

Inhibition of the Jagged-1/Notch pathway increases the hematopoiesis-supportive activity of mesenchymal stem cells



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Notch signaling pathway (1)

- Notch is expressed in CD34+ hematopoietic precursors (Milner et al. 1994, Blood)
 - Human Jagged-1 is expressed by marrow stromal cells (Li et al. 1998, Immunity)
- Interactions between stromal and hematopoietic cells include Jagged1-Notch signaling
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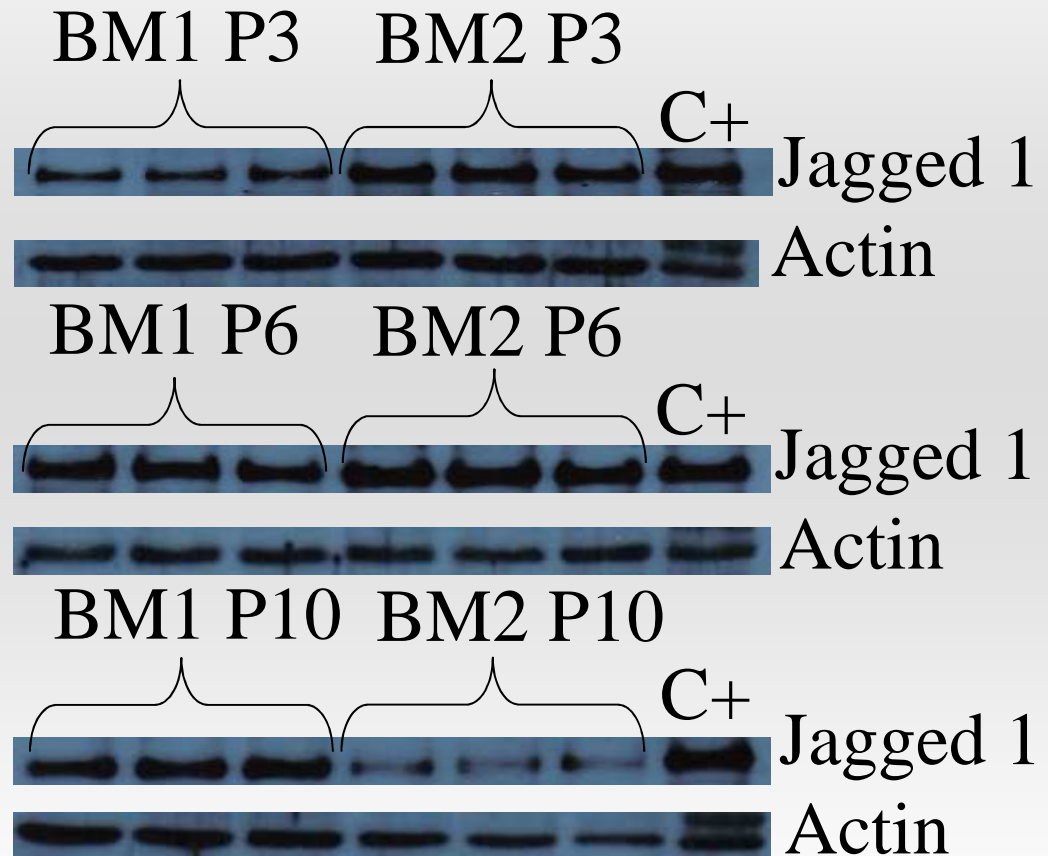
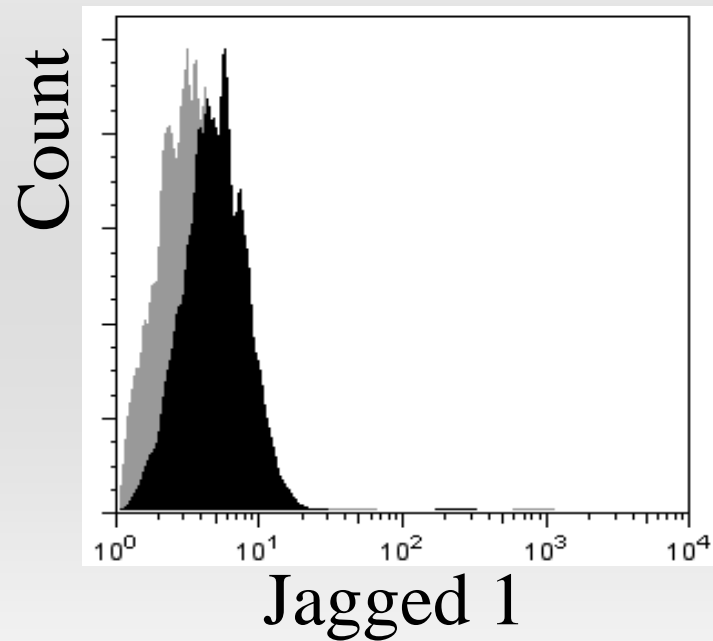
Notch signaling pathway (2)

- Exposure to soluble or bound Notch ligands inhibit myelopoiesis from most hemopoietic cell lines and HPCs (Walker et al 1999, Stem Cells)
 - No myelopoietic defect from bone marrow retrovirally transduced with a constitutively active form of Notch (Pui et al. 1999, Immunity)
 - Jaggeds do not impede myelopoiesis (Karanu et al. 2003, Leukemia), **but Dll-1 does** (Ohishi et al. 2001, Blood et Ohishi et al. 2002, J. Clin. Invest.)
- Notch signaling in multipotent HSC are largely unknown

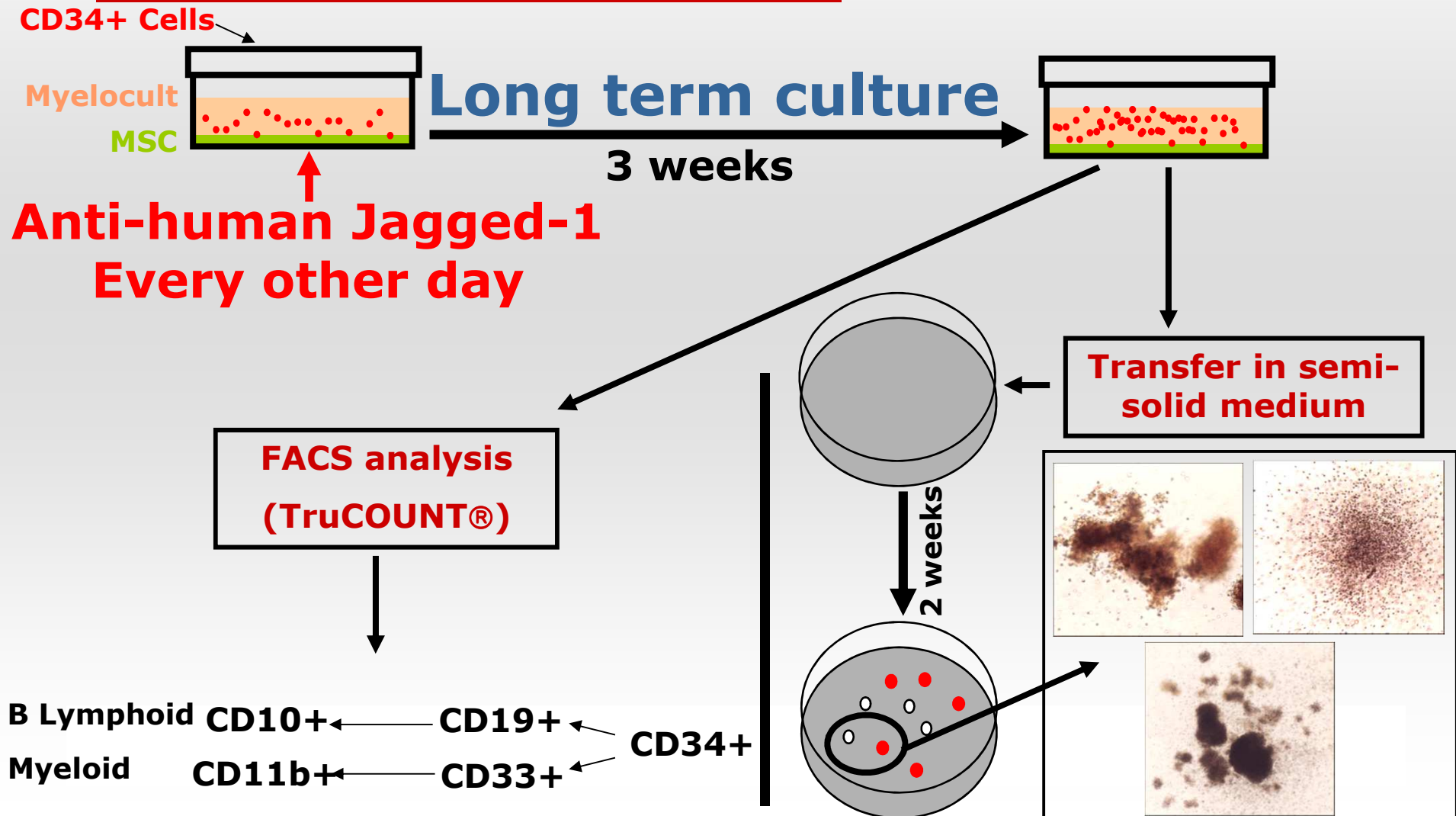
Aim of the study

- MSC are able to support hematopoiesis ex vivo by providing components of the extracellular matrix and essential growth signals allowing proliferation and differentiation of hematopoietic stem cells
- Aim = determining the contribution of the Notch/Jagged-1 pathway in the ex vivo hematopoiesis-supportive activity of MSC

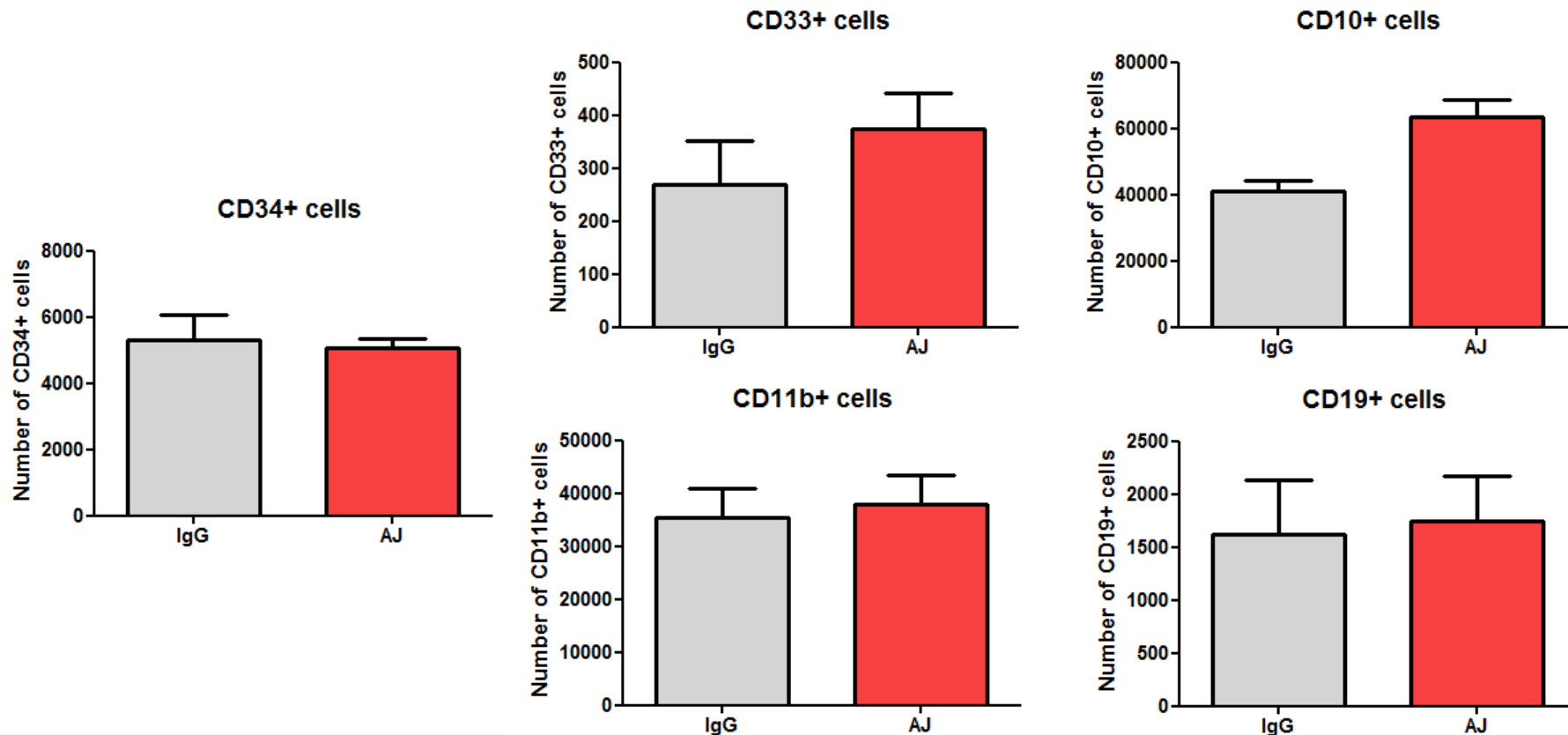
Expression of Jagged-1 by MSC



MSC support of LTC-IC (1)

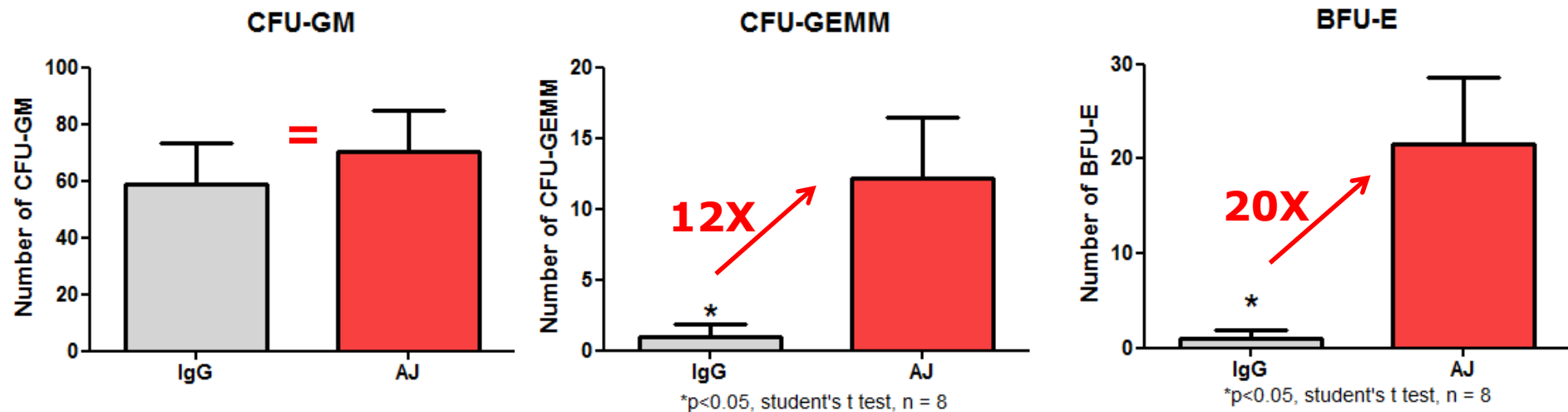


MSC support of LTC-IC (2)



Compared to culture with control IgG, outgrowth of lymphoid and myeloid cells, as well as expansion of CD34+ cells, were not affected by Jagged-1 inhibition

MSC support of LTC-IC (3)

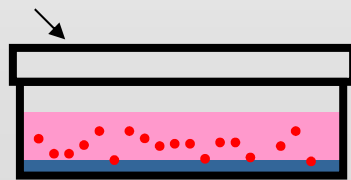


Compared to culture with control IgG, the number of CFU-GEMM and BFU-E was significantly increased by Jagged-1 neutralisation

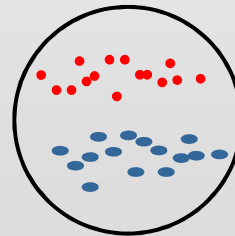
MSC support of SRC (1)

1 10^5 CD34+ cells

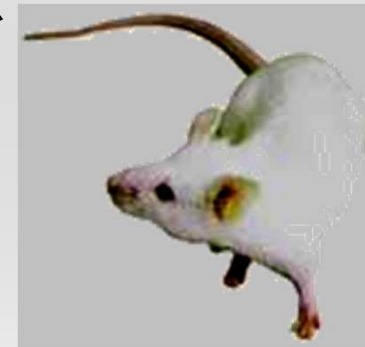
MSC



**1 week co-culture
No added cytokines**



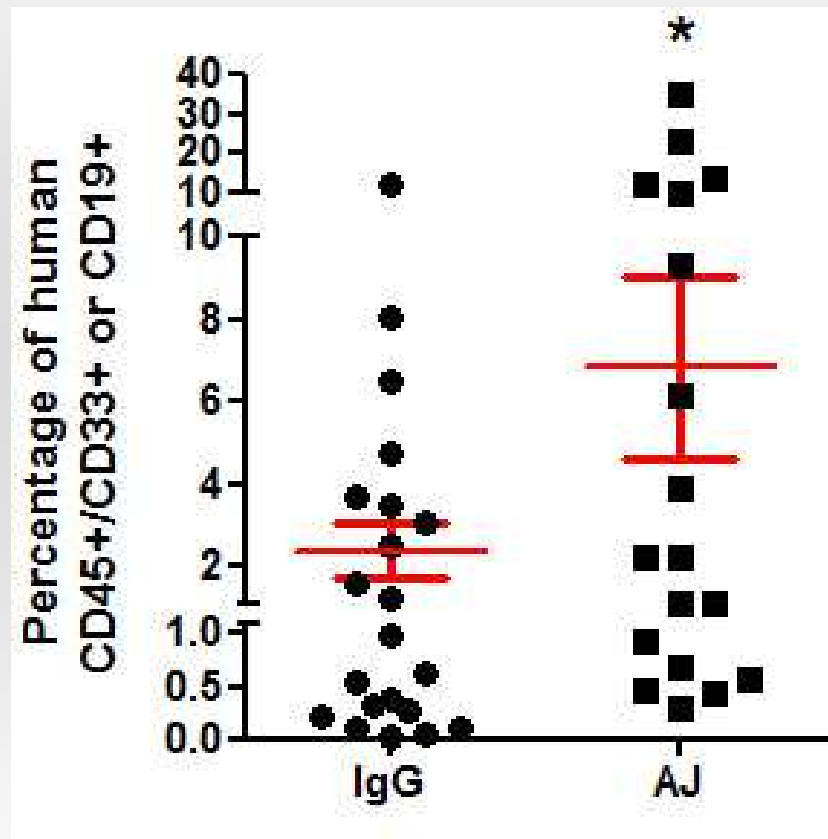
NOD/SCID mice
Irradiation: 3 Gy



**With anti-human Jagged-1
Every other day**

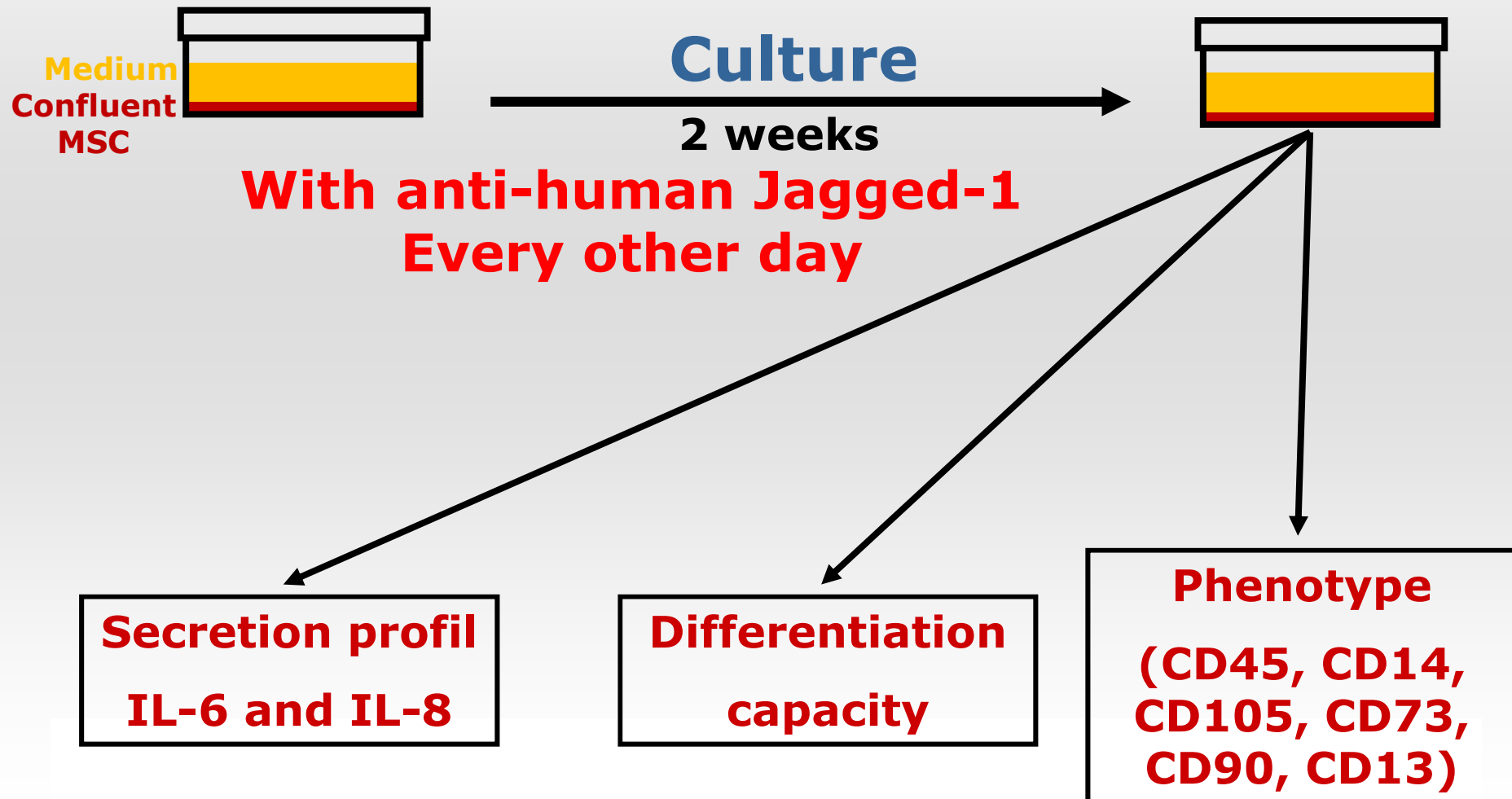
repopulation (6 weeks)
% human chimerism:
FACS analysis: CD45

MSC support of SRC (2)

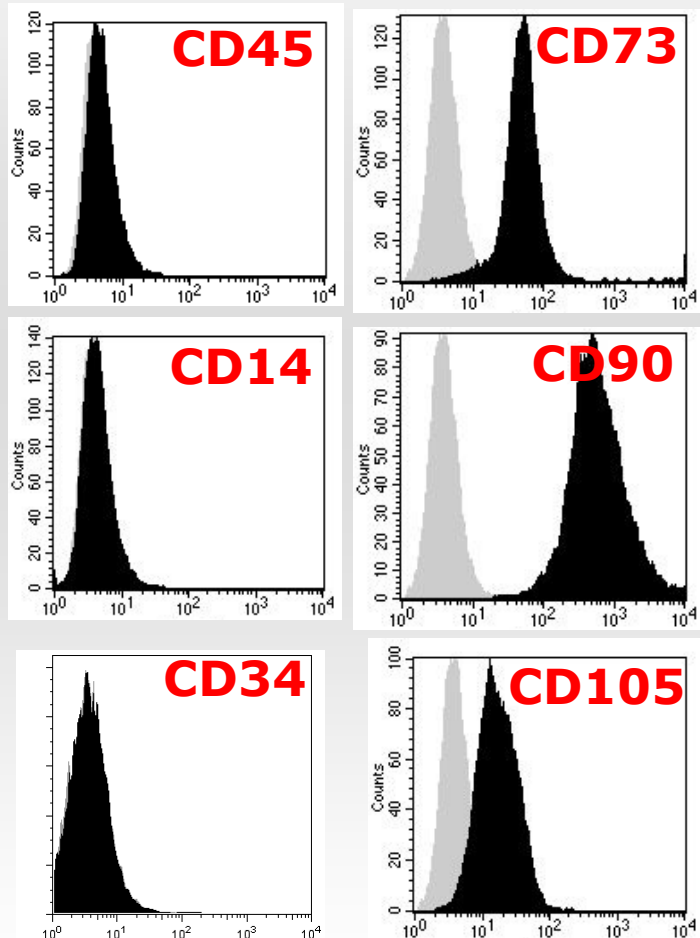


Compared to infusion of CD34+ cells cultured in the presence of control IgG, NOD/SCID mice repopulating activity was increased by Jagged-1 neutralisation ($p < 0.05$)

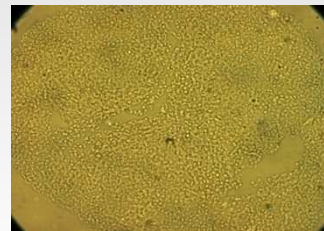
Direct or indirect effects ? (1)



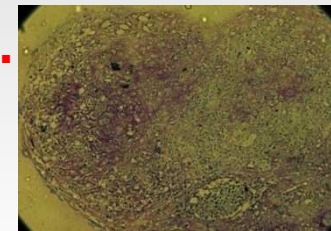
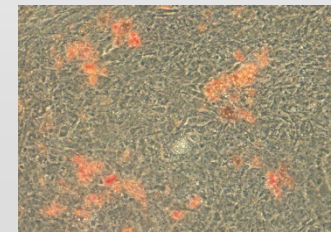
Direct or indirect effects ? (2)



MSC in control medium



MSC in induction medium



adipogenesis

dexamethasone
indomethacin
insulin

osteogenesis

ascorbic acid
 β -glycerophosphate
dexamethasone

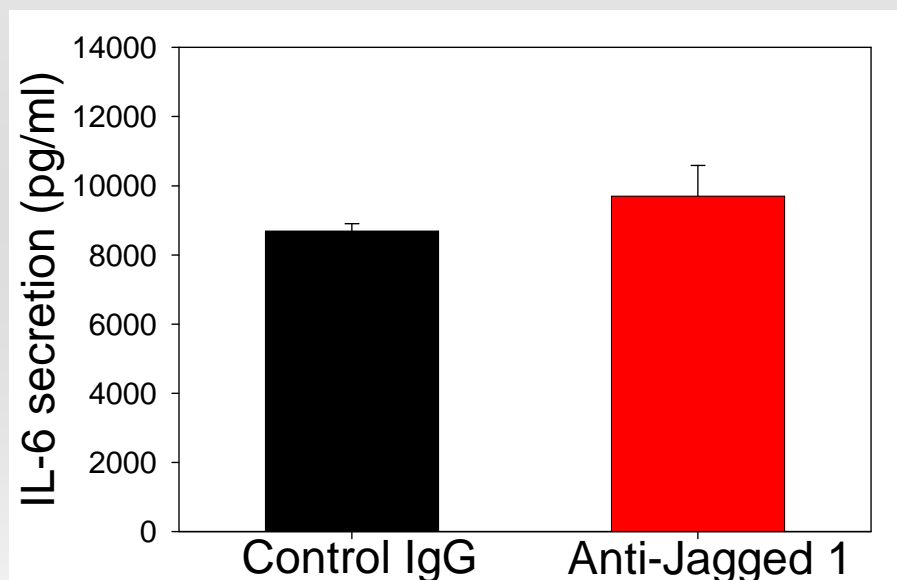
chondrocytic diff.

TGF β 3
ascorbic acid
insulin

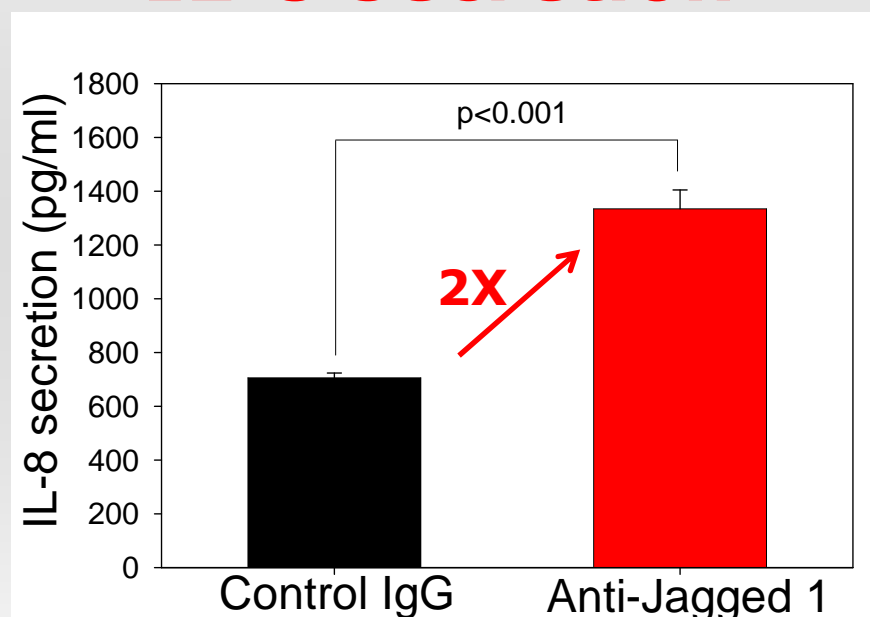
Phenotype, adipogenic, chondrogenic and osteogenic differentiation capacity of MSC were not affected by Jagged-1 inhibition

Direct or indirect effects ? (3)

IL-6 secretion



IL-8 secretion



We noted a 2-fold increase of IL-8 secretion ($p < 0.001$) in the presence of anti-human Jagged-1
In contrast, IL-6 secretion did not significantly change

Conclusions

- In our conditions, the Jagged-1/Notch pathway **inhibits** the supportive activity of MSC toward NOD/SCID-repopulating cells
 - This is not paralleled by changes in the phenotype or differentiation potential of MSC but **may be related to inhibition of IL-8 secretion**
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