

8th Swiss Apoptosis Meeting 2014





Training course on "Concepts and Methods in Programmed Cell Death"

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Organized by:

Thomi Brunner (Konstanz), Hans-Uwe Simon (Bern), Mario Tschan
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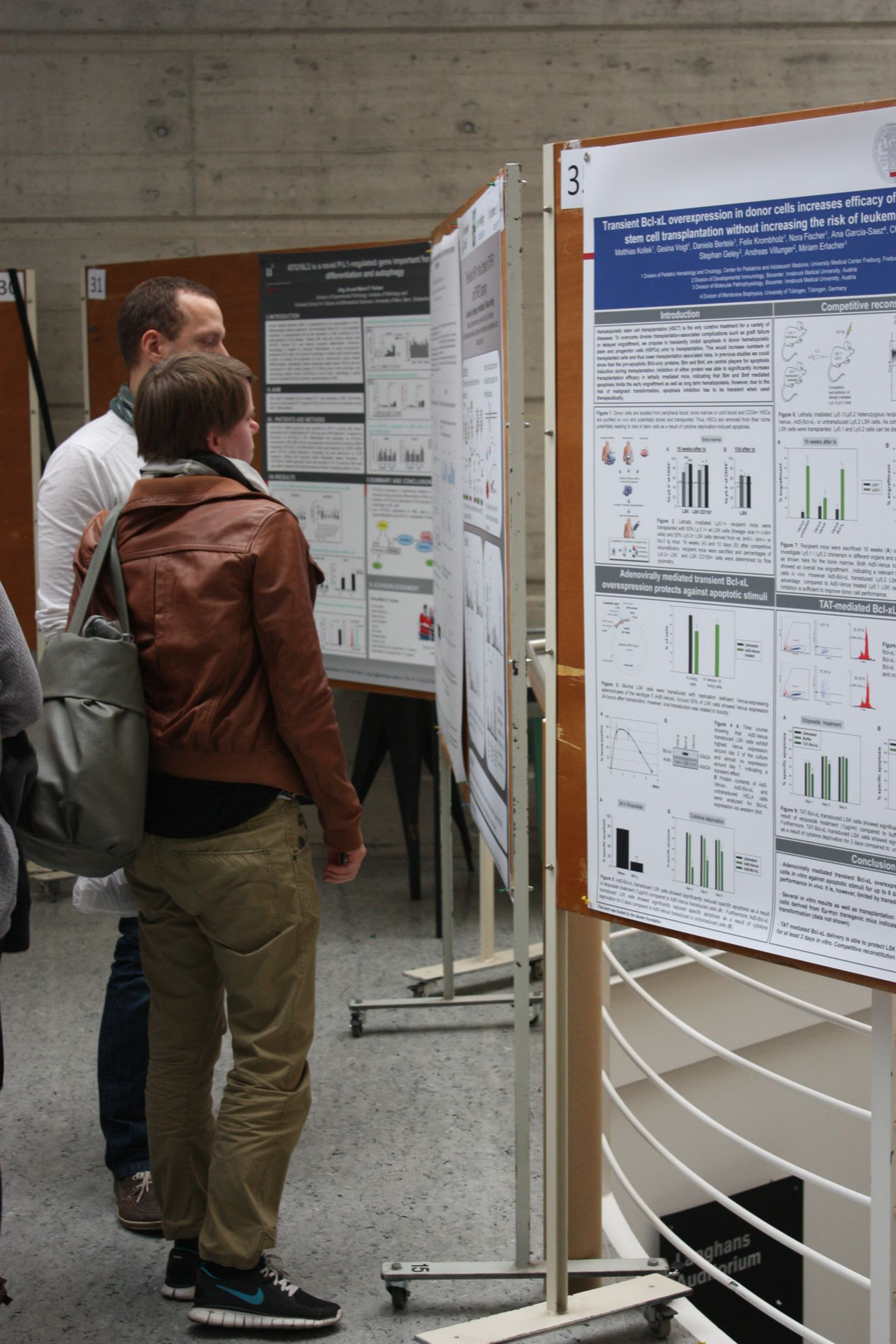












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Transient Bcl-xL overexpression in donor cells increases efficacy of stem cell transplantation without increasing the risk of leukemia

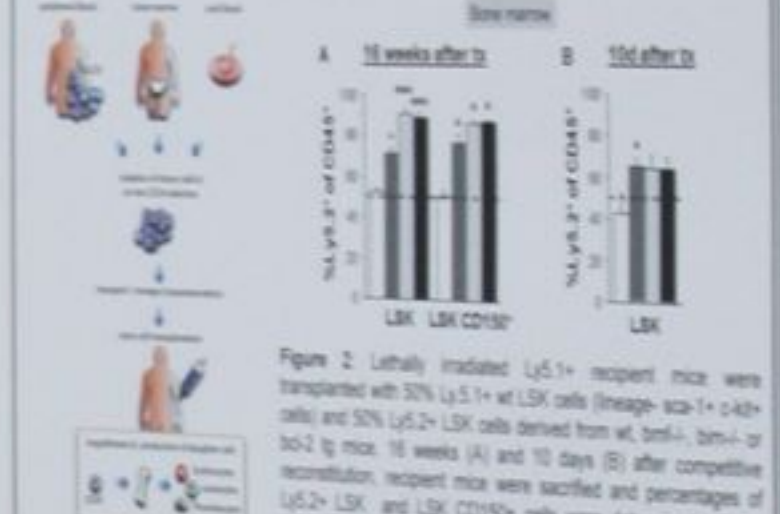
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Introduction

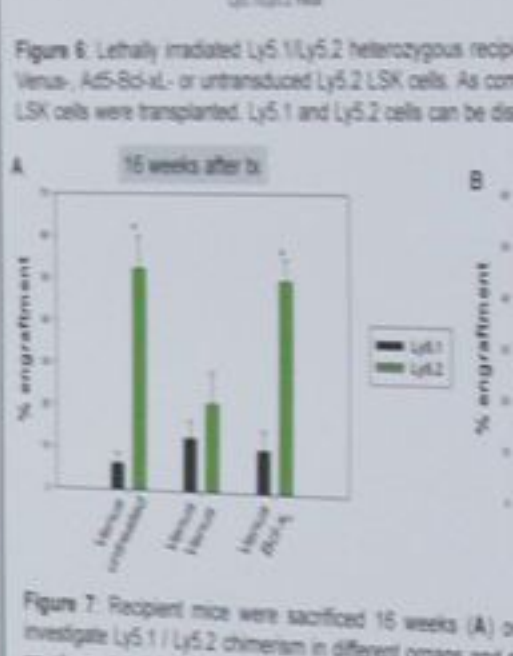
Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for a variety of diseases. To overcome diverse transplantation-associated complications such as graft failure or delayed engraftment, we propose to transiently inhibit apoptosis in donor hematopoietic stem and progenitor cells (HSPCs) prior to transplantation. This would increase numbers of transplanted cells and thus lower transplantation-associated risks. In previous studies we could show that the pro-apoptotic Bcl-2 family proteins, Bim and Bmf, are central players for apoptosis induction during transplantation. Inhibition of either protein was able to significantly increase transplantation efficacy in lethally irradiated mice, indicating that Bim and Bmf mediated apoptosis limits the early engraftment as well as long term hematopoiesis. However, due to the risk of malignant transformation, apoptosis inhibition has to be transient when used therapeutically.

Figure 1: Donor cells are isolated from peripheral blood, bone marrow or cord blood and CD34⁺ HSCs are purified *ex vivo* and potentially stored and transported. Thus, HSCs are removed from their niche potentially leading to loss of stem cells as a result of cytokine deprivation-induced apoptosis.



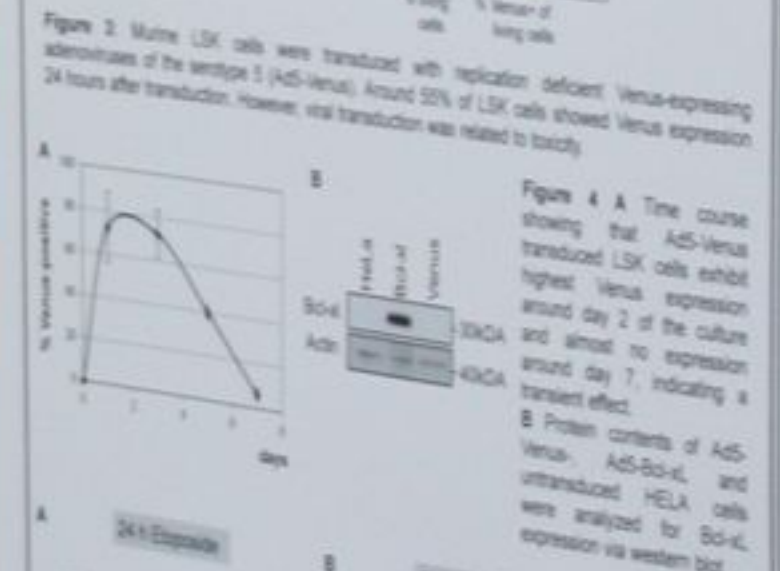
Competitive reconstitution

Figure 2: Lethally irradiated Ly5.1/Ly5.2 heterozygous recipient mice were transplanted with 50% Ly5.1⁺ wt LSK cells (lineage-sca-1⁺ × 2 × 10⁴ cells) and 50% Ly5.2⁺ LSK cells derived from wt, bcl-xL^{-/-}, bcl-xL^{+/+} or bcl-xL^{+/+} mice. 16 weeks (A) and 10 days (B) after competitive reconstitution, recipient mice were sacrificed and percentages of Ly5.2⁺ LSK and LSK CD135⁺ cells were determined by flow cytometry.



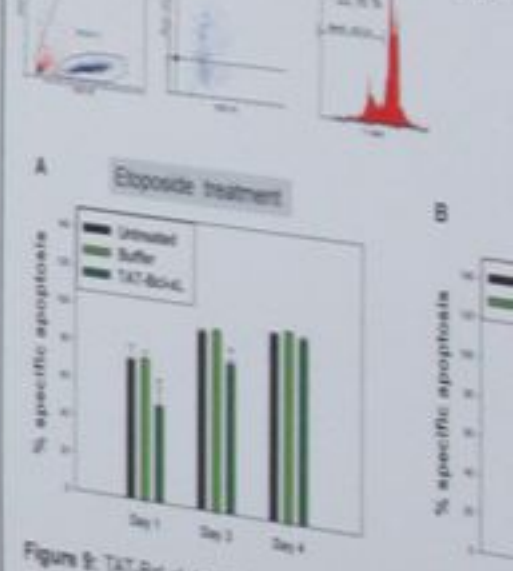
Adenovirally mediated transient Bcl-xL overexpression protects against apoptotic stimuli

Figure 3: Mature LSK cells were transduced with replication deficient Ad5-Venus or Ad5-Bcl-xL. Around 50% of LSK cells showed Venus expression 24 hours after transduction. However, viral transduction was related to toxicity.



TAT-mediated Bcl-xL

Figure 4: Time course showing that Ad5-Venus transduced LSK cells exhibit highest Venus expression around day 2 of the culture and almost no expression around day 7, indicating a transient effect.



Conclusion

- Adenovirally mediated transient Bcl-xL overexpression in donor cells increases efficacy of stem cell transplantation without increasing the risk of leukemia.
- Several *in vitro* results as well as transplantation results derived from Eμ-myc transgenic mice indicate that transient Bcl-xL overexpression is sufficient to improve donor cell performance.
- TAT mediated Bcl-xL delivery is able to protect LSK cells for at least 2 days *in vitro*. Competitive reconstitution