WU-NK-101, An Allogeneic Memory NK Cell, for the Treatment of Relapse or Refractory (R/R) Acute Myeloid Leukemia (AML)

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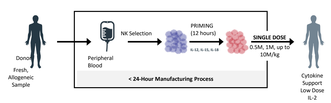
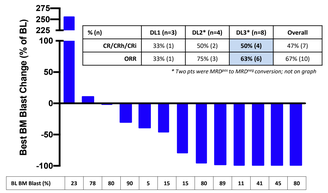
INTRODUCTION

- Memory NK cells have emerged as being well tolerated with demonstrated clinical activity in the treatment of AML
- Memory NK cells are significantly more potent than conventional NK cells (cNK), with enhanced activity and functional persistence
- WU-NK-101 is an "off-the-shelf" Memory NK cell therapy product derived from healthy donor cells, generated using Wugen's proprietary MONETA™ process and scaled for commercial utility

Cytokine-induced Memory-like NK Cells in Patients With Acute Myeloid Leukemia (AML) or Myelodysplastic Syndrome (MDS) NCT#01898793



Treatment Related Adverse Events (TRAEs)	Population (n=15)*	
	All [n(%)]	Grade ≥ 3 [n(%)]
Increased bilirubin	1 (6.7%)	0 (0%)
Sinus tachycardia	1 (6.7%)	0 (0%)
Hypoxia	1 (6.7%)	0 (0%)
Dyspnea	1 (6.7%)	0 (0%)
Confusion	1 (6.7%)	0 (0%)
Lethargy	1 (6.7%)	0 (0%)
Chills	1 (6.7%)	0 (0%)
Anorexia	1 (6.7%)	0 (0%)
Fatigue	1 (6.7%)	0 (0%)
Alopecia	1 (6.7%)	0 (0%)



% (n)	DL1 (n=3)	DL2* (n=4)	DL3* (n=8)	Overall
CR/CRh/CRi	33% (1)	50% (2)	50% (4)	47% (7)
ORR	33% (1)	75% (3)	63% (6)	67% (10)

* Two pts were MRD^{neg} to MRD^{pos} conversion; not on graph

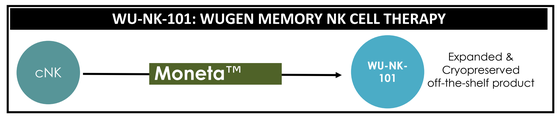
Learnings

- Well-tolerated and safe
  - **No** TRAEs Grade ≥ 3, No CRS, no ICANS, No GvHD
- Active in vivo against AML

Limitations

- One donor to one patient
- Only single dose possible for most patients
- Limits a comprehensive treatment paradigm

Berrien-Elliott *et al. Cancer Discov* (2020) 10 (12): 1854–1871



Previously frozen NK cells were derived from healthy donor whole blood and activated and expanded using proprietary process (MONETA) and cryopreserved to get an off-the-shelf NK cell product

OBJECTIVE

To characterize WU-NK-101 and evaluate its anti-tumor activity in preclinical models of AML

METHODS

- WU-NK-101 cells were phenotypically characterized using multidimensional mass cytometry (CyTOF)
- Functionality was evaluated using in vitro cytotoxicity assays against AML cell lines (TF-1, THP-1, and HL-60)
- Cells were characterized in conventional and tumor microenvironment (TME)-aligned media
- Proteomic analysis of WU-NK-101 was performed using tandem-mass spectrometry
- In vivo efficacy was evaluated in NSG mice bearing THP-1 AML tumors

CONTACT INFORMATION

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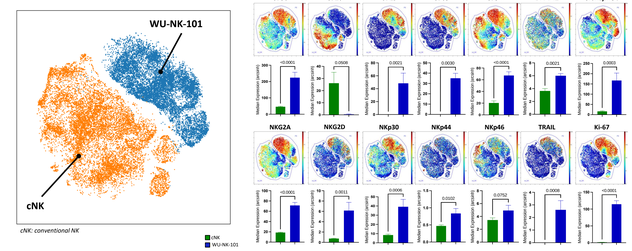
RESULTS

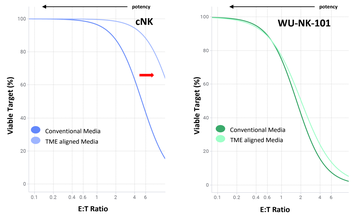
WU-NK-101 has a Unique Phenotype

WU-NK-101 is poised for improved activation and anti-tumor activity

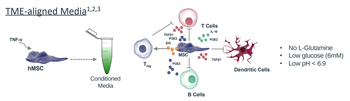


Viably frozen WU-NK-101 (n=4) and purified NK cells from healthy donors (n=8) were thawed and assessed by mass cytometry. Briefly, cells were stained for viability using cisplatin (Enzo Life Sciences), according to standard protocol. NK cells were identified as CD45^{+}CD14^{-}CD19^{-}CD3^{-}CD56^{+} and median expression of the indicated markers assessed.

WU-NK-101 Cytotoxicity is not Hampered in Adverse/Immunosuppressive TME



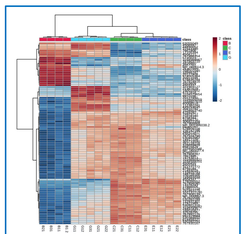
WU-NK-101 cytotoxicity is not hampered by adverse TME as compared to cNK cells; mEC50 2.0 vs 16.3 (*p= 0.018)*, respectively.



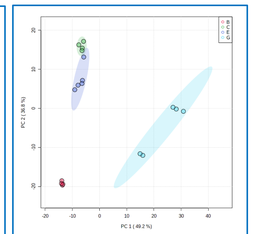
Conventional NK cells (CD3^{neg}/CD56^{pos}) and paired WU-NK-101 cells were generated from healthy donors (n=5) and frozen. Cells were then thawed and co-cultured with HL-60 cells at various E:T ratios for 48 hours. Percent viable, was normalized to control (no NK added). TME-aligned media is an acidic, and hypoglycemic conditioned media obtained from TNF-α-stimulated human mesenchymal stem cells (hMSC), and contains high levels of nitric oxide, PGE2, IDO, IL-10, TGFb1, and other immune suppressive agents, as previously published[1,2,3]. *(mean fits represented in the graphs)*

Samples analyzed by proteomics	
Group B	HL-60
Group C	WU-NK-101 conventional media
Group E	WU-NK-101 + HL-60 co-cultured in **conventional media**; magnetic CD56+ NK isolation
Group G	WU-NK-101 + HL-60 co-cultured in **TME-aligned media**; magnetic CD56+ NK isolation

6654 proteins identified at 1% FDR

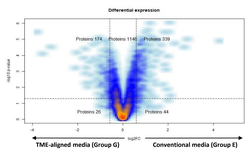


Unsupervised clustering shows grouping of samples based on culturing conditions



Principal component analysis distinguishes differences in protein expression in these 4 groups

KEGG Pathway Analysis Shows Enrichment of Distinct Metabolic Pathways Upon WU-NK-101 Activation in Conventional vs. TME-Aligned Media

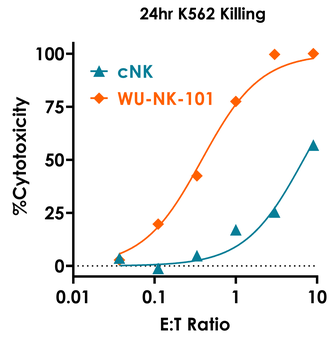


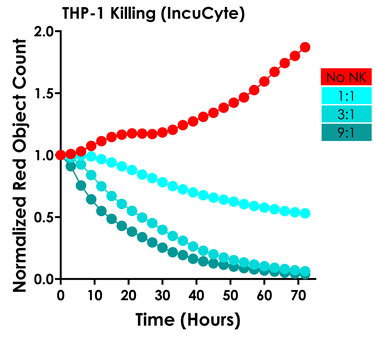
P-Value 0.05;
Fold Change 1.5

Enriched Metabolic Pathways – TME aligned media	adj .p-value
Steroid biosynthesis	0.0019
Folate biosynthesis	0.0047
Amino sugar and nucleotide sugar metabolism	0.0049
Metabolic pathways	0.0051
Alanine, aspartate and glutamate metabolism	0.0087
D-Glutamine and D-glutamate metabolism	0.0109
Nitrogen metabolism	0.0320

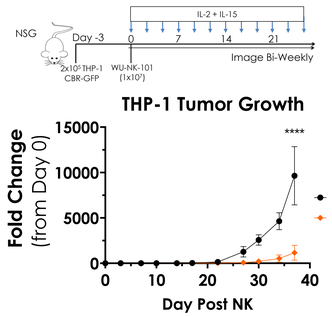
Enriched Metabolic Pathways –conventional media	adj .p-value
Pentose phosphate pathway	0.0003
Glycolysis / Gluconeogenesis	0.0020
Amino sugar and nucleotide sugar metabolism	0.0310
Metabolic pathways	0.0339
Fructose and mannose metabolism	0.0339

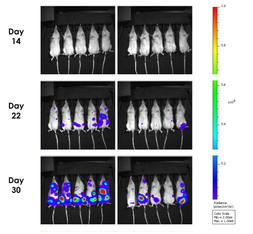
WU-NK-101 Exhibits Robust *In-vitro* and *In-vivo* Activity Against Myeloid Leukemia Models





NK cells were assessed for cytotoxic capacity in vitro using luciferized K562 or NucLight Red-labeled THP-1 cells at the indicated E:T ratios.





NK cells were assessed for efficacy in THP-1-bearing NSG mice, as described in the figure above. A subgroup was also dosed multiple times, with improved efficacy. Tumor growth was measured by BLI.

CONCLUSIONS

- WU-NK-101 cells have unique phenotype indicative of improved activation and cytotoxicity
- In culture conditions that mimic the TME, an immunosuppressive, acidic, and hypoglycemic environment, cNK cell function was strongly inhibited, while WU-NK-101 maintained its enhanced cytotoxic activity
- Proteomic analysis revealed that glucose metabolism pathways were augmented at basal state in conventional media, whereas amino acid metabolism was upregulated in hypoglycemic and nutrient depleted TME conditions, suggesting that WU-NK-101 cells are highly resistant to immunosuppression and can overcome adverse TME
- In vivo activity of WU-NK-101 was confirmed in an AML THP-1 xenograft model, where a single dose effectively reduced tumor burden relative to vehicle controls
- Repeated dosing further enhanced efficacy in vivo. The data presented here augur well for R/R AML patients who will be treated in an upcoming clinical study, WUN101-01
- WU-NK-101 overcomes several limitations associated with NK cell therapy in AML and furthermore displays enhanced trafficking, metabolic fitness and functionality within the TME

REFERENCES

1) Samsonraj *et al.* Stem Cells Transl Med. 2017 Dec;6(12):2173-2185. 2) Sasser *et al.* Cancer Lett. 2007 Sep 8;254(2):255-64. 3) Studebaker *et al.* Cancer Res. 2008 Nov 1;68(21):9087-95. 4. Sato *et al.* Clin Cancer Res 2020;26:2573-2581