Synopsis NK4AML

Rationale:

Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults and the incidence increases with age. Although complete remission (CR) can be achieved after treatment with intensive chemotherapy and/or hypomethylating agents (HMA) with or without consolidation with allogeneic stem cell transplantation (allo-SCT), prognosis remains poor due to high relapse rates. Relapse occurs in 20-80% of patients, depending on the genomic risk classification and response to the induction chemotherapy. Persistent minimal residual disease (MRD) prior to allo-SCT has been associated with increased relapse rates and decreased survival. Currently, only 35-40% of younger and 5-15% of older AML patients are alive 5 years after diagnosis. Therefore, novel therapies are needed to induce deeper remission before allo-SCT in more patients. Allogeneic natural killer (NK) cell-based immunotherapy is a promising, relatively non-toxic adjuvant therapeutic approach for AML. Various studies have shown that allogeneic NK cell infusion following lymphodepleting conditioning is well-tolerated and exerts anti-leukemia activity. However, further enhancement of its therapeutic efficacy is warranted. To improve NK cell adoptive immunotherapy in cancer patients, we developed a unique GMP-compliant culture system for the generation of large numbers of highly functional NK cells from umbilical cord blood (UCB)-derived CD34+ hematopoietic stem and progenitor cells (HSPC). These UCB-NK cell products display high purity, without T cell contamination, and potent anti-leukemia activity. We have demonstrated that infusion of these allogeneic UCB-NK cells is feasible, safe and well-tolerated in older AML patients, who were ineligible for allo-SCT (PLMA25 study) (1). Recently, we adapted the UCB-NK cell culture protocol resulting in a more mature 'second generation' UCB-NK cell product with enhanced cytolytic anti-tumor activity, IFNγ secretion potential and superior maturation potential *in vivo*. In addition, *in vivo* NK cell persistence and expansion could be further boosted through cytokine co-administration. Based on validation studies, we can generate between 1-3 x 10^9 NK cells from >2 x 10^6 UCB-derived CD34+ cells during maximal 5 weeks of culture. In this phase I/IIa study, we propose to combine UCB-NK cell adoptive immunotherapy with subcutaneous (SC) administration of IL-2 adjuvant therapy to eliminate refractory or relapsed AML, in order for patients to become eligible for allo-SCT.

Objective:

The study is divided in two phases.

The primary objective of phase I of the study is to evaluate the safety and toxicity of the infusion of *ex vivo*-expanded UCB-NK cells, both with and without SC IL-2, following a non-myeloablative immunosuppressive conditioning regimen in patients with AML or MDS with excess blasts-2 (EB-2). In this phase I part, we will determine the safety of IL-2 combined with our second generation UCB-NK cell product.

The primary objective of phase IIa of the study is to evaluate the effect of UCB-NK cell adoptive immunotherapy in combination with SC IL-2 following a non-myeloablative immunosuppressive conditioning regime on disease activity in patients with AML/MDS-EB-2. For both phases of the study secondary objectives include 1) evaluation of the *in vivo* lifespan and expansion potential of the donor

NK cells following adoptive transfer, 2) exploration of the functional activity of the donor NK cells in peripheral blood (PB) and bone marrow (BM) and 3) evaluation of IL-2 serum levels and plasma cytokine concentrations pre- and post-administration of SC IL-2. An extra secondary objective for the phase IIa of the study is the number of patients bridged to transplant with this treatment protocol.

Study design:

This is a prospective phase I/IIa study.

The first phase is a safety study in twelve patients. The second phase of the study is designed as a Simon's optimal two-stage single-arm phase IIa study, comprising seventeen patients. Prior to NK cell infusion, patients will receive cyclophosphamide and fludarabine (Cy/Flu) based lymphodepleting chemotherapy. On day 0, patients will receive a fixed dose of 1.0-3.0 x 10^9 allogeneic UCB-NK cells. In phase I of the study patients will receive UCB-NK cells without SC IL-2, with lower dose SC IL-2 or with higher dose SC IL-2 (n=3 per treatment group and n=6 in the highest tolerable dose). After establishing the safety of UCB-NK cells combined with SC IL-2, we will continue with phase IIa of the study, with ten patients in the first stage (including the six patients from phase I with comparable IL-2 dose) and if clinical efficacy is achieved an additional seven patients in the second stage.

Study population:

AML patients (de novo and secondary) or MDS patients with excess blasts-2 (MDS-EB-2) aged ≥ 18 years who have stable or at least non-rapidly progressive disease with or without disease controlling medication and who are (at time of inclusion) ineligible for allo-SCT. Patients on immunosuppressive treatment, with active infections that require specific therapy and patients with severe renal, pulmonary or myocardial impairment will be excluded.

Intervention:

All twenty-three patients will receive allogeneic UCB-NK cells in a fixed dose of 1.0-3.0 x 10^9 after completing standard conditioning therapy consisting of Cy/Flu on days -5 to -3 (three days). In phase I of the study 3 patients (first cohort) will receive UCB-NK cells without SC IL-2, 3 patients (second cohort) will receive UCB-NK cells with SC IL-2 in a low dose and 6 patients (third cohort) UCB-NK cells with SC IL-2 in a higher dose. IL-2 will be administered to the second and third cohort of patients in a fixed dose of 3.0 x 10^6 or 6.0 x 10^6 units, respectively, starting 4 hours after NK cell infusion and given every other day for 6 doses in total. In phase IIa of the study, 17 patients will receive UCB-NK cells combined with 6 doses of the highest tolerable SC IL-2 dose. The patients will be monitored for clinical toxicity, biological parameters in PB and BM and disease activity.

Main study parameters/endpoints:

During the phase I safety study, patients will be evaluated intensively for toxicity caused by the NK cell infusions, whether or not followed by SC IL-2, using the CTCAE toxicity criteria and graft versus host disease (GvHD) classification criteria, defining dose limiting toxicities (DLTs).

For phase IIa of the study, clinical response to therapy is the main study parameter and will be defined according to European Leukemia Network (ELN) response criteria by day +28 post UCB-NK cell administration (2).

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

The Cy/Flu treatment dosage is lower as in the study we previously performed with UCB-NK cells in elderly AML patients (PLMA25 study), with expected hematologic toxicity, but no severe toxicity (1).

In other clinical studies up to $3x10^7$/kg body weight peripheral blood or cord blood derived allogeneic NK cells have been administered to patients with relapsed or refractory AML (1, 3, 4). The NK cell infusions were well-tolerated without clinical signs of cytokine release syndrome (CRS) or evidence of induction of GvHD. In this new study, we use an adapted culture protocol for expanding CD34+ progenitor-derived NK cells resulting in more potent UCB-NK cells. Although these optimized UCB-NK have an increased potency we do not expect them cells to induce severe side effects.

SC IL-2 administration up to 9×10^6 units was well-tolerated in earlier studies and IL-2 side effects occurrence and severity were dose related (3-6). Common side effects consist of constitutional symptoms, hematologic toxicity and local reactions at the injection site. In studies using comparable dosing and dose interval as we plan to use, no IL-2 specific adverse reactions were registered, except of mild erythema at the injection site (4-6). In this new study we will examine our second generation allogeneic UCB-NK cells combined with SC IL-2 for safety and efficacy. Patients will be screened for (serious) adverse events ((S)AEs) and GvHD, defining DLTs.

For follow-up PB samples (pre-study (during intake and at day -6), before NK cell infusion (day 0), at 4 hours, day 2, 4, 7, 14, 21, 28 after NK cell infusion and 3 and 6 months in case no allo-SCT is given) and BM aspirates (pre-study, 7 and 28 days after NK cell infusion) will be collected. Patients will be asked to complete a patient diary for injection site reactions after SC IL-2 administration. UCB units stored in the Cord Blood Bank Nijmegen will be used to enrich CD34+ cells for *ex vivo* expansion and differentiation of NK cells.

This study investigates NK cell adoptive therapy in a group of patients with a poor prognosis. Current therapies result in only 20% complete response in refractory or relapsed AML/MDS-EB-2 patients. As NK cell infusion has exerted promising anti-leukemia activity in the past, with achieving up to 50% CR in poor prognosis AML patients with refractory or relapsed disease, this protocol can serve as potential bridge toward allo-SCT in our study population.