

ENHANCED SURVIVAL OF LETHALLY-IRRADIATED MICE WITH HSC RECONSTITUTION IN COMBINATION WITH THE E-SELECTIN ANTAGONIST, GMI-1271 (UPROLESELAN)

Abstract #
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Abstract

The involvement of the adhesion molecule E-selectin and its interactions with E-selectin ligands as pertains to hematopoietic stem cells (HSC) and transplantation has been investigated (Winkler et al. 2012; Winkler et al. 2014). These preclinical studies focused on the role of E-selectin and the use of uproleselan, an E-selectin antagonist, during HSC mobilization in harvesting procedures of donors to accelerate recovery in transplant recipients. However, the impact of E-selectin and uproleselan administration on transplant recipients was less clear. In the current investigations we assessed the survival outcome of bone marrow depleted mice when reconstituted with HSC in combination with uproleselan. Twenty-four hours post irradiation (6Gy x2), cohorts of mice (n=10/group) were injected i.v. with 1x10⁶ bone marrow cells (study day 0) from congenic donors using three i.p. dosing regimens with 40 mg/kg uproleselan. These regimens were: (a) bid on study days 0 and 1; (b) bid on study days 1 and 2; and (c) bid on study day 1 only. Control groups in this study included irradiated mice alone (expected survival = 0%), non-irradiated mice alone (expected survival = 100%), and irradiated, reconstituted mice (no uproleselan). The survival of mice was determined over the course of the study (Day 0 to 30). Treatment with uproleselan as part of the transplant regimen significantly increased the median survival time (MST) of mice compared with the control group – the MST of mice treated with uproleselan and HSC was >30 days with 80-90% of mice alive at study completion. In contrast, the MST of irradiated mice (no transplant) was 11.5 days with no survivors at study conclusion. The MST of mice irradiated and transplanted with congenic HSC was 9 days with 40% survival on day 30. The impact of uproleselan on survival represented a >233.3% increase in life span. Flow cytometric analysis in all surviving mice on day 30 showed that the mean percentage of CD45.1+ cells from donor congenic mice was approximately 90% (blood and bone marrow) indicating that all surviving mice were successfully reconstituted. In summary, we report on a novel therapeutic use of inhibitors of E-selectin, such as uproleselan, which results in the increased survival of mice when combined with HSC transplantation for reconstitution of depleted and compromised bone marrow. The impact on increased host survival could extend to the use of peripheral blood and stem cell transplantations as a therapeutic option in various malignancies where curative intent is intended.

Background

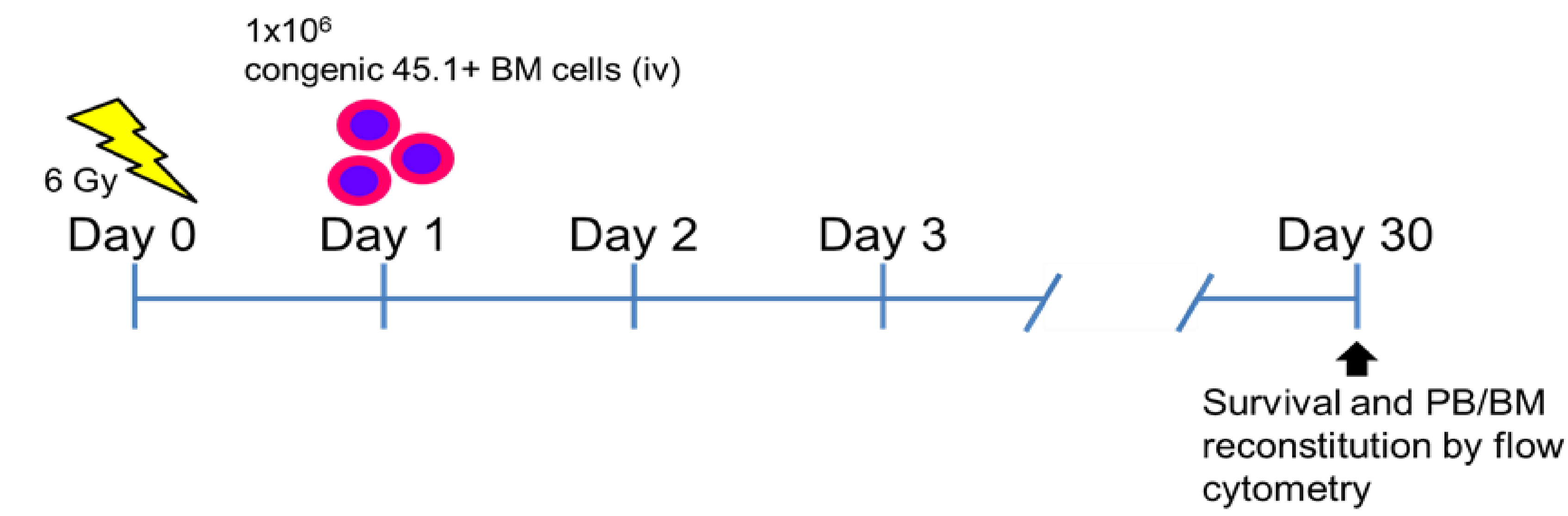
The impact of the endothelial cell adhesion molecule E-selectin and the use of uproleselan, an E-selectin antagonist, during HSC mobilization during harvesting procedures of donors to accelerate recovery in transplant recipients has been demonstrated (Winkler et al., 2012; Winkler et al. 2014). These studies demonstrated:

- a novel function for E-selectin that involved the differentiation the differentiation of otherwise dormant HSC with the induction of lineage commitment. GMI-1271 (now known as uproleselan), an antagonist of E-selectin interaction with E-selectin ligands, enhances HSC quiescence.
- therapeutic blockade of E-selectin in vivo with uproleselan specifically augments the mobilization of HSC with highest self-renewal potential following G-CSF administration, and markedly improves subsequent engraftment and reconstitution in mice.

Noteworthy, the above transplantation studies focused the role of E-selectin and the use of uproleselan during HSC mobilization in current harvesting procedures of donors to accelerate recovery in transplant recipients. The current studies were done to investigate the impact of E-selectin and uproleselan administration on transplant recipients.

Results

Figure 1. Experimental Model to Determine Hematopoietic Reconstitution of Lethally Irradiated C57/BL6 (CD45.2+) mice with CD45.1+ congenic B6.SJL cells



Group	N	Radiation	Transplant	Uproleselan (40 mg/kg/IP injection)	Parameters Assessed
1	10	+	-	-	Survival, weight, and reconstitution of PB and BM
2	10	+	+	-	
3	10	+	+	Day 0 and 1; q12h	
4	10	+	+	Day 1 and 2; q12h	
5	10	+	+	Day 1; q12h	
6	3	-	-	-	

The study protocol used in this invention disclosure is summarized in Figure 1. Cohorts of C57BL/6 mice (n=10/group) were sorted into study groups on Day 0. Mice in groups 1-5 were irradiated with two treatments of 6Gy on day 0 (6 hours apart) with a RadSource RS-2000 irradiator utilizing a 160 kV, 4.2 kW x-ray source, to deplete bone marrow. Twenty-four hours post irradiation mice in groups 2-5 were intravenously injected with 1x10⁶ cells from B6.SJL donor mice. In groups 3-5 three treatment regimens with uproleselan were evaluated for survival, change in body weight and reconstitution with donor CD42.1 cells. Control groups in this study included irradiated mice alone (expected survival = 0%), non-irradiated mice alone (expected survival = 100%), and irradiated, reconstituted mice (no uproleselan) to determine survival.

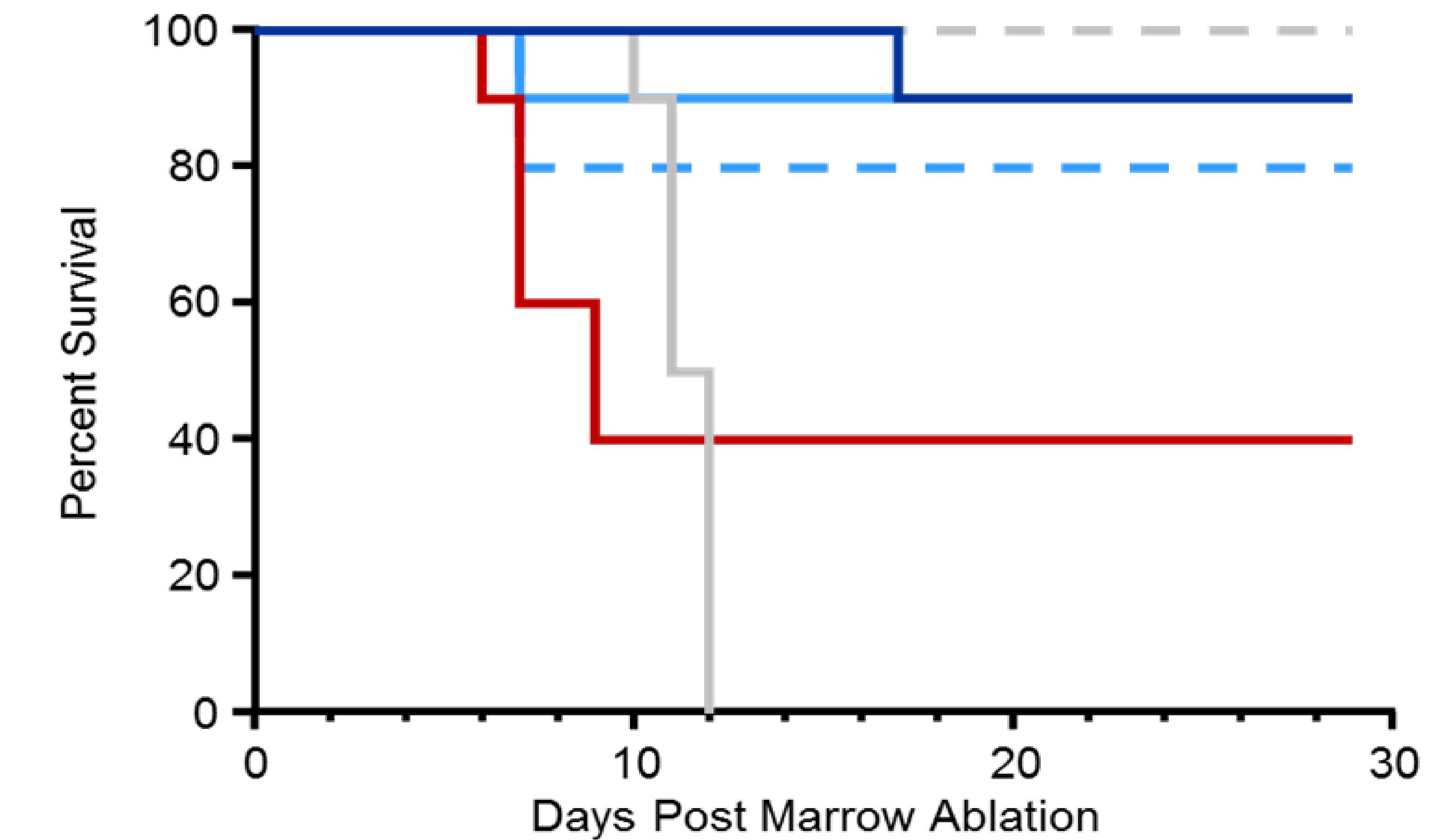
Figure 2. Uproleselan (GMI-1271) does not Inhibit Engraftment or Expansion of Donor HSCs in Recipient Mice

Bone marrows were collected from individual surviving mice at study conclusion (Day 30) and the percentage of CD45.1+ donor cells and residual CD45.2+ recipient cells was determined by flow cytometry using PE-CD45.1 and APC-CD45.2. The results are expressed as % cells positive for each marker.

TREATMENT		%CD45.1 CELLS (DONOR)	%CD45.2 CELLS (RECIPIENT)
Group 2	mean	94.2	6.9
	SD	1.9	3.0
Group 3	mean	93.6	5.8
	SD	1.6	1.4
Group 4	mean	93.2	5.6
	SD	1.7	1.6
Group 5	mean	88.9	10.4
	SD	5.8	9.1
Group 6	mean	0.2	97.3
	SD	0.0	0.5

Results (cont.)

Figure 3. Uproleselan (GMI-1271) Enhances Survival of Bone Marrow Depleted, Reconstituted Mice



GROUP	ABLATE BM (Day 0)	TRANSPLANT (Day 1)	GMI-1271	MST (Days)	% SURVIVAL	P vs. transplant alone (survival)
1	+	-	-	11.5	0	
2	+	+	-	9	40	
3	+	+	(Day 0 and 1; q12h)	>30	90	0.015
4	+	+	(Day 1 and 2; q12h)	>30	90	0.023
5	+	+	(Day 1; q12h)	>30	80	0.078
6	-	-	-	>30	100	

Conclusions

- Administration of the E-selectin antagonist, uproleselan, with HSC transplantation of compromised, bone marrow depleted mice does not impact reconstitution and results in increased survival
- The impact on increased host survival could extend to the use of peripheral blood and stem cell transplantations as a therapeutic option in various malignancies where curative intent is intended