# PUTATIVE PREDICTORS OF RESPONSE TO WU-NK-101, AN ALLOGENEIC, ENHANCED MEMORY (ML) NATURAL KILLER (NK) CELL THERAPY PRODUCT, FOR RELAPSED/REFRACTORY (R/R) ACUTE MYELOID LEUKEMIA

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## INTRODUCTION

- Immune effector cells in the tumor microenvironment (TME), including CD8+ T cells and NK cells, are dysfunctional
- Strategies to enhance NK-cell activity prior to adoptive transfer into patients with AML have yielded inconsistent clinical benefit
- Cytokine-induced memory-like (CIML) NK cells have shown clinical activity in patients with relapsed/refractory AML, with a CR/CR with partial/incomplete hematologic recovery rate of 47% and a median duration of response of 9.4 months after a single infusion
- WU-NK-101 are activated to promote a cytokine-induced memory-like gene expression profile, expanded, and frozen to create an off-the-shelf cell therapy

## AIM

- To identify bone marrow gene expression signatures associated with outcome after the infusion of cytokine-induced memory-like NK cells in 15 patients with R/R AML (**NCT#01898793**)
- To functionally characterize WU-NK-101, a novel cell therapy derived from healthy-donor NK cells

## METHOD

- Bone marrow (BM) aspirates and trephine biopsies for immune GE profiling (IO360® panel, n = 750 genes; NanoString Technologies) were collected at baseline (BL) and on treatment (OT) from 15 patients who received CIML-NK cells on study **NCT#01898793**
- Machine learning was used to select “features” (genes) correlating with response
- Cell phenotypes were evaluated using flow and mass cytometry
- NK-cell cytotoxicity was assayed in either conventional (N-; 20% IMDM) or TME-aligned media
- Protein expression was studied using mass spectrometry-based proteomics
- Cell trafficking to the TME was measured by tracking fluorescently-labeled WU-NK-101 cells in NSG mouse tumors

## RESULTS

We initially profiled 15 bone marrow samples on the nCounter® platform from NanoString Technologies (Seattle, WA) and we used machine learning (LASSO regularized regression) to select a parsimonious gene set that correlated with response to NK cells (**Fig. 1**).

The gene expression score was significantly higher at baseline in responders to NK therapy and correlated with the duration of response (Fig. 2A). Next, we inferred the abundance of 22 immune cell types leveraging immune deconvolution with CIBERSORT. This analysis showed a higher frequency of both innate and adaptive immune cells in responders (**Fig. 2B**).

Metabolic profiles reflecting enhanced glucose and amino acid consumption were significantly enriched in on-treatment BM samples from responders as compared to non-responders (**Fig. 3**).

We next aimed to characterize the phenotype and function of WU-NK-101. **Fig. 4** shows that WU-NK-101 expressed higher levels of activation and cytotoxicity markers as compared to conventional CD3$^{neg}$CD56$^{pos}$ NK cells. The immune phenotype of WU-NK-101 is consistent with the memory-like phenotype that has been associated with long-term in vivo persistence and enhanced *in vivo* functionality.

WU-NK-101 also up-regulated amino acid, glucose and nutrient transporters as compared to conventional NK cells, as summarized in this radar plot, suggesting enhanced metabolic adaptability (**Fig. 5**).

Finally, when tested in an NSG mouse model bearing SKOV3 tumors, WU-NK-101 migrated to the tumor bed and to the bone marrow. As highlighted in panels C and D, bone marrow engraftment at 24 hours averaged 16.5% in our model. These data favorably compare with a recent publication where only 3% of infused NK cells localized to the BM one day following transfer (**Fig. 6**).

A study evaluating WU-NK-101 in R/R AML will start enrollment in Q1-2023. The study design is presented in **Fig. 7**.

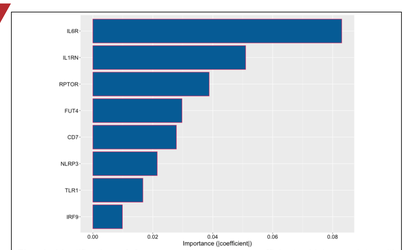



*Fig. 1. Identification of 8 genes with non-zero coefficient which correlate with response to CIML-NK cells (glmnet package in R). Genes are ranked by variable importance (VIP).*

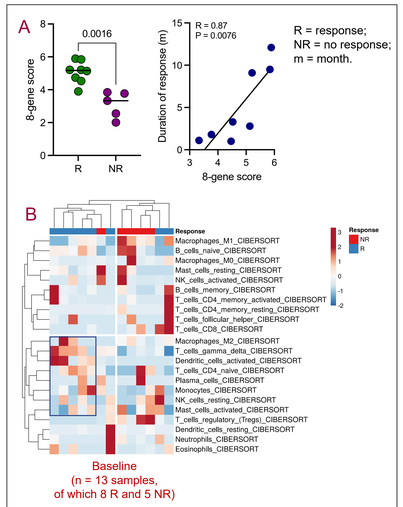



*Fig. 2. **Panel A**: Correlation between the 8-gene LASSO score and duration of response to CIML-NK cells. **Panel B**: Immune deconvolution of the TME with CIBERSORT (unsupervised hierarchical clustering; Euclidean distance). The blue box highlights immune cell types that were more abundant in responders compared to non-responders.*

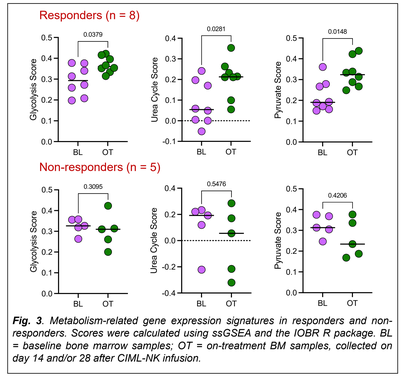



*Fig. 3. Metabolism-related gene expression signatures in responders and non-responders. Scores were calculated using ssGSEA and the IOBR R package. BL = baseline bone marrow samples; OT = on-treatment BM samples, collected on day 14 and/or 28 after CIML-NK infusion.*

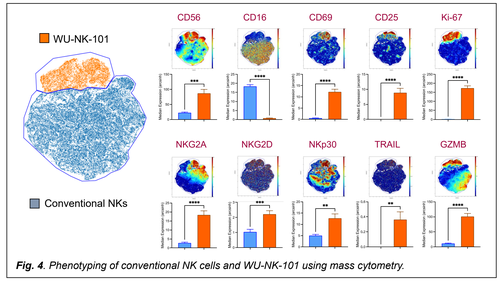



*Fig. 4. Phenotyping of conventional NK cells and WU-NK-101 using mass cytometry.*

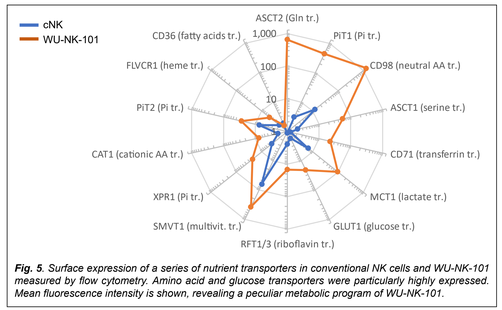



*Fig. 5. Surface expression of a series of nutrient transporters in conventional NK cells and WU-NK-101 measured by flow cytometry. Amino acid and glucose transporters were particularly highly expressed. Mean fluorescence intensity is shown, revealing a peculiar metabolic program of WU-NK-101.*

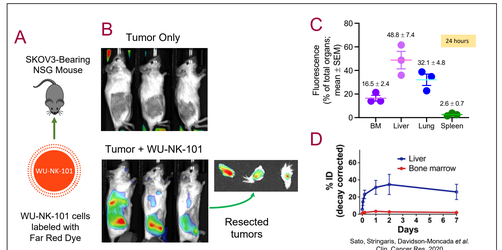
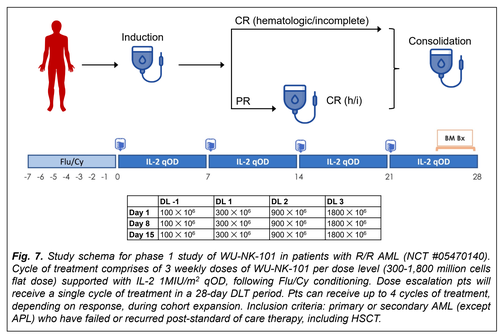



*Fig. 6. **Panel A**: Establishment of SKOV3 tumors in NSG mice. **Panel B**: Adoptive transfer of Far Red Dye-labeled WU-NK-101 and their localization to resected tumors. **Panel C**: Trafficking and localization of WU-NK-101 to the bone marrow and tumor bed. **Panel D**: Benchmarking of WU-NK-101 homing to the TME against recently published data.*




| | DL -1 | DL 1 | DL 2 | DL 3 |
|---|---|---|---|---|
| Day 1 | 100 × 10⁶ | 300 × 10⁶ | 900 × 10⁶ | 1800 × 10⁶ |
| Day 8 | 100 × 10⁶ | 300 × 10⁶ | 900 × 10⁶ | 1800 × 10⁶ |
| Day 15 | 100 × 10⁶ | 300 × 10⁶ | 900 × 10⁶ | 1800 × 10⁶ |

*Fig. 7. Study schema for phase 1 study of WU-NK-101 in patients with R/R AML (NCT #05470140). Cycle of treatment comprises of 3 weekly doses of WU-NK-101 per dose level (320-1,800 million cells flat dose) supported with IL-2 1MIU/m² qOD, following Flu/Cy conditioning. Dose escalation pts will receive a single cycle of treatment in a 28-day DLT period. Pts can receive up to 4 cycles of treatment, depending on response, during cohort expansion. Inclusion criteria: primary or secondary AML (except APL) who have failed or recurred post-standard of care therapy, including HSCT.*

## CONCLUSIONS

- An 8-gene tumor microenvironment immune signature showed excellent predictive ability for response to CIML-NKs
- In responders, CIML-NK infusion was associated with tumor microenvironment modulation and with metabolic re-programming
- Compared to cNK cells, WU-NK-101 had enhanced anti-tumor activity, trafficked to the BM, and showed metabolic flexibility, potentially mitigating the adverse effects of the highly immuno-suppressive AML tumor microenvironment
- WU-NK-101 may represent an effective treatment modality for relapsed/refractory AML
- A phase 1 study of WU-NK-101 is being developed in this setting

## REFERENCES

Berrien-Elliott MM, Cashen AF, Cubitt CC, **et al**. Multidimensional analyses of donor memory-like NK cells reveal new associations with response after adoptive immunotherapy for leukemia. *Cancer Discov*. 2020; 10 (12):1854-71.

Sato N, Stringaris K, Davidson-Moncada JK, **et al**. In vivo tracking of adoptively transferred natural killer cells in Rhesus macaques using $^{89}$Zirconium-Oxine cell labelling and PET imaging. Clin. Cancer Res. 2020; 26: 2573-81.

Rutella S, Vadakekolathu J, Mazziotta F, **et al**. Immune dysfunction signatures predict outcomes and define checkpoint blockade-unresponsive microenvironments in acute myeloid leukemia. *J. Clin. Invest.* 2022; 132 (21): e159579.

Vadakekolathu J, Minden MD, Hood T, **et al**. Immune landscapes predict therapeutic resistance, immunotherapy response and clinical outcomes in acute myeloid leukemia. *Sci. Transl. Med.* 2020; 12: eaaz0463.

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