Combination and integration to redirect NK cells for cancer immunotherapy

Impact Paragraph

Cancer is a leading cause of death that threatens the life quality of human beings all over the world [1]. Traditional treatments have achieved great success in early diagnosed cancer, but have poor outcome in the advanced disease, especially in refractory and relapse stages where metastasis tumors exist [2,3]. Immunotherapy is an emerging therapy that aims to boost the immune system to eliminate the tumor cells and has been considered as the scientific breakthrough in 2013 [4]. NK cells are potent killer cells against cancer cells and can be further genetically modified to express a chimeric antigen receptor (CAR), which will be a powerful alternative effector cell for cancer therapy [5,6]. In the current thesis, we continue our group's previous experience and try to generate a natural killer (NK) cell-based immunotherapy for hematologic and solid tumors.

1 Target group

In advanced stages of cancer, morbidity and mortality are high due to cancer cells becoming insensitive to traditional treatments, such as chemotherapy and radiotherapy [7]. Of note, an estimated 19.3 million new cancer cases and almost 10 million cancer deaths occurred in 2020 [1]. These patients are in need of an effective therapy to control the progress of their disease and alleviation of suffering. Immunotherapy was recently introduced as a new treatment modality that also shows promising results in advanced disease [8]. CAR-NK cells are an example of immunotherapy and have gained interest due to their effectiveness and favourable safety profile. Although the current CAR-NK may only be effective in some hematologic malignancies, there are also some effects on solid tumors reported. Combination therapy with cytokine and checkpoint blockade antibodies may further improve the outcome of NK cell-based therapy.

2 Innovation and implementation

Throughout my PhD project, we aimed to develop CAR-NK and CRISPR/Cas9 genetically modified NK cells as an advanced therapeutic medicinal product (ATMP) for cancer treatment. We attempted to generate a flexible strategy for making CAR-NK cells and also to systematically test additional mechanisms to enhance NK cell mediated anti-cancer effects via antibodies or CRISPR/Cas9 technology. These generated optimization strategies can be extended further than our model diseases, breast cancer and multiple myeloma (MM), as we expect that the prerequisites for effective signalling are universal for NK cells. We have successfully expanded human NK cells within a GMP facility that can be used in clinical trials for solid tumor patients, rendering a much cheaper ATMP (**Chapter 2~4**) compared to the current CAR-T cells (see below). Moreover, we also applied CRISPR/Cas9 to introduce genetic modifications, thereby disrupting the NKG2A inhibitory receptor on NK cells to boost the persistence and efficiency of killing tumor cells in an immunosuppressive tumor microenvironment (TME) (**Chapter 5**). Combined with the extensive immunotherapy experience in our laboratory, we expect that this knowledge will be easily adaptable and applicable for the development of other anti-tumor strategies in the coming future.

Even though CAR-engineered lymphocytes are currently only studied in the context of cancer, they can also be used to control diseases, such as infections and auto-

immune disorders. Given the rapid increase in antibiotic resistance, the development of alternative strategies is highly relevant. As NK cells have the intrinsic capacity to recognize and eliminate virus-infected cells (such as SARS-CoV-2), their responses might be further enhanced by equipping them with a CAR [9]. Such cells could be applied in patients in the intensive care units, outbalancing the cost for long stay at the ICU ward. Till May 2021, there are five CAR-T ATMP products approved by the U.S. Food and Drug Administration (FDA) for hematologic malignancy, while there are currently no CAR-T cells registered as effective therapy for solid tumors. Moreover, in some CAR-T infused patients, several severe side-effects have been reported, including a cytokine storm that may even be lethal. Here, NK cells have several advantages over T cells. Firstly, NK cells are strong killer cells to cancerous target cells which can be generated in a GMP-compliant large-scale expansion in only 2 weeks. Secondly, NK cells lack the T cell receptor (TCR), which allows for the use of allogeneic NK cells, resulting in an “off-the-shelf” product. The TCR on T cells are the major molecule involved severe graft versus host disease (GVHD), which will not occur in case of NK cells. Moreover, several clinical trials have demonstrated that infusion of allogeneic NK cells to both hematologic and solid tumors are safe and feasible, and even induces graft-versus-leukaemia cytotoxicity [10-12]. This favourable profile of NK cells broadens the applicability of NK cells in clinical trials and daily clinical practice.

3 Social and economic relevance

With increasing numbers of patients being treated with ATMP and achieving encouraging outcomes, we can expect that more ATMP cellular products will be approved by the FDA or European Medicine Agency (EMA). The policies related to the ATMP will lift limitations on genetic modified cellular products to guarantee that more cancer patients can get appropriate and safe immunotherapy. These policies will also encourage companies to take part in the development of ATMPs. Together, this will lead to positive effects on the whole society by improving the health and welfare of all human beings.

Besides the encouraging social effects of these ATMPs, the economical aspect needs to be considered. One of the big hurdles of the distribution of these ATMPs is the economic burden [13]. The cost of the two FDA-approved anti-CD19 CAR-T cell therapy products are $475,000 (Tisagenlecleucel; Novartis, Basel, Switzerland) and $373,000 (Axicabtagene Ciloleucel; Gilead/Kite Pharma, Foster City, CA, USA) per patient, with an estimated amount of $1 million per patient when costs with medical tests and hospitalization are included [14]. There are several studies conducted to evaluate the cost-effectiveness of the CAR-T in diffuse large B cells lymphoma (DLBL) and some models were made to predict how to reduce the cost [15,16]. If the price remains at this level, many patients will not be able to afford this therapy, especially in countries with a weaker economic status. As mentioned before, CAR-NK is an alternative effector cell source that most likely will be cheaper than CAR-T therapy.

Another approach for reducing the price of immunotherapy is to decrease the cost associated with CAR-NK production [14,17], such as using a non-viral transfection method to replace the current costly lentiviral approach, and produce the “off-the-shelf” CAR-NK products. The already mentioned severe cytokines release storms (CRS) that have often been reported in CAR-T infused patients, will also give an additional economic burden. The first Phase I/II CD19 CAR-NK expanded from cord blood

elucidated that these cells are safe and effective for B cell lymphoma patients without severe side effects that have been reported in CAR-T therapy [12]. Thus, CAR-NK may further diminish the costs of ATMP products. Of note, one study proposed that decentralized ATMP production would allow even further cost reductions due to easier logistics and reduced cell preservation fees [18]. With the development of ATMP production technology, more people, or societies in general, ultimately can afford CAR-NK therapy.

Our group has investigated NK cells for more than 20 years and has developed several products for malignant disease. Our group leaders, Prof. Dr G.M.J. Bos and Dr. W.T.V. Germeraad, have established the start-up company CiMaas that transfers these novel cellular therapies to clinical application. CiMaas has the protocols, procedures and equipment in a B-grade clean room that that allows to produce GMP grade, expanded NK cells to clinical applicable numbers of 3E10 within 2 weeks. The infrastructure established by CiMaas together with Maastricht University Medical Center (MUMC+) will also be capable of testing CAR-NK cells in clinical trials in the future. Following the model of CiMaas, we believe more and more hospitals and institutes will transform and implement novel immunotherapies in clinical trials and will become a reality in cancer treatment.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A*, et al.* Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021. doi:10.3322/caac.21660.
2. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: From t cell basic science to clinical practice. Nat Rev Immunol. 2020; 20:651-668. doi:10.1038/s41577-020-0306-5.
3. Xu X, Li T, Shen S, Wang J, Abdou P, Gu Z*, et al.* Advances in engineering cells for cancer immunotherapy. Theranostics. 2019; 9:7889-7905. doi:10.7150/thno.38583.
4. Galluzzi L, Vacchelli E, Bravo-San Pedro JM, Buqué A, Senovilla L, Baracco EE*, et al.* Classification of current anticancer immunotherapies. Oncotarget. 2014; 5:12472-12508. doi:10.18632/oncotarget.2998.
5. June CH, Sadelain M. Chimeric antigen receptor therapy. N Engl J Med. 2018; 379:64-73. doi:10.1056/NEJMra1706169.
6. Dunbar CE, High KA, Joung JK, Kohn DB, Ozawa K, Sadelain M. Gene therapy comes of age. Science. 2018; 359:eaan4672. doi:10.1126/science.aan4672.
7. Myers JA, Miller JS. Exploring the nk cell platform for cancer immunotherapy. Nat Rev Clin Oncol. 2020. doi:10.1038/s41571-020-0426-7.
8. Miller JS, Lanier LL. Natural killer cells in cancer immunotherapy. Annu Rev Cancer Biol. 2019; 3:77-103. doi:10.1146/annurev-cancerbio-030518-055653.
9. Ma M, Badeti S, Chen CH, Pinter A, Jiang Q, Shi L*, et al.* Car-nk cells effectively target the d614 and g614 sars-cov-2-infected cells. bioRxiv. 2021. doi:10.1101/2021.01.14.426742.
10. Khatua S, Cooper LJN, Sandberg DI, Ketonen L, Johnson JM, Rytting ME*, et al.* Phase i study of intraventricular infusions of autologous *ex vivo* expanded nk cells in children with recurrent medulloblastoma and ependymoma. Neuro Oncol. 2020; 22:1214-1225. doi:10.1093/neuonc/noaa047.
11. Ciurea SO, Schafer JR, Bassett R, Denman CJ, Cao K, Willis D*, et al.* Phase 1 clinical trial using mbil21 *ex vivo*-expanded donor-derived nk cells after haploidentical transplantation. Blood. 2017; 130:1857-1868. doi:10.1182/blood-2017-05-785659.
12. Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R*, et al.* Use of car-transduced natural killer cells in cd19-positive lymphoid tumors. N Engl J Med. 2020; 382:545-553. doi:10.1056/NEJMoa1910607.
13. Lyman GH, Nguyen A, Snyder S, Gitlin M, Chung KC. Economic evaluation of chimeric antigen receptor t-cell therapy by site of care among patients with relapsed or refractory large b-cell lymphoma. JAMA Netw Open. 2020; 3:e202072. doi:10.1001/jamanetworkopen.2020.2072.
14. Chicaybam L, Bonamino MH, Luckow Invitti A, Bortman Rozenchan P, de Luna Vieira I, Strauss BE. Overhauling car t cells to improve efficacy, safety and cost. Cancers (Basel). 2020; 12. doi:10.3390/cancers12092360.
15. Thielen FW, van Dongen-Leunis A, Arons AMM, Ladestein JR, Hoogerbrugge PM, Uyl-de Groot CA. Cost-effectiveness of anti-cd19 chimeric antigen receptor t-cell therapy in pediatric relapsed/refractory b-cell acute lymphoblastic leukemia. A societal view. Eur J Haematol. 2020; 105:203-215. doi:10.1111/ejh.13427.
16. Lin JK, Muffly LS, Spinner MA, Barnes JI, Owens DK, Goldhaber-Fiebert JD. Cost effectiveness of chimeric antigen receptor t-cell therapy in multiply relapsed or refractory adult large b-cell lymphoma. J Clin Oncol. 2019; 37:2105-2119. doi:10.1200/jco.18.02079.
17. Couchoud C, Fagnoni P, Aubin F, Westeel V, Maurina T, Thiery-Vuillemin A*, et al.* Economic evaluations of cancer immunotherapy: A systematic review and quality evaluation. Cancer Immunol Immunother. 2020; 69:1947-1958. doi:10.1007/s00262-020-02646-0.
18. Ran T, Eichmüller SB, Schmidt P, Schlander M. Cost of decentralized car t-cell production in an academic nonprofit setting. Int J Cancer. 2020; 147:3438-3445. doi:10.1002/ijc.33156.