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WU-NK-101, A Best-in-Class Memory NK cell, Has Key Features Vital for Adoptive Cell Therapy (ACT) in Solid Tumors (ST)

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Introduction

Desirable features for successful ACT include:

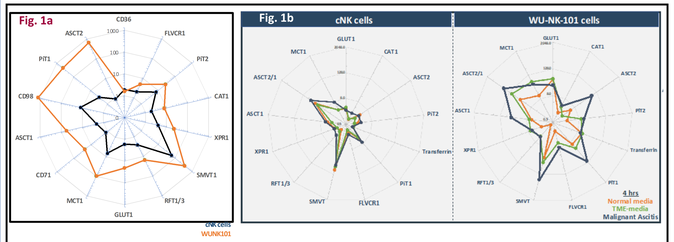
1. Effective migration into tumors.
2. Overcoming immunosuppression and dearth of nutrients to survive/function within the tumor microenvironment (TME).
3. Not restricted by antigen exclusivity/impervious to antigen escape.
4. Engagement to the recipient's immune system.

- While engineering is a solution to many of these limitations, WU-NK-101(WUNK) is a non-engineered cytokine-reprogramed, expanded, cryopreserved off-the-shelf NK cell product derived from PBMCs, with a specialized phenotype to inherently overcome those challenges.
- We have previously shown that WUNK traffic to and persist in tumor models, which is enhanced by mAb combination. Additionally, compared to conventional NK(cNK), WUNK demonstrated improved cytotoxicity[A549 (NSCLC): 0% *vs.* 79%, *p=0.0001* and LoVo (CRC) 0% *vs.* 57%, *p=0.01*, respectively] (Rutella *et al.*,ESMO 2022).

Methods

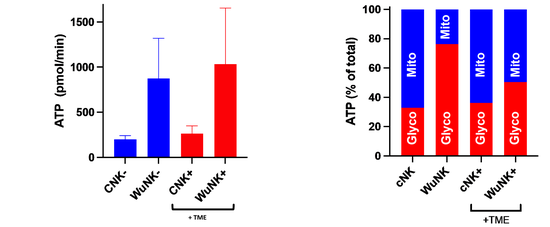
- Assays were performed in conventional (N) media (pH 7, glucose 11 mM), or two TME models (TME- aligned media - pH 5.9, glucose 6 mM, immune suppressive agents, *e.g.*, PGE2, TGFb1, and ascites derived from patients with malignancy.
- Cell phenotypes were evaluated under different media conditions using flow cytometry.
- Bioenergetics assays (Seahorse XF, Seahorse T Cell Metabolic Profiling Kit; Agilent) under different media conditions.
- Evaluated cytotoxicity under different media conditions using 2D and 3D models.
- 3D cytotoxicity was assessed against primary CRC surgical samples. Tumor samples were mechanically digested, followed by chemical digestion (cocktail of collagenases and DNase enzymes were added to promote matrix digestion). The final cell pellet was resuspended in tumor growth media and embedded in a 3D matrix (Cultrex® Basement Membrane Extract - laminin, collagen IV, entactin, and heparan sulfate proteoglycans) that mimics in vivo tumor microenvironment.
- Xenograft models were established by intravenous (IV) administration of MDA-MB-231 (TNBC). Engraftment of humanized immune system was achieved by intraperitoneal administration of hPBMC's. WUNK treatment was administered by intravenous injection and tumor growth was determined over time by bi-weekly bioluminescent imaging.
- Biopsies from patients that received CIML-NK (Cytokine Induced Memory NK cells) on study NCT01898793 were interrogated for immune cell infiltration by immunohistochemistry (IHC).

Results

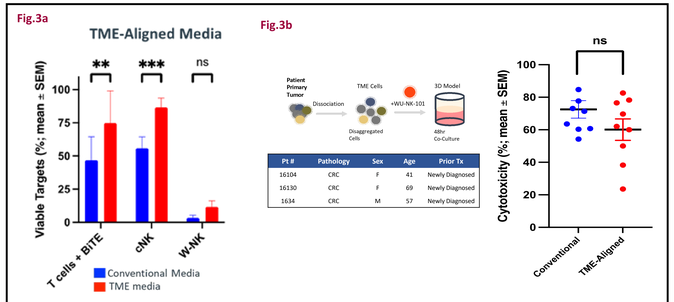


WUNK express higher levels of cell surface nutrient transporters.

Data summarized in a radar plot (**Fig. 1a**) compared to conventional (c)NK cells. When exposed to different models of TME (TME aligned media, or malignant ascites) WUNK adapted to nutrient content of the media by modifying the receptor expression in contrast to cNK. The WUNK phenotype exhibits improved nutrient utilization in nutrient-poor environments, suggesting metabolic flexibility.

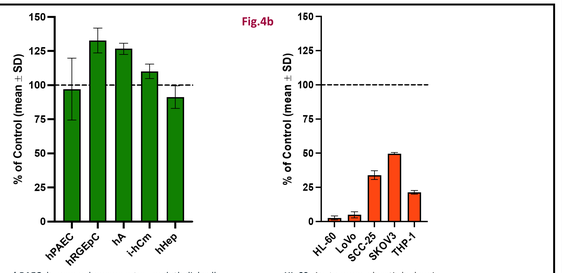


WUNK showed robust ATP production, compared to cNK cells in both N- and TME-media (**Fig. 2a**). ATP production was mainly glycolytic, as previous described by Warburg (**Fig. 2b**). In hypoglycemic TME-aligned media, WUNK metabolism shifted from glycolytic to mitochondrial ATP production, suggesting metabolic resilience.



Cytotoxic activity was preserved in TME-aligned media (acidic and hypoglycemic-conditioned media) compared to cNK and T-cells (WUNK: 96.5% vs. 88.2%, *p=0.45*; cNK: 44.1% *vs.* 13.5%, *p=0.009;* T-cell: 53.2 % *vs.* 25.2%, *p=0.041*, TME *vs*. conventional media, respectively; **Fig. 3a**). Resistance to TME was further confirmed in native-TME-aligned 3D assays from primary tumor surgical samples (**Fig. 3b**).

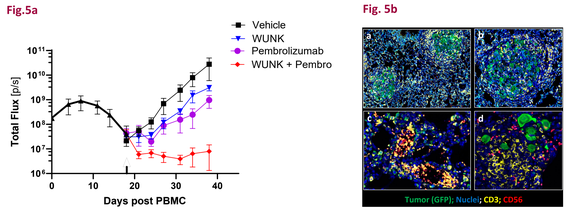
Results



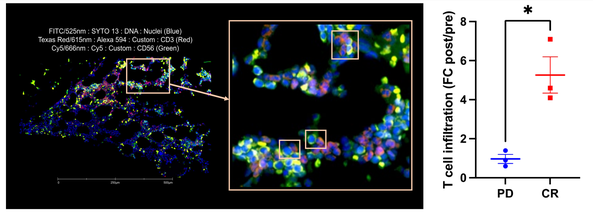
hPAEC: human pulmonary artery endothelial cells
hRGEpC: human renal glomerular epithelial cel
hA: human astrocytes
i-hCm: iPSC-derived human cardiomyocyte
hHep: human hepatocytes

HL-60: Acute promyelocytic leukemia
LoVo: Colorectal adenocarcinoma
SCC-25: Squamous Cell Carcinoma of the head and neck
SKOV3: Ovarian adenocarcinoma
THP-1: monocytic leukemia

WUNK demonstrate cytotoxicity against malignant, but not normal human cells. A panel of malignant and normal human cells were made to express NucLight Red or stained with CytoLight Rapid Red Dye to enable IncuCyte-based tracking. Target cells were co-incubated with WUNK at 9:1 effector to target (E:T) ratio for 72 hours and remaining target cells were analyzed by an IncuCyte Live-Cell Analysis System. Data are expressed as mean ± SEM; n = 6.



WUNK and T-cell activity synergize to control tumor growth. MDA-MB-231 (TNBC) were engrafted into humanized mice. When administered at the point of immune escape (D18), WUNK and pembrolizumab administered as a monotherapy provided modest tumor control. However, when WUNK was administered in combination with pembrolizumab, synergistic control over tumor growth was observed which translated into increased survival (**Fig. 5a**). IHC demonstrated increased T-cell infiltration into the TME, and tumor control when combined with WUNK in a humanized mouse model (**Fig. 5b**), suggesting that WUNK and T cells synergize to elicit anti-tumor activity.



Treatment with CIML-NK increased T-cell infiltration in TME. Post CIML-NK immunofluorescent images (GeoMx DSP platform; NanoString Technologies) of patient BM biopsy [CD123 (Green) CD3 (Red) and nuclei (blue)]. D28 showed increased CD3 infiltration and clustering within TME, which was associated with improved outcomes.

Phase 1b Study of WU-NK-101 with Cetuximab for Advanced and/or Metastatic Colorectal Cancer (CRC) and Advanced and/or Metastatic Squamous Cell Carcinoma of Head and Neck (SCCHN)

Objectives

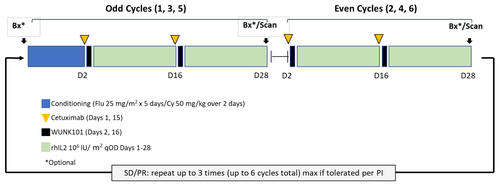
1. To characterize the safety, tolerability, DLT, and MTD or MAD and define the recommended Phase 2 dose.
2. To investigate the preliminary anti-tumor activity (ORR, and PFS).

Inclusion Criteria

1. Patients must have a histologically confirmed diagnosis of advanced and/or metastatic CRC that has failed or progressed beyond first or higher line standard of care therapy including cetuximab combination, 5-FU based regimens, or checkpoint inhibitors alone or in combination.

Or...

2. Patients must have a histologically confirmed diagnosis of SCCHN that has failed or progressed beyond first or higher line standard of care therapy including cetuximab alone or in combination, regimens that include checkpoint inhibitors alone and in combination.



Conclusions

- WUNK expresses higher level of cell surface nutrient transporters which adapt to nutritional content of the TME, suggesting **metabolic adaptability**. WUNK showed robust ATP production which was mainly glycolytic in N-media. In hypoglycemic media, ATP production shifted from glycolytic to mitochondrial ATP production, suggesting **metabolic resilience**. This was further highlighted by a shift from glucose to amino acid catabolism, suggesting **metabolic flexibility** (Rutella *et al.*, ESMO 2022).
- WUNK may overcome antigen loss, a common challenge limiting ACT efficacy, owing to the multiple mechanisms of target antigen recognition by NK cells. Furthermore, WUNK did not induce cytotoxicity of healthy human tissues, including PBMCs, indicating that WUNK are not indiscriminate killers. This observation may predict low myelosuppression and a favorable safety profile.
- Memory NK cells may potentiate the recipient's immune system offering the prospect of long-term anti-tumor activity.
- In summary, we show that WUNK cells exhibit features that overcome challenges for ACT and enhance anti-tumor activity, suggesting the promise of NK cell therapy for solid tumor indications.
- The presented data augurs positively for patients to be treated in the upcoming Phase 1/b clinical study (NCT#05674526) and may reshape adoptive cellular therapy in colorectal carcinoma and other EGFR-expressing tumors.

Conflict of Interest

John Muth employee of Wugen Inc.

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