Refining natural killer cell-based immunotherapy

VALORIZATION

The 2018 GLOBOCAN data estimated that there would be around 18.1 million new cancer cases and 9.6 million cancer deaths worldwide in 2018. Although several types of cancer have a better chance of remission than other types after surgery/chemo-/radiotherapy, cancer is mostly still an incurable disease. Multiple myeloma (MM), a plasma cell malignancy, is an example of a malignancy with a low survival-rate, regardless of an increased progression-free survival in the last few years due to the discovery of new drugs besides the conventional chemotherapy. NK cell-based immunotherapy might be a promising alternative therapy for MM patients as recent studies showed that NK cells in MM patients are often dysfunctional. In addition, recent clinical trials in MM patients using either autologous or allogeneic *ex vivo*-expanded NK cells showed that it is safe and feasible. However, the clinical efficacy reported was still limited.

One of the possible limitations of the clinical efficacy of an NK cell-based immunotherapy could be the under representation of the tumor microenvironment in an *in vitro* testing. In this thesis (chapter 2 and 5) we showed that the addition of selected factors (hypoxia, lactate, PGE2) could negatively affect NK-cell killing against MM cells. In another study in this thesis (chapter 7), another factor (low glucose) on the other hand, seemed to be beneficial for NK cells. Given these results, a development of a more representative *in vitro* model of tumor microenvironment resembling *in vivo* (patient's) tumor microenvironment would be pivotal to better predict the effect of the treatment *in vivo*. This could reduce unnecessary (further) developments of treatment which are unlikely to give a desirable effect in patients.

To be able to mediate an antitumor response in the tumor microenvironment, NK cells need to be properly activated. We showed in this thesis that a combination strategy of KIR-ligand mismatched NK cells with a monoclonal antibody resulted in a better killing of tumor cells in the presence of selected biochemical factors mimicking the tumor microenvironment. At the same time, more importantly, we showed that the administration of an antibody had two less desirable outcomes, 1) the expression of the target antigen had to be high on the target cells otherwise the addition of the monoclonal antibody did not enhance NK cells killing against tumor cells, 2) when the target antigen is also expressed on NK cells, using the monoclonal antibody could be detrimental for NK cells as a result of fratricide (NK-NK killing). To overcome these unnecessary "side effects", several strategies could be considered. First, proper timing between the antibody administration and the injection of NK-cell adoptive transfer. Second, pretreatment of NK-cells with a $F(ab)_2$ fragment of the antibody

prior to injection. Third, development of a bi-specific or tri-specific antibody to better target tumor cells. Fourth, development of an antibody which could still trigger an antibody-dependent cell-mediated cytotoxicity (ADCC) on NK cell despite the low/intermediate level of antigen expressed on the target cells. A proper design of an antibody is highly crucial for the success of a combination therapy with an NK cell-based immunotherapy.

In this thesis, we also described that a factor that potentially contributes to the success of antibody therapy is the CD16a (FcγRIIIa) receptor. A few polymorphisms of the *FCGR3A* gene, the gene encoding the CD16a receptor, have been described to influence the binding of CD16a to an antibody. We are the first to provide an extensive overview of *FCGR3A* gene polymorphisms using the 1000 Genome project database. Additionally, we also successfully developed two gene-sequencing methods to detect a full-length *FCGR3A* gene polymorphisms: a Sanger based and a MinION based, a novel sequencing method. From this study, both the overview and the sequencing methods together could be used to: 1) further investigate the functional relevance of *FCGR3A* gene polymorphisms on NK-cell ADCC capacity, 2) design an antibody or CAR-NK cells, 3) select an NK-cell donor. A major advantage of the nanopore MinION strategy would be that it enables faster analysis of full length gene polymorphism. Due to the possibility to barcode individual samples or genes, it also enables the simultaneous analysis of samples which may reduce the costs of this technology.

Another key limiting factor for an NK cell-based immunotherapy is to obtain sufficient numbers of functional NK cells for the infusion to a cancer patient. Although there is no consensus for the minimum or maximum number of NK-cells that can or should be injected to a patient, current clinical trials have gone up to 10^8 NK cells/kg. An advantage of donor NK cell-based immunotherapy is that donor NK cells could be *ex vivo*-expanded as an off-the-shelf product and hence a readily available product. As NK cells do not attack healthy cells infusion of allogeneic NK cell is safe and KIR-ligand mismatched NK cells seemed to be the better effector cells. Therefore, a universal "perfect" NK-cell donor could be selected based on the genotype; that is when donor NK cells express all licensed KIRs. Our group has been working for years to develop an optimized protocol and technology to expand NK cells *ex vivo* aiming to produce 10^{10} NK cells. At CiMaas, a spin-off company founded by Prof. dr. G. M. J. Bos and Dr. W. T. V. Germeraard, it is now possible to manufacture a GMP-grade *ex vivo*-expanded NK-cell product.

One of the very promising strategies to use those expanded NK cells is by combining them with a haploidentical stem cell transplantation. Our group is currently leading the first Phasel/II multicenter study where MM patients receive a haploidentical stem cell transplantation and donors are selected by the Transplantation Immunology lab, based on the presence of a KIR-ligand mismatch. Although the study has not been completed yet, it demonstrated the feasibility and safety of the approach. From the study, we also learned that it takes 30-60 days before mature NK cells are circulating in the patient. To further enhance clinical responses, haplo-SCT could be combined with infusion of NK cells form the same donor expanded according to the CiMaas protocol. The expanded NK cells could potently mediate their anti-tumor responses in the first two months while persistence of the response will result from donor NK cells that developed from the stem cell graft. This procedure has been shown to be very efficient by our close collaborators at Cytosen and MD Anderson. Based on the results described in this thesis, ADCC or blocking antibodies could be combined with haplo-SCT and NK infusion to further potentiate the response. A further development for these *ex vivo*-expanded NK cells would include molecular modifications of the receptors (termed chimeric antigen receptor or often abbreviated CAR) to better target tumor cells. As an off-the-shelf product, *ex vivo*-expanded NK cells would be stored in the freezer for storage. Previous studies, however, have reported that NK cell viability decreased after a freeze thawing procedure. Therefore, a further technology optimization/development on the freeze-thawing protocol/technology might be necessary and interesting aspect to develop.

In summary, data presented in this thesis serve as a confirmation and follow up of our previous studies as well as a starting point and foundation of our subsequent studies. Altogether, the findings described in this thesis contribute to the refinement of an NK cell-based immunotherapy which may contribute to novel treatment options for MM patients that provide curative responses and preferably have a low level of toxicity.