Harm *et al.*: *Intrinsic Activity*, 2023; **11**(Suppl.1):A2.21
doi:10.25006/IA.11.S1-A2.21
published online: 22 September 2023

INTRINSIC ACTIVITY

27th Scientific Symposium of the Austrian Pharmacological Society

Vienna, 29–30 September 2023

MEETING ABSTRACT

A2.21

Uncovering new NK-cell checkpoints in the context of triple-negative breast cancer

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Background: Breast cancer is the most common cancer diagnosed in women worldwide. Screening programs for early detection of primary tumours are widely implemented and have improved the outcome of breast-cancer patients. However, metastasis to distant organs is the main cause of death of these patients, which underlines the need for novel therapeutic approaches. Natural killer (NK) cells are able to kill metastasizing cells and limit distant metastasis. However, their function is often suppressed by the cancer environment, for example through the activation of immune checkpoints. These immune checkpoints are key negative regulators of cytotoxic lymphocytes and are important targets for restoring their activity. Furthermore, immune checkpoint inhibitors started a new era of immune therapy. Although inhibition of the checkpoint pathway programmed cell death protein 1 (PD-1) / programmed cell death protein ligand 1 (PD-L1) was a breakthrough in many cancer types, its success in breast cancer remains limited. The goal of this project is to identify changes in the NK-cell receptor repertoire in the course of metastatic triple-negative breast cancer (TNBC).

Methods: We used a previously established mouse model for TNBC metastasis to obtain blood from metastasis-bearing mice at the timepoint of euthanization. Blood NK cells were subjected to single-cell RNA sequencing using the 10x Genomics technique, and to mass spectrometry to identify subpopulations with exhausted signatures. As breast cancer is known to modify NK cells, we expect to uncover new checkpoints, which, when inhibited, unleash NK-cell responses against metastasizing cells. Identified targets were validated *in vitro* and *in vivo* and blocking antibodies of these targets were used in the course of the metastasis mouse model, which should limit metastasis formation.

Results: An *in vitro* system for the co-cultivation of NK cells with TNBC cells was established to mimic the changes in NK-cell surface receptors in the course of TNBC. After co-cultivation of a human NK cell line and primary murine NK cells with the respective TNBC cell line, both human and murine NK cells showed decreased cytotoxicity. Co-cultivation of primary human NK cells with a TNBC cell line resulted in an altered surface receptor expression, which was also shown in TNBC patients.

Discussion: These results show that our co-cultivation system can be used as an *in vitro* tool to validate changes in NK-cell surface receptor expression.

Keywords: NK cells – triple-negative breast cancer – immune checkpoints

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