EFFICIENT EX-VIVO EXPANSION OF ADAPTIVE NKG2C+/CD57+ NK CELLS FROM CMV-POSITIVE DONORS USING DENDRITIC CELLS DERIVED FROM THE ACUTE MYELOID CELL LINE DCONN

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Background Human cytomegalovirus (CMV) infection profoundly affects NK cell compartment, as documented by the presence of a long-lived adaptive NK cell subset co-expressing NKG2C and CD57. Several features render adaptive NK cells a potentially attractive contributor to the efficacy of mAb-based therapeutic strategies and predict a lower sensitivity to immunosuppressive signals in the tumor microenvironment. Moreover, a recent work showed that CAR-transduced adaptive NK cells exhibit superior effector functions when compared to other NK subsets. The requirements for adaptive NK cell expansion ex vivo have however not been fully characterized. Ex-vivo expansion of NKG2C+ memory NK cells can be achieved by coculturing NK cells from CMV-positive subjects with CMV-infected fibroblasts + IL-2, HLA-E-transfected cell lines + IL-15, or IgG-opsonized tumor cells + IL-2. However, the reported fold-expansion after 14-21 days in culture in vitro is generally below 30-fold.

Methods PBMCs isolated from buffy coat of CMV-positive healthy donors. NK cells were co-cultured with or without DCON-derived mature DCs (DCOne mDCs) in the presence of cytokines for 2 weeks. After 1 week, cells were restimulated by the addition of DCON-derived mature DCs and cytokines. NK cell proliferation, viability and phenotype were monitored on day 7 and 14.

Results DCOne-derived mature DCs (high expression of HLA-DR, CD40/80/86 and CD83) were found to highly express CD58 and CD155, two ligands known to participate in adaptive NK cell activation and expansion. When these tumor cell/dendritic cell “hybrids” were co-cultured for 2 weeks with isolated NK cell from CMV-positive healthy donors, a selective (figure 1) and strong (>200-fold) median expansion (figure 2) of adaptive NKG2C+/CD57+ NK cells was found when cocultures were performed in medium supplemented with IL-2 or IL-15. Characterization of the functional profile, including ADCC, cytokine production and long-time survival, after target cell interaction by these expanded NKG2C+/CD57+ NK cells is ongoing.

Conclusions The presented data indicate that DCOne-derived mature DCs are endowed with the capability to promote strong and selective ex vivo expansion of adaptive NKG2C+/CD57+ NK cells from healthy CMV+ donors. Such expanded NK cells could potentially be used for adoptive immunotherapy, including combinations with tumor-targeting antibodies, in different hematological and solid tumor indications.