Cancer immunotherapies

Impact paragraph

Cancer is a leading cause of death worldwide. Although improvements have been made including earlier diagnosis and better treatment, cancer remains a high burden for society. One in four men and one in five women were diagnosed with cancer in 2018 and about 60% of cancer patients die from the disease [1]. Therefore, it is highly desirable to design therapies that will lead to higher cure rates of cancer. Immunotherapies are considered one of the major breakthroughs in the treatment of cancer and present promising approaches for cancers that are currently incurable [2]. Immunotherapies comprise various strategies that harness and strengthen the power of immune cells to better recognize and destroy tumor cells. But even within one patient, tumor cells can be highly heterogeneous cell populations and some tumor cells evade the destruction by immune cells and continue to grow. Tumor growth is further supported by the tissue surrounding the tumor, the TME. Therefore, one challenge of immunotherapies is to stimulate the immune cells sufficiently to detect and eliminate all tumor cells without off-target toxicity. NK cells are the immune cell type that we focused on in this thesis. In our research group, NK cell-based cancer immunotherapies have been studied for more than a decade and, continuing on our research groups' experiences, we assessed in this thesis how donor-derived NK cells can be further optimized to improve their anti-tumor responses against two cancer types, multiple myeloma and breast cancer.

Scientific impact

To enhance the anti-tumor efficacy of cytokine-activated NK cells, we described the following main principles in this thesis:

1. Selection of NK cell donors with a KIR-HLA ligand mismatch to prevent inhibitory signaling of NK cells through HLA class I
2. Combine KIR-HLA ligand mismatched NK cells with monoclonal antibodies that target tumor cells and that trigger ADCC in NK cells to potentiate NK cell activation
3. Increase NK cell activation and IFN-γ production through ADCC-triggering antibodies that are directed against tumor-supporting cells in the TME, such as macrophages

Importantly, the anti-tumor efficacy of highly activated NK cells was not diminished either by hypoxia or by low glucose levels, both of which are factors known to occur in the TME. As the TME is highly complex and contains multiple mechanisms that can reduce NK cell efficacy, it is desirable for future research to include more complex models that reflect the patient situation as closely as possible.

For solid tumors, we additionally need to acquire more knowledge about the spatial distribution of NK cells because increasing evidence supports that the complexity and diversity of the TME influences immunotherapy responses. As a first step towards NK cell distribution in breast cancer, we quantified and profiled NK cells in situ in breast cancer cohorts and found low infiltration of endogenous NK cells in

comparison to other immune cell subsets, suggesting that NK cell-based therapies need to include strategies that enhance NK cell infiltration into tumors.
During the years of my PhD studies, I have presented and discussed the scientific results on several national and internal conferences. Moreover, the scientific findings of this thesis are published in peer-reviewed and open-access journals and are thus available to the scientific community. The research presented in this thesis was supported by local donations through the Kankeronderzoeksfonds Limburg (Cancer Research Foundation Limburg), illustrating which positive impact local donations are having on advancing cancer research. And partly based on the findings in this thesis, a research grant of about 1.5 million Euros was recently obtained from Health~Holland and KWF, allowing new PhD students to further explore the development of effective NK cell treatment in breast cancer. For this goal, our institute will closely collaborate with the University of Utrecht to combine knowledge and work towards the goal of higher cure rates for breast cancer.

Target group
The scientific studies were performed with the goal to improve therapy approaches for cancer patients. While the results presented in this thesis were specifically tested for multiple myeloma and breast cancer, the findings could be relevant for a broader spectrum of cancer types and patients: NK cell-based therapies have potential for blood cancers other than multiple myeloma, in which their effectiveness has been demonstrated in preclinical and clinical studies, and recently, advances with NK cell-based approaches have also been made for solid tumors [3,4]. In this thesis, we for example demonstrated that the principle of combining KIR-HLA ligand mismatched donor NK cells with monoclonal antibodies could be extended from MM to breast cancer. Since it resulted in more potent NK cells in both MM and BC, the concept may be beneficial for patients with other tumor types that retain HLA class I expression.
However, we also observed differences in the level of NK cell responses, which might depend on the NK cell donor or the tumor cell or most likely a combination thereof. To select the optimal immunotherapy approach for each patient, better patient stratification will be required and can be supported by extensive profiling of both tumor and the TME. Good patient stratification will ensure that patients only receive treatments they respond to and consequently, money will not be wasted on ineffective treatments and, importantly, the quality of life for cancer patients will improve as they do not need to suffer from unnecessary treatments that often come with severe side effects.

Innovation
NK cells are most commonly derived from peripheral blood, either from the patient or from a healthy donor and the infusion of donor-derived NK cells has been demonstrated to be safe in several clinical trials. Moreover, donor NK cells might be advantageous as demonstrated by the enhanced NK cell activity of KIR-ligand mismatched NK cells in this thesis. Independent of the source, NK cells need to be

expanded to reach the high number used in clinical trials (up to $1x10^8$ NK cells/kg bodyweight). Producing the required numbers of NK cells for clinical trials needs to be performed in laboratories that fulfill the guidelines of good manufacturing practices. The fact that donor-derived NK cells are safe provides the opportunity for the generation of an "off-the-shelf" NK cell product, derived from large scale expansions of one donor. Such "off-the-shelf" NK cell products are considered much more cost effective than isolating NK cells from the patient itself and therefore represent a major advantage over T cell-based therapies, where donor-derived therapies are much more challenging due to GVHD mediated by donor T cells.

The results of this thesis might be relevant for guiding the design of clinical trials e.g., the selection of the best NK cell product for the patient, and can additionally be relevant for biotechnology companies that focus on the development, production, and medical application of NK cell products. The expansion process of NK cells provides a wide range of opportunities to improve the final NK cell product, for instance through cytokine stimulation, small interfering RNAs, or genetic modifications of NK cells. Based on the results of this thesis, it seems relevant to ensure sufficient IFN-γ production, sufficient expression of activating receptors including CD16 expression, and to interfere with inhibitory signaling. Moreover, the expansion process can provide possibilities to promote NK cell migration to solid tumors, which seems to be required based on the results of this thesis. In our research group, Prof. Dr. Gerard Bos and Dr. Wilfred Germeraad have founded CiMaas, a spin-off company from Maastricht University, with the goal to achieve better cure for cancer patients. One of the clinical product lines at CiMaas is the generation of donor-derived NK cells for cellular therapy. Protocols are available at CiMaas to produce NK cell products in accordance with the applicable Good Manufacturing Practices and to expand up to 10^{10} NK cells. To translate the novel therapy approaches into clinical practice, CiMaas closely collaborates with Maastricht University and the Maastricht University Medical Center to start clinical trials. Such collaborative networks of researchers and clinicians in university, hospital, and biotechnological companies are very valuable to support the translation of new therapeutic approaches into clinical practice.

Overall, with various approaches being in development and clinical trials showing promising results, immunotherapies are on the way to becoming more prominent therapeutic approaches in the future. Our research results provided small building blocks to create effective NK cell-based immunotherapies and we envision that our findings can eventually contribute to improved cancer immunotherapies.

References

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