CAR-MEDIATED TARGETING OF NK CELLS OVERCOMES TUMOR IMMUNE ESCAPE CAUSED BY ICAM-1 DOWNREGULATION

1,2Jiri Eitler*, 2,3Wiebke Rackwitz, 4Natalie Wotschel, 4Venugopal Gudipati, 4Johannes Huppa, 2Paola Ortiz Montero, 5Laurent Boissel, 5Hans Klingemann, 6Winfried Wels, 2,3Torsten Tonn. 1Faculty of Medicine Carl Gustav Carus, Dresden, Germany; 2German Red Cross Blood Donation Service North-East, Dresden, Saxony, Germany; 3Faculty of Medicine Carl Gustav Carus, Dresden University of Technology, Dresden, Saxony, Germany; 4Institute for Hygiene and Applied Immunology, Medical University of Vienna, Vienna, Austria; 5ImmunityBio Inc, Culver City, CA, USA; 6Frankfurt Cancer Institute, Goethe University, Frankfurt am Main, Hesse, Germany

Background Antitumor activity of natural killer (NK) cells can be enhanced by specific targeting with therapeutic antibodies that trigger antibody-dependent cell-mediated cytotoxicity (ADCC) or genetic engineering with chimeric antigen receptors (CARs). Nevertheless, despite continued presence of the target antigen, some tumors can escape antibody or CAR-NK cell treatment, with the underlying mechanisms only poorly understood. While the importance of ICAM-1/LFA-1 interaction for natural cytotoxicity of NK cells has been previously shown, its impact on ADCC induced by the ErbB2 (HER2)-specific antibody trastuzumab and ErbB2-CAR-mediated cytotoxicity against breast cancer cells has not yet been investigated.

Methods NK-92 cells expressing high-affinity FcγRIIIa (haNK) in combination with trastuzumab or ErbB2-CAR engineered NK-92 cells (NK-92/5.28.z) as well as primary human NK cells combined with trastuzumab or modified with the ErbB2-CAR were employed to investigate the effects of ICAM-1 downregulation on breast cancer cells on NK cell cytotoxicity. Blockade of the ICAM-1/LFA-1 interaction significantly reduced cell killing and cytokine release during trastuzumab-mediated ADCC against breast cancer cells, while pretreatment with 5-aza-2' -deoxycytidine (5AZA) induced ICAM-1 upregulation and reversed NK cell resistance. In contrast, CAR-NK cell-mediated cytotoxicity did not rely on ICAM-1/LFA-1 interaction, and was not impaired by reduction of ICAM-1 expression on target cells or blockade of LFA-1 on NK cells (figure 1). In degranulation experiments, trastuzumab alone did not sufficiently activate NK cells but required additional LFA-1 stimulation, while activation of the ErbB2-CAR in CAR-NK cells induced efficient degranulation independent of LFA-1. TIRF single molecule imaging revealed that CAR-NK cells formed an irregular immunological synapse with tumor cells that excluded ICAM-1. Mechanistically, the absence of ICAM-1 did not affect cell-cell adhesion during ADCC but rather resulted in decreased signaling via Pyk2, which was restored by CAR-mediated targeting. Furthermore, while stimulation of the inhibitory NK cell checkpoint molecule NKG2A markedly reduced trastuzumab- and ICAM-1-mediated NK cell activation, CAR-NK cells were only marginally affected.

Conclusions We identified downregulation of ICAM-1 expression on breast cancer cells as a critical mechanism mediating escape from trastuzumab-triggered ADCC. Importantly, CAR-NK cells were able to overcome ICAM-1-based resistance as well as NKG2A-mediated inhibition, which may be relevant for the development of more effective NK cell-based cancer immunotherapies.

Ethics Approval Primary cells from healthy donors were obtained from the German Red Cross Blood Donation Service under an Ethics Review Board-approved protocol number EK138042014.

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Abstract 442-Q Figure 1 Schematic representation of the main findings: Downregulation or loss of ICAM-1 on cancer cells leads to escape from antibody-targeted cytotoxicity of NK cells (ADCC). This can be reversed by pre-incubation of cancer cells with 5-aza-2' -deoxycytidine (5AZA) and further enhanced by production of TNF-α by activated NK cells. In contrast, CAR-targeting of NK cells is independent of ICAM-1 expression levels. Mechanistically, CAR signaling bypasses the LFA-1 signaling through the Pyk2 pathway, resulting in efficient NK cell degranulation and killing of the otherwise resistant cancer cell.