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.

Results 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.