# In vitro generation of human hematopoietic cells

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### Organisation

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**Geographical Area** Flemish Region


## SCOPE OF THE METHOD

| | |
|---|---|
| **The Method relates to** | Human health |
| **The Method is situated in** | Basic Research |
| **Type of method** | In vitro - Ex vivo |
| **Specify the type of cells/tissues/organs** | Human hematopietic 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

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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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