## Research Strategy: Erik Manting

# Memory NK cells and their therapeutic potential

Immunological memory, the ability of the immune system to respond rapidly and provide protection against previously encountered pathogens, for long has been described as a specific feature of the adaptive immune system. In summary, an initial encounter with a pathogen such as a viral infection leads to a rapid expansion of naïve T cells that bind to a corresponding antigen and their transformation into effector cells, capable of killing the virus-infected cells.

This phase is followed by a contraction phase and the survival of only a small population of memory T cells, which persist over time through self-renewal, ready to expand and be re-activated in case of a subsequent infection. A similar process has been described for B cells, which produce protective antibodies that bind to pathogens and in this way help clear infections. Immunological memory, or active immunity, is the only long-lasting form of immunity and provides the basis for vaccination as an effective preventive treatment to provide long-term protection against disease.

A relatively new finding in immunology is the phenomenon that immunological memory is not exclusive to the adaptive immune system, but also that natural killer (NK) cells possess the capacity to transform into memory cells. NK cells are part of the innate immune system, traditionally described as the first line of defence against infections, which is not dependent on the recognition of specific antigens and does not contribute to the formation of immunological memory. However, NK cells exposed to viral infections can transform into cells that persist over long periods of time through self-renewal and can be quickly reactivated upon secondary infection[1].

A virus particularly linked to memory NK cells formation is cytomegalovirus (CMV), a herpes virus with the largest known viral genome. As a result of long evolutionary pressure and co-evolution with their hosts, cytomegaloviruses possess many pathways to avoid destruction by the immune system[2]. In response, NK cells develop into effective memory cells, capable of suppressing recurring CMV reactivation.

The discovery of memory NK cells explains a remarkable observation in the treatment of blood-borne tumours: the not yet fully understood link between CMV infection and survival benefit following hematopoietic stem cell transplant (HSCT or bone marrow transplant). In patients suffering from blood-borne tumours such as acute myeloid leukaemia (AML), chronic myeloid leukaemia, and multiple myeloma, a reduced relapse rate following HSCT was observed in patients with CMV reactivation. Increasing evidence points out that the improved graft-versus-leukaemia effect is not due to the virus itself, but instead to the concomitant expansion of memory NK cells that contribute to the immune control over residual cancer cells[3].

These findings have led to the search for methods to produce memory NK cells for therapeutic purposes, particularly using cytokines and genetically engineered cancer cell lines as feeder cells to stimulate their outgrowth[4]. The administration of *ex vivo* expanded memory-like NK cells following HSCT has delivered promising signs of clinical efficacy in an early-stage trial in paediatric AML[5].

At Mendus AB, a clinical-stage oncology company, we have established a production method for the expansion of memory NK cells based on so-called leukaemic-derived dendritic cells. Using a proprietary leukaemic production cell line (DCOne) and production process, we manufacture cells that endogenously express cancer cell ligands for memory NK cells, combined with mature dendritic cell surface molecules without the need for genetic engineering. By adding these cells as feeder cells in culture with NK cells from CMV-positive donors, we have observed consistent, selective expansion of memory NK cells. The outcome is a very efficient method to produce therapeutic quantities of memory NK cells, which can be manufactured from HSCT donor material and developed as a post-transplant treatment in blood-borne tumours.

In allogeneic HSCT, the therapeutic administered NK cells are immunologically matched with the engrafted immune system, which supports their longevity, but unmatched with residual cancer cells. This improves their effectiveness as tumour cell killers, because inhibitory signals related to the recognition of self are missing in the NK-tumour cell interaction.

A tempting thought is to expand the reach of memory NK cell-based therapies beyond the post-HSCT setting based on their robust *in vivo* persistence, expansion capacity and selective cytotoxicity toward virus-infected or cancerous cells. Their effectiveness can be enforced by monoclonal antibodies or NK cell engagers, arming and guiding the NK cells toward their specific target, opening up new therapeutic applications, including solid tumour indications.

Based on the interest to develop safe and effective cellular immunotherapies based on NK cells, the manufacturing of NK cells from different sources including donor blood, cord blood, cell lines and induced pluripotent stem cells has evolved. Despite promising initial results with some of these approaches, there remains a need to improve persistence and predictability of the anti-tumour response of the administered NK cells. The possibility to specifically manufacture therapeutic quantities of memory NK cells from HSCT donor or patient blood could overcome these hurdles in specific therapeutic settings, as described above.

*References:* 1. Sun and Lanier, Eur J Immunol. 2009; 39(8): 2059. 2. Boeckh and Geballe, J Clin Invest. 2011;121(5):1673. 3. Cichocki *et al.*, JCI Insight. 2019;4(2):e125553. 4. Haroun-Izquierdo *et al.*, JITC. 2022;10:e005577. 5. Bednarski *et al.*, Blood. 2022; 139(11):1670

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