

# In vitro generation of human hematopoietic cells

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## Contact person

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## Organisation

**Name of the organisation** Ghent University (UGent)

**Department** Diagnostic Sciences

**Country** Belgium

**Geographical Area** Flemish Region

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Basic Research
<b>Type of method</b>	In vitro - Ex vivo
<b>Specify the type of cells/tissues/organs</b>	Human hematopoietic stem and progenitor cells

## DESCRIPTION

### Method keywords

human HPCs

in vitro differentiation of hematopoietic cells

OP9-coculture

MS5-coculture

ATO system

organoid culture

FTOC

### **Scientific area keywords**

immune deficiency

leukemia

human hematopoiesis

stem cells

gene editing

### **Method description**

Better understanding of molecular mechanisms controlling both normal and malignant human hematopoiesis will lead to a more efficient therapy of immune deficiencies and lymphoid leukemias. Therefore, human hematopoietic progenitor cells (HPCs) are differentiated *in vitro* towards distinct hematopoietic lineages, with or without perturbation conditions such as gene targeting, viral transductions, specific compounds or blocking antibodies. Our lab has a broad expertise in the differentiation of human T cell progenitors, for which 3 different *in vitro* techniques are available:

- 1) Fetal thymic organ cultures (FTOCs), using fetal thymic lobes from NOD/SCID mice as 3D-micro environment allowing human HPCs to differentiate towards T cells. ;
- 2) OP9-coculture system, using OP9 mouse stromal cells with or without specific Notch ligands as a 2D-layer to culture human HPCs on ;

3) Artificial Thymic Organoid (ATO) cultures, using Notch expressing MS5 mouse stromal cells in combination with human HPCs in 3D aggregates.

Furthermore, OP9- and MS5-cocultures are used in order to differentiate HPCs towards myeloid cells (dendritic cells, monocytes, granulocytes), B cells, NK cells and both erythrocytes and megakaryocytes. Differentiation of different cell types is determined using flow cytometry.

### **Lab equipment**

Biosafety cabinet level 2 ;

Tissue culture incubator ;

Centrifuge ;

Flow cytometer.

### **Method status**

Internally validated

Published in peer reviewed journal

## **PROS, CONS & FUTURE POTENTIAL**

### **Advantages**

These techniques allow to study normal or aberrant differentiation of human hematopoietic stem cells in conditions of genetic or other perturbations *in vitro*. It permits a kinetic and quantitative analysis of human hematopoietic differentiation which is difficult *in vivo*.

### **Challenges**

The challenge of *in vitro* differentiation systems is reproducing the *in vivo* environment in which different hematopoietic cells arise. Although FTOCs and the ATO system offer a close physiological background, the use of OP9 or MS5 stromal cells also allows us to generate distinct hematopoietic cells resembling their *in vivo* counterparts. Gene targeting in human HSCs is still inefficient.

### **Modifications**

More efficient gene targeting in human HSCs is still desired for genetic studies, as well as further modifications that lead to a closer resemblance of the *in vivo* environment.

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### References

Taghon T et al. Blood 2002; 99(4):1197-204.

Schmitt TM et al. Immunity 2002; 17(6):749-56.

Van de Walle I et al. Blood 2011; 117(17):4449-59.

Seet CS et al. Nat Methods 2017; 14(5):521-530.

Montel-Hagen A et al. Cell Stem Cell 2019; 24(3):376-389.

### Links

[lab website](#)

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