metastasis. These data provided a platform for advanced therapeutic strategies by targeting lymphatic vessels as a microenvironmental factor and we will next establish plan for clinic application by using safety materials such as peptide and monoclonal antibodies in targeting FLT4.

416

Off-the-shelf ipsc derived car-nk immunotherapy for solid tumors

N.R. Boyd*, M. Tiedemann, K. Cartledge, M. Cao, V. Evtimov, R. Shu, T. Nguyen, N. Nguyen, I. Nisbet, R. Boyd, A. Trounson
Cartherics, Richmond, Victoria, Australia

Background & Aim The clinical impact of Chimeric Antigen Receptor T cell (CAR-T) technologies on hematological malignancies have revolutionized cancer treatment. However, current autologous CAR-T therapies face major roadblocks for mass adoption. These include high-cost, patient-specific manufacturing, inconsistent CAR-T yield and function due to inherently depleted patient immune systems, and life-threatening adverse events. NK cell therapies have the potential to overcome at least some of these deficiencies. NK cells utilize multiple anti-cancer receptors without risk of graft versus host disease. However, they have reduced longevity *in vivo,* which may necessitate multiple infusions, increasing the risk of their rejection by the patient. Both CAR-T and CAR-NK cells are subject to checkpoint blockades and other mechanisms of immunosuppression that diminish their killer function *in vivo*.

To address these key deficiencies, we developed 'off-the-shelf', genetically-enhanced CAR-NK cells via induced pluripotent stem cells (iPSCs). Utilization of iPSCs as a renewable source for NK cells allows for consistent, precisely-defined immunotherapy and the capacity to gene-edit in multiple anti-cancer modes of action, which we have taken advantage of. The CAR-iNK cells can be cryopreserved, delivered on-demand for each patient, and crucially enable a major reduction in manufacturing cost.

Methods, Results & Conclusion Our manufacturing system is highly scalable, completely xeno- and feeder-free, yielding $\sim 10^5$ iNK cells from a single iPSC in less than 30 days. The iPSCs are gene-edited to carry a CAR (specific for the adenocarcinoma neoantigen TAG72) and deleted of immune suppression gene(s) to enhance NK longevity and efficacy. Unlike nearly all current allogeneic NK therapies, our iPSCs are derived from rare triple homozygous HLA donors, reducing the risk of host-mounted rejection of these iNK cells. The manufactured CAR-iNK cells display potent, on-target cytotoxic functionality against multiple ovarian cancer cell lines *in vitro* but do not kill cells from fresh healthy tissue. They are presently being evaluated *in vivo*. Our process provides a near limitless, on-demand supply of standardized 'off-the-shelf' CAR-iNK cells, with potential application to a variety of cancers, sufficient to treat many patients using a single manufactured product.

417

Live cell imaging of lytic granule motility in anti-ErbB2 CAR NK cells and FcR NK cells plus Herceptin towards ErbB2+ breast cancer cells

J. Eitler[1], N. Wotschel[1], H. Klingemann[3], W.S. Wels[2], T. Tonn[1,*]
[1] Experimental Transfusion Medicine, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany; [2] Georg-Speyer-Haus, Frankfurt am Main, Germany; [3] Nantkwest Inc., Culver City, California, United States

Background & Aim Upon encountering a susceptible target, NK cells mediate directed cytotoxicity by exocytosis of lytic effector molecules such as perforin and granzymes. The steps leading to NK cell granule exocytosis are highly regulated. Granule exocytosis is preceded by convergence of granules to the microtubule organizing center (MTOC) and subsequent polarization of the MTOC and granules to the immunological synapse (IS). In case of antibody-dependent cell-mediated cytotoxicity (ADCC), it has been shown that signaling through the Fc receptor is critical to polarize MTOC and granules to the IS with otherwise resistant targets.

Methods, Results & Conclusion Here we used spinning disk confocal microscopy for live cell imaging to analyze granule-mediated NK cell cytotoxicity in ErbB2-targeted CAR expressing NK-92 cells (NK-92/5.28.z) and research-grade high affinity FcR expressing NK-92 cells plus Herceptin™ towards ErbB2-positive breast cancer cells (MDA-MB-453), which are resistant to parental NK-92. Interestingly, unmodified NK-92 cells in combination with MDA-MB-453 cells showed granule convergence to the MTOC, but failed to polarize MTOC and granules to the IS. In contrast, retargeting by either CAR or mAb/FcR towards the ErbB2 antigen on MDA-MB-453 enabled granule polarization to the IS resulting in highly effective cytotoxicity. Granule polarization was rapid in both the CAR and high affinity FcR expressing NK-92 cells after cell-cell contact was initiated. These observations suggest that retargeting of NK-92 cells by either transgenic CAR or high affinity FcR expression in combination with tumor-specific antibodies confers tumor cell lysis by enabling the otherwise impaired MTOC and granule polarization to the IS which resembles the physiological exocytosis cascade observed in naturally occurring ADCC.

418

Fully automated integrated process for depletion of T cells with or without subsequent purification of NK cells from apheresis using the CliniMACS Prodigy

A. Mekes*, M. Nitsche, J. Kostyra, M. Sohmen, S. Mueller, M.E. Luevano, N. Nickel, N. Moeker
Miltenyi Biotec B. V. & Co. KG, Bergisch Gladbach, Germany

Background & Aim Donor-derived natural killer (NK) cells mediate graft-versus-leukemia effects low risk in inducing alloreactive graft-versus-host-disease and are used as component of T cell-depleted donor lymphocyte infusion (DLI) or purified. As clinical grade purification of NK cells requires two semi-automated separation procedures, including multiple manual hands-on steps, we developed an integrated fully automated process enabling either preparation of T cell-depleted DLI or clinical grade purification of NK cells within a single automated process on the CliniMACS Prodigy.

Methods, Results & Conclusion The novel integrated CliniMACS Prodigy LP-3-56 System uses the CliniMACS Prodigy Tubing Set 320 for an fully automated procedure with integrated labeling and washing steps for both, CD3 and CD56 separation enabled through an integrated lytic rinsing sequence allowing subsequent CD56 enrichment in the same tubing set. By users choice the depletion of $CD3^+$ cells will be performed with or without subsequent enrichment of $CD56^+$ cells. For both strategies target cells are prepared with user-chosen formulation solution (e. g. sodium chloride or NK MACS medium).

The new application software effectively depletes 9.6×10^9 $CD3^+$ cells, and enriches 4.5×10^9 $CD56^+$ cells from up to 40×10^9 total white blood cells.

Inhouse evaluation runs with leukapheresis products (n= 29 in total, n=17 with CD3 depletion, n=12 with CD3 depletion and CD56 enrichment) were performed. After CD3 depletion, mean log depletion of T cells was 4.0. The mean recovery of NK cells was 89% with an average NK:T ratio-increase of 9509 fold. After CD3 depletion and CD56 enrichment, mean log depletion of T and B cells was 4.2 and 2.7, respectively. Purity of NK cells was 90.7% on average with a mean recovery of 49%. The majority of non-NK cells after the process were monocytes with a mean frequency of 5.9%. Functionality of purified NK cells was proven by their ability to lyse AML cells, to secrete cytokines after co-culture, and to perform antibody dependent cell-mediated cytotoxicity (ADCC) against Raji cells, using human CD20 antibody.