Stammzellen Zelltherapie Transplantation

CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN

Expansion of CD3 negative CD56 positive NK Cells for repeated clinical application in a GMP Compliant Process Using a Novel Bioreactor System

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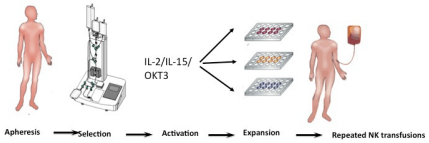
BACKGROUND

✓Natural Killer (NK) cells show high cytotoxic activity against tumour cells, act as major effector population of antibody dependent cytotoxicity (ADCC), and contribute to the anti-leukemic effect in haploidentical transplantation by killing target cells, which lack MHC class I molecules, without inducing graft-versus-host-disease.

✓The haploidentical transplantation program established at our institution[1] comprises CD34-selected stem cells and an additional transfer of high numbers of CD3-/CD56+ NK cells.

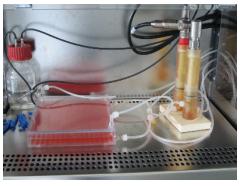
METHODS

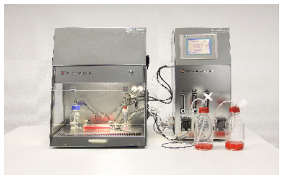
NK cells were obtained from peripheral blood mononuclear cells from healthy donors (n = 6) by CD56+selection and CD3-depletion by magnetically labelled bead technology. Suspensions of purified CD56+CD3-NK cells (Median 2x10e6 cells; CD3+ T cells < 3%) were transferred into the disposable bioreactor system.



✓After sedimentation, NK cells were cultured under continuous laminar flow for a median of 28 days (range 25 – 40) without feeder cells. SCGM-medium was supplemented with humam albumin 10%, IL-2 500 U/ml and IL-15 50ng/ml

✓Feeding rates, pH and pO2 values as well as temperature were controlled automatically by the bioreactor system.





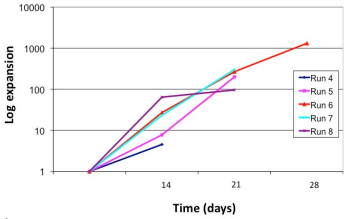
Controls and Read-out:

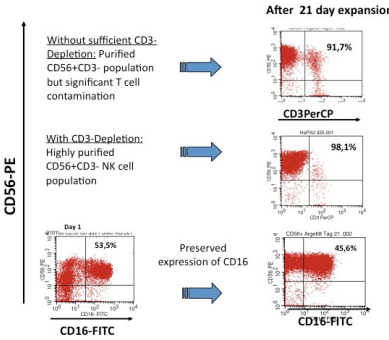
✓Harvested NK cells were analysed for puritiy, NK cell phenotype and natural cytotoxicity against K562 cell line (in standard calcein cytotoxicity assay.

✓ADCC was analysed using the anti-CD20-antibody Rituximab and the CD20+ lymphoma cell line Raji.

RESULTS

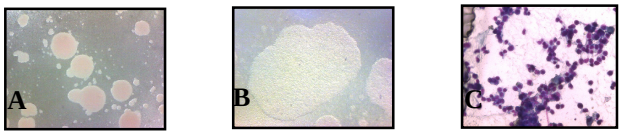
✓CD3-/CD56+ cells expanded 250 to 400-fold. Further expansion by more than 100-fold was possible in a larger disposable bioreactor.





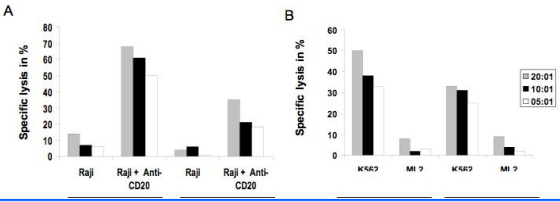
✓The mean NK cell purity was > 95% (range 98-100 %) if the initial purity of > 97% CD3-/CD56+ NK cells was achieved.

✓Viability was > 80% directly after harvest from the reactor.



Microscopic images of NK cells sedimented to the bottom of the biareactor vessels (A,B). NK cells start to form cellular 3D agglomerates after several days (A). Giemsa stained NK cells showing some extracellular matrix formed by the NK cells (C),

Expanded NK cells showed a very strong lysis (median of 42 % lysis of K562 cells at E/T ratio of 10:1, range 17-55%) compared to freshly isolated NK cells (median 27%, range 15-41%). Analysis of ADCC demonstrated high lytic activity (median 57%, range 20-70%) towards otherwise resistant lymphoma cells.



CONCLUSION

✓With a novel bioreactor system we succeeded in mass expansion of effective NK cells under GMP compliant conditions, allowing high dose and repeated treatments.

✓The availability of nearly unlimited numbers of activated NK cells with high lytic activity against various tumour targets in vitro offers new treatment options for leukemia patients receiving haploidentical transplantats by strengthening of the anti-leukemic effect of the approach.