A phase I study of allogeneic Natural Killer Cell Therapy generated from Cord Blood Hematopoietic Stem and Progenitor Cells in Elderly Acute Myeloid Leukemia Patients

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Introduction
Elderly AML patients have a poor prognosis due to high relapse rates following standard therapy. Natural Killer (NK) cell alloreactivity has found to significantly control relapse in AML in the HLA-mismatched haploidentical allogeneic SCT setting. Moreover, allogeneic NK cell infusions has shown to induce CR in patients with advanced AML. As a consequence, adoptive NK cell transfer may be a promising treatment for elderly AML patients, who are unfit or ineligible for allo-SCT. Most clinical studies explored CD3+CD56+ NK cell products enriched from leukapheresis of haploidentical donors containing low numbers of T cells that could have contributed to the observed therapeutic effects and potentially induced GVHD. Therefore, we have developed a GMP-compliant cell culture system for the generation of highly functional NK cells from umbilical cord blood (UCB)-derived CD34+ progenitor cells, without T cell contamination. Here, we report the feasibility, safety, toxicity and efficacy data of the first in man trial with this unique UCB-NK cell product.

Study design and intervention
A phase I dose escalation study was performed in elderly AML patients who achieved morphologic CR (<5% BM blasts) after standard treatment. Prior to NK cell infusion, patients received immunosuppressive conditioning consisting of cyclophosphamide (900 mg/m²/day) and fludarabine (100 mg/m²/day) for 4 consecutive days. On day 0, cohorts of 3 patients have received 3×10⁴, 1×10⁵ and 3×10⁶ allogeneic NK cells per kg body weight generated ex vivo from CD34+ UCB cells obtained from a partially HLA-matched allogeneic UCB unit. No systemic IL-2 was to be given and if in vivo UCB-NK cell persistence and expansion could be obtained without cytokine support.

Results
Patient & UCB donor characteristics
Seventeen elderly AML patients have been included of which 30 were treated with Flu/Cy and allogeneic UCB-NK cells (see table below). All patients were in morphologic CR1 (except UPN6 who was in CR3) after 2-3 standard chemotherapy courses (n=7), or 1 chemotherapy course followed by subsequent azacitidine or decitabine treatment (n=3). CD3+ T cells (x10⁴/kg) were 46.9 (SD 0.00), CD3+NK cells (x10⁴/kg) were 12.8 (SD 8.3), CD56+NK cells (x10⁴/kg) were 2.6 (SD 0.99), CD34+ UCB cells (x10⁶/kg) were 2818 (SD 2818) and Donor HLA-DQ, D1/ D2 crossmatch were shown.

Characteristics infused UCB-NK cell products
Ex vivo generated UCB-NK cells have a highly activated phenotype and expressed more than 70% NKGD2, NKp30, NKp44, NKp46 and CD244. The CD16 receptor was expressed 211±20% (range 5-34%).

Donor chimerism after UCB-NK cell transfer
Donor chimerism was measured by Q-PCR for discriminating DNA polymorphisms. A temporary repopulation and persistence of UCB-NK cells could be detected in PB between days 1 and 8 post transfusion, which was associated with increased IL-15 plasma levels. Donor chimerism increased with higher doses of infused UCB-NK cells, and donor chimerism up to 3.5% was found in BM at day 7/8.

Conclusion
GMP-compliant UCB-NK cell products containing up to 3×10⁶ NK cells/kg body weight can be safely infused in non-transplant eligible AML patients following immunosuppressive chemotherapy, showing no NK cell related toxicity. Infused UCB-NK cells repopulate, mature and migrate to BM without IL-2 infusion.

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