231 DIFFERENTIAL EFFECTS OF TARGET LIGANDS AND RECEPTOR ARCHITECTURE UPON NKG2D CAR T CELL ACTIVATION

[1]Jennifer Bolsee, [1]Caroline Lonez, [1]Benjamin Violle, [2]Sophie Agaugue, [1]Thuy Nguyen, [1]Jerome Marijsse, [1]Eytan Breman*. [1]*Celyad Oncology, Mont-Saint-Guibert, Belgium*; [2]*Imcheck Therapeutics, Marseille, France*

Background The NKG2D receptor binds to eight stress-induced ligands (NKG2DL): the major histocompatibility complex class I chain-related A and B (MICA/B) and the UL16 binding protein family (ULBP1–6). These ligands are absent from most normal tissues, but frequently expressed in various types of tumors, making NKG2D a promising tool for cancer immunotherapy. The binding affinity of NKG2D to all its ligands is not completely known, and it is likely that different ligands elicit distinct responses, moreover the high polymorphic nature of MICA/B implies different lytic consequences.

Methods We created different chimeric antigen receptor (CAR) T-cells containing the full length human NKG2D fused to the CD3zeta signaling domain (as a type I or II protein), and assessed the interaction of the NKG2D CAR T-cells with: i) cells combining differential levels of multiple-NKG2DLs or ii) cell lines expressing single NGK2DLs.

Results We first assessed the NKG2DLs expression in healthy and cancer tissues (from multiple solid tumor indications), where we could confirm expression was limited to cancer tissues (with no expression visible on normal healthy tissues), with differing staining patterns depending on the ligand. Next, we assessed the ability of CAR T-cells to bind to NKG2DLs and activate. Interestingly, while all CAR T-cells activated similarly, the levels of cytokine secretion was not similar between the NKG2DLs assessed, indicating that the NKG2DLs do lead to the same activation pathway but not to the same degree. Furthermore, in all instances the type I NKG2D secreted higher levels of cytokines in comparison to the type II. Next, we assessed cytolytic activity of NKG2D CAR T-cells against cancer cell lines expressing different NKG2DLs combination and could confirm a more pronounced role for MICA/B rather than ULBP1–6. Interestingle, even slight polymorphisms led to clear differences in both cytokine and lytic activity of NKG2D CAR T-cells. While, both type I/II led to potent cytolytic activity, cell persistence and proliferative capacity was clearly enhanced in the type I NKG2D.

Conclusions NKG2D-receptor architecture plays a major role in NKG2D CAR T mediated activity. Type I based structure enhanced cellular persistence and proliferation in comparison to type II, independent of the NKG2DLs. Furthermore, while all different polymorphisms of the NKG2DLs led to CAR T activity, the levels of CAR T activity were not similar between the different conditions. Indicating that affinity does most likely plays a role in CAR T-cell mediated activity.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0231