332 WU-NK-101, AN OFF-THE-SHELF MEMORY NK CELL PRODUCT, REVERSES IMMUNE CHECKPOINT INHIBITOR (ICB) RESISTANCE WITH SYNERGISTIC ANTI-TUMOR ACTIVITY

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Background ICB have shown remarkable clinical efficacy with durable responses in many cancers. However, many patients are resistant, or develop resistance to ICB. For these patients' treatment options are limited. Thus, treatments to overcome ICB resistance are necessary.

WU-NK-101 (WUNK) is a PBMC-derived, cytokine-reprogrammed, expanded, and cryopreserved off-the-shelf memory-like (ML) NK cell product. WUNK exhibits enhanced cytotoxicity, metabolic fitness/flexibility, and resistance to tumor microenvironment (TME) immunosuppression compared to conventional NK cells.[1 2] WUNK cells are active against multiple solid tumor cell lines *in vitro* and demonstrate additive cytotoxicity in combination with ICB. Furthermore, ML-NK cell treatment *in vivo* promotes T cell migration/activation in the TME, IFN-γ and chemokine release, and MHC-I and PD-L1 upregulation on residual tumor cells.[3]

Methods Herein, we investigate the utility of WUNK in reversing ICB, namely pembrolizumab, resistance. We report that WUNK therapy rescues pembrolizumab failure and show that combination of WUNK and ICB synergize leading to increased cytotoxicity in a novel xenograft mouse model of immune escape.

Results MDA-MB-231 TNBC cells and human PBMC's were co-engrafted in NSG MHC-I/II dKO mice. At baseline 88.7 ±02% of tumor cells expressed MHC-I molecules and 7.6 ±0.4% were positive for PD-L1. Tumor engraftment was noted on day 2, while the presence of hPBMC in the circulation was confirmed by D7 with continued expansion (1000-fold) through day 40. Initial tumor control, ~40-fold decrease in tumor burden, was noted between D7–16. However, after two weeks, tumor growth resumed and correlated with a 68% decrease in MHC-1 and ~52% increase of PD-L1 positivity in tumor cells indicating escape from immune surveillance. Administration of pembrolizumab (5mg/kg dose) at the time of immune evasion (D18) resulted in tumor growth inhibition (*TGI=13.8%; f*igure 1). Remarkably, co-administration of WUNK with pembrolizumab resulted in a synergistic activity, significantly reducing tumor growth (*TGI=94.4%; f*igure 1) and improving overall survival. Further histological analysis of tumor tissue confirmed enhanced infiltration of T-cells, and interaction between ML-NK cells within the TME.

Conclusions In summary, using a novel model of ICB resistance, our study demonstrates that treatment with WUNK led to increased infiltration and activation of T-cells into the TME. Furthermore, WUNK synergized with ICB to enhance anti-tumor activity and rescue pembrolizumab resistance leading to a durable adaptive response. Overall, these findings provide a strong rationale for exploring the combination of WUNK with ICBs clinically to overcome immune evasion and improve patient outcomes.

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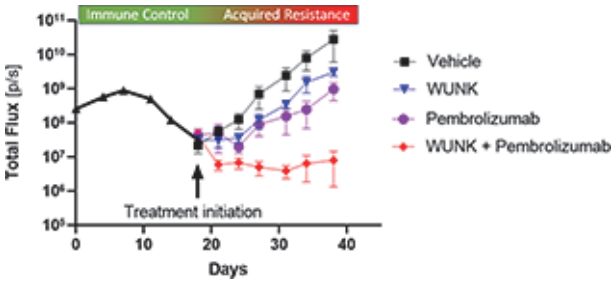

Abstract 332 Figure 1 In vivo xenograft model of immune surveillance and escape. Engrafted human PBMCs initially control MDA-MB-231 tumor growth before immune evasion occurs (Day 18) and tumor growth resumes. Treatment initiation at the time of immune evasion resulted in synergistic anti-tumor activity when WUNK was combined with Pembrolizumab compared to each delivered as a monotherapy.

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