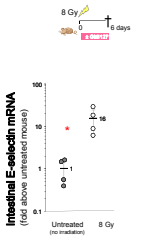


Fig 12. Intestinal E-selectin mRNA increases 16-fold after irradiation

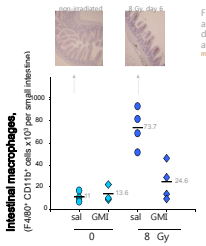


Fig 13. Macrophages are recruited to damaged intestine after irradiation. F4/80 macrophage staining (brown).

Fig 14. Flow cytometry analysis on small intestine digestions.