# Multi-methodologic dissection of human Natural Killer cell ontogenesis to study the contribution of unconventional NK cell subsets and other Innate Lymphoid Cells (ILCs)

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## Abstract body:

Natural Killer (NK) cell maturation remains unclear in humans. Although the Bone Marrow (BM) niche is a major site for the initial phases of NK cell ontogenesis, hereupon, committed precursors reach secondary lymphoid tissues to differentiate in $CD56^{bright}CD16^{neg}$ ($CD56^{bright}$) and eventually $CD56^{dim}CD16^{pos}$ ($CD56^{dim}$) NK cells. Recently, we described an additional $CD56^{dim}CD16^{neg}$ ($unCD56^{dim}$) subset, whose role in the ontogenesis is unknown.

Herein, we integrated several techniques to dissect NK cell ontogenesis: first we set up an autologous *ex-vivo* BM-niche by co-culturing BM-derived mesenchymal stromal cells with FACS-sorted NK cell precursors (NKPs), for 28 days. We observed that NKPs differentiate into ILC-resembling cells, and then into $CD56^{bright}$ NK cells and $unCD56^{dim}$.

Through *in-silico* analysis of single-cell RNA sequencing data, we confirmed $CD56^{bright}$ cells as ancestors for both $unCD56^{dim}$ and $CD56^{dim}$ cells.

Lastly, we investigated NK cell immune reconstitution after hematopoietic stem cell transplantation as a model of *in vivo* NK cell differentiation, by applying single-cell based unbiased pipelines to multi-parametric flow-cytometry data. This allowed us to appreciate fully differentiated $unCD56^{dim}$ NK cells.

In conclusion, we demonstrated that ILC-like cells are involved in the initial steps of human NK cell ontogenesis and that $unCD56^{dim}$ cells likely represent a more differentiated NK cell subset.