Comparison of clonogenic potential of Lin⁻c-Kit⁺ cells from normal and regenerating bone marrow

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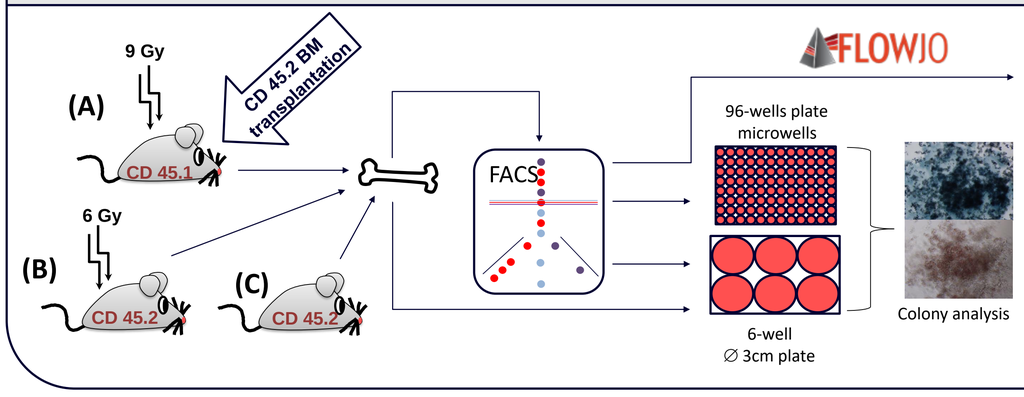
Aim of the study

Bone marrow (BM) regenerating after transplantation or after submyeloablative irradiation of mice is outcompeted when co-transplanted with normal bone marrow (Harrison and Astle, 1982). We asked to which extent this „weakness“ of regenerating BM is caused by decreased numbers of repopulating cells or possible higher dependence of repopulating cells on the natural haematopoietic microenvironment. To resolve this question, we analysed regenerating bone marrow for the content of immature Lin⁻c-Kit⁺ cells and compared the clonal efficiency of the cells in standard *in-vitro* clonogenic assays.

Conclusion

- Lin⁻c-Kit⁺ cells from the transplanted cells (A) are approximately twice weaker in *in-vitro* clonal efficiency as compared to the same cells from normal BM
- Lin⁻c-Kit⁺ cells from the cells that survived irradiation (B) are also significantly weaker in *in-vitro* clonal efficiency
- Cells surviving irradiation generated large endogenous spleen colonies
- Regenerating bone marrow depends more on the natural haematopoietic microenvironment compared to normal BM

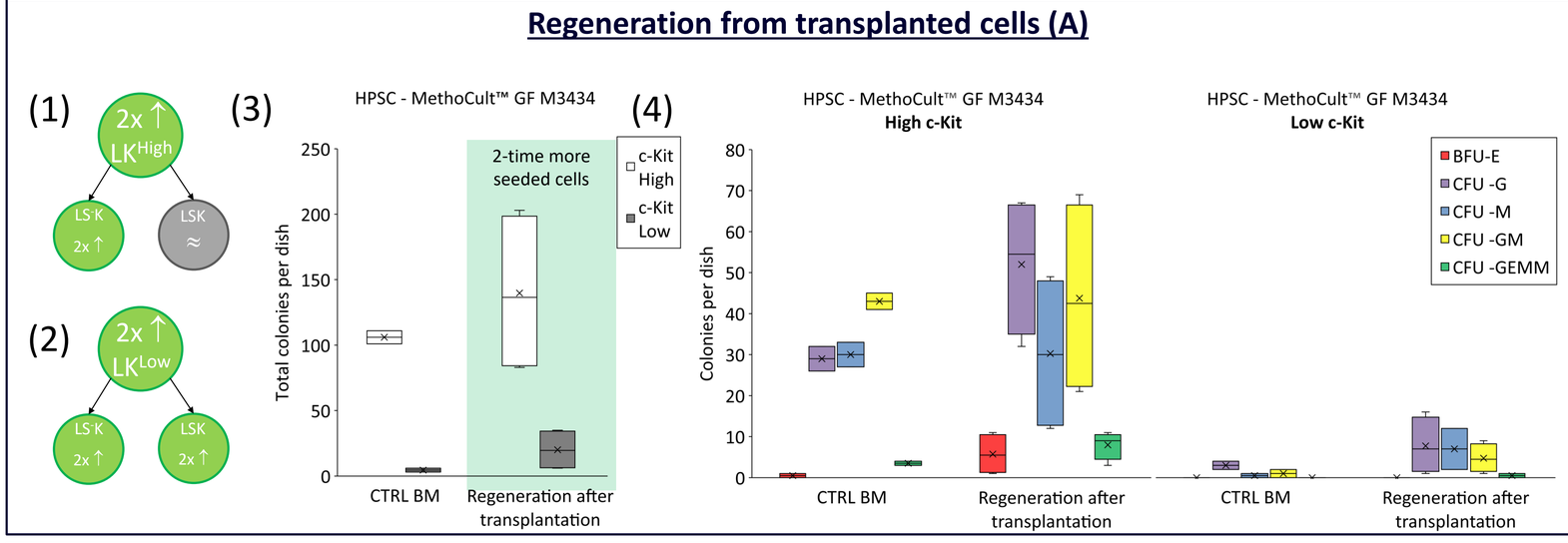
Materials and methods



- B6.SJL-Ptprc^a Pepcb/BoyJ 9 Gy (lethally) irradiated mice with syngeneic bone marrow cells (A)
- C57Bl/6NJ 6 Gy (sublethally) irradiated mice (B) or control (C)
- γ irradiation ^{60}Co
- Bone marrow (BM) transplantation
- BD FACSAria™ IIu analysis and sorting
- MethoCult™ GF M3434, SF M3436 & M3334 culture media

Results

Regeneration from transplanted cells (A)



9 Gy (lethally) irradiated mice **transplanted** with syngeneic BM cells

Cells sorted based on Lin⁻c-Kit^{High} or Lin⁻c-Kit^{Low} population

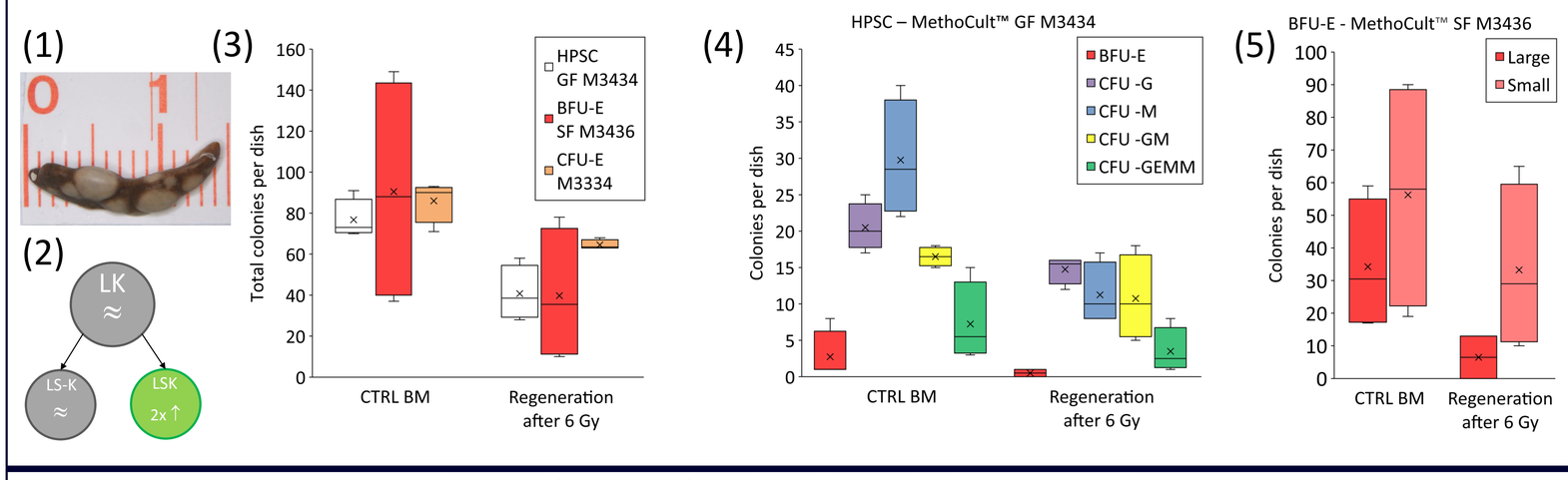
BM analysed **14 days** after transplantation

(1) Graphical representation of flow cytometry data analysis of c-Kit^{High} or (2) c-Kit^{Low} plated cells comparison to control
(2x ↑ - two fold higher number of plated cells) (≈ - equivalent amount of plated cells)

(3) Decreased *in-vitro* growth ability observed in all HPSC colony types (correction for different number of seeded Lin⁻c-Kit⁺ cells

(4) Detailed HPSC colony-types analysis in MethoCult™ GF M3434 medium

Regeneration from surviving cells (B)



6 Gy (sublethally) irradiated mice
– spontaneous regeneration

Whole BM (non-sorted) cells

Normal or regenerating BM cells **14 days** after irradiation

(1) Large endogenous spleen colonies 13 days after 6 Gy irradiation

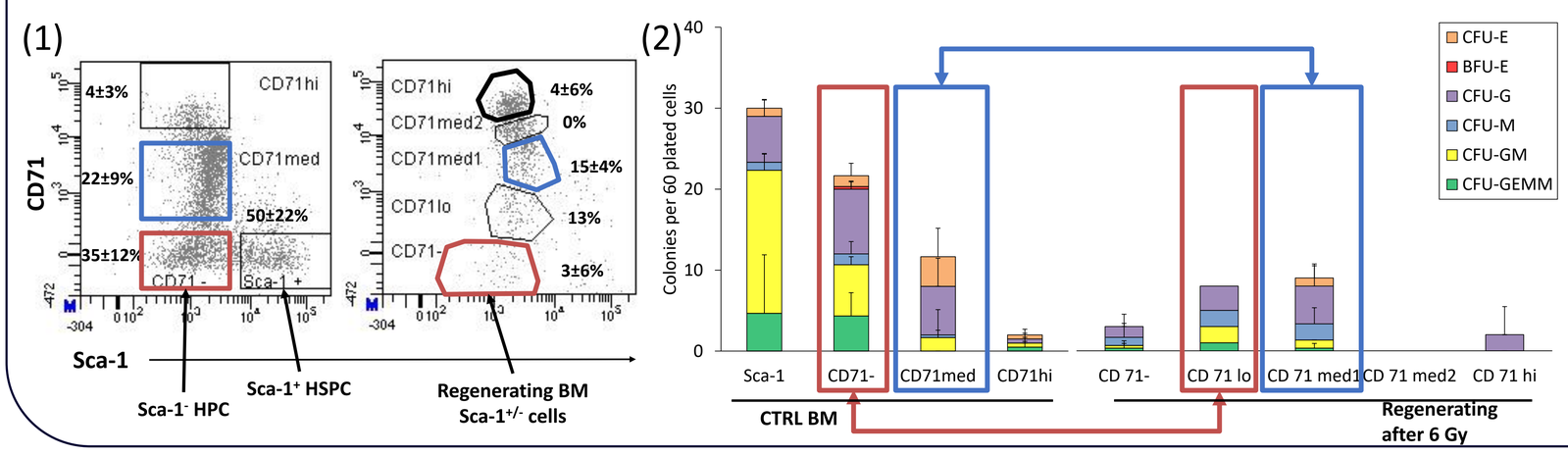
(2) Graphical representation of flow cytometry data analysis of seeded cells
(2x ↑ - two fold higher number of plated cells) (≈ - equivalent amount of plated cells)

(3) Decreased *in-vitro* growth ability observed in all colony types

(4) Detailed HPSC colony-types analysis in MethoCult™ GF M3434 medium

(5) Detailed BFU-E colony-size analysis in MethoCult™ SF M3436 medium

Plating efficiency of single cells seeded in microwells (B)



6 Gy (sublethally) irradiated mice
– spontaneous regeneration

Cells sorted based on presented gating strategy (1)

Normal or regenerating BM cells **14 days** after irradiation

(1) Sorting strategy based on CD71 and Sca-1 antigens expression

(2) Changed *in-vitro* growth ability is observed only in CD71^{Low} population

Acknowledgements

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