**Thrombopoietin mimetic therapy alleviates radiation-induced vascular injury in a bone marrow transplant mouse model**

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**Supplementary Introduction**

**JNJ-26366821:**

JNJ-26366821 (TPOm), a pegylated synthetic peptide distinct from endogenous thrombopoietin, is being developed to address platelet recovery failure after HSC transplantation[1]. It has shown promising efficacy in improving survival outcomes and hematopoietic recovery in mice exposed to radiation[2]. The intervention enhances recovery of indicators such as BM colony forming units, megakaryocytes, and FMS-like tyrosine kinase 3 ligand (FLT3-L). TPOm treatment in ear veins of radiation exposed mice has shown it’s potential in reducing associated vascular leakage and lowering inflammation, as observed through a decrease in leukocyte-endothelial cell interactions[3]. Additionally, in a pilot study using a rat model of prostate radiation therapy, TPOm treatment exhibits significant vascular protective effects, preventing changes in the penile artery cross-sectional area induced by radiation therapy[4]. These findings suggest the potential of JNJ-26366821 to reduce toxicities from radiotherapy and safeguard crucial microvascular structures for tissue function. Studies also indicate that JNJ-26366821 treatment increases megakaryopoiesis without affecting malignant myeloid proliferation in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)[1]. This selective effect is particularly valuable in BMT, where achieving a balance between hematopoietic recovery and controlling malignancy is crucial[5].

**Supplementary Result**

**Total number of bone marrow cells:**

The total number of bone marrow cells was evaluated in TPOm and PBS treated mice on days 7, 14, and 30 post-BMT. As shown in Figure S1, the TPOm treated mice exhibited a significant increase in the total number of bone marrow cells compared to the PBS treated mice on day 30.



**Figure S1**: Total number of Bone marrow cells in transplanted mice at D7, 14 and D30 post-BMT. There is a significant difference in bone marrow cells in TPOm and PBS treated mice on day 30. Data are expressed as mean ± SEM.

**Supplementary 1: Key resource table**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Reagent type (species) or resource** | **Designation** | **Source or reference** | **Identifiers** | **Additional information** |
| Chemical compound | Sucrose | Sigma-Aldrich | Cat#: S0389 | Final conc: 30% w/v |
| Chemical compound | O.C.T Compound | Fisher | Cat#: 23-730-571 |  |
| Chemical compound | DMSO | Corning | Cat#: 25-950-CQC | Final conc: 10% v/v |
| Chemical compound | Triton X-100 | Sigma-Aldrich | Cat#: T8787 | Final conc: 0.5% v/v |
| Chemical compound | Hoechst 33342, trihydrochloride. trihydrate | Life Technologies | Cat#: H3570 | IF (1:2000) |
| Other | Horse Serum | Gibco | Cat#: 16050130 | Final conc: 5% v/v |
| Antibody | Anti-mouse VEGFR2 (Goat polyclonal)  | R&D Systems | Cat#: AF644 | IF (1:100) |
| Antibody | Alexa Fluor 594 anti-mouse CD45.1 (mouse monoclonal) A20 | Biolegend | Cat#: 110750 | IF (1:100) |
| Antibody | Alexa Fluor 488 AffiniPure F(ab')₂ Fragment Donkey Anti-Goat IgG (H+L) | Jackson ImmunoResearch | Cat#: 705-546-147 | IF (1:500) |
| Software | Volocity | Quorum Technologies |  | v6.5.1 |
| Software | Prism | GraphPad |  | v9.4.1 |
| Software | Bitplane Imaris | Oxford Instruments |  | v9.6.0 |

**Table S1.** Key resource table for the whole-mount immunofluorescence imaging and analysis.

**Supplementary References**

1. Adrianzen-Herrera, D., et al., *The thrombopoietin mimetic JNJ-26366821 increases megakaryopoiesis without affecting malignant myeloid proliferation.* Leukemia & Lymphoma, 2020. **61**(10): p. 2453-2465.

2. Kumar, V.P., et al., *Mitigation of total body irradiation-induced mortality and hematopoietic injury of mice by a thrombopoietin mimetic (JNJ-26366821).* 2022. **12**(1): p. 3485.

3. Ashcraft, K.A., et al., *Application of a novel murine ear vein model to evaluate the effects of a vascular radioprotectant on radiation-induced vascular permeability and leukocyte adhesion.* 2018. **190**(1): p. 12-21.

4. Ashcraft, K.A., et al., *Clarifying the relative impacts of vascular and nerve injury that culminate in erectile dysfunction in a pilot study using a rat model of prostate irradiation and a thrombopoietin mimetic.* 2019. **103**(5): p. 1212-1220.

5. Pinho, S. and P.S.J.N.r.M.c.b. Frenette, *Haematopoietic stem cell activity and interactions with the niche.* 2019. **20**(5): p. 303-320.