



# WU-NK-101, an Enhanced Memory Natural Killer (NK) Cell Therapy, with Cetuximab (Ctx) for the Treatment of Advanced Colorectal Cancer (CRC)

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## INTRODUCTION

- CRC is the 4<sup>th</sup> leading cause of global cancer-related deaths, and novel therapeutic strategies for advanced CRC are urgently needed.
- Adoptive cell therapy (ACT) is effective in treating hematological malignancies; however, ACT in solid tumors is hindered by:
  1. **Target antigen identification.**
  2. **Survival in the tumor microenvironment (TME) due to immunosuppressive signals and scarcity of nutrients.**
  3. **Restricted migration into tumors.**
- NK cells are central to anti-tumor immunity and can directly eliminate tumor cells without prior sensitization. Through cytokine reprogramming, NK cells gain memory-like (ML) features that augment their anti-tumor potential.
- WU-NK-101 is a cytokine-reprogrammed, expanded, cryopreserved, off-the-shelf NK cell product derived from peripheral blood mononuclear cells, with no additional engineering.

## AIM

- To advance an adoptive cell and immuno-oncology combination therapy for the treatment of advanced and/or metastatic colorectal cancer (**NCT#XXXXXX**)

## METHODS

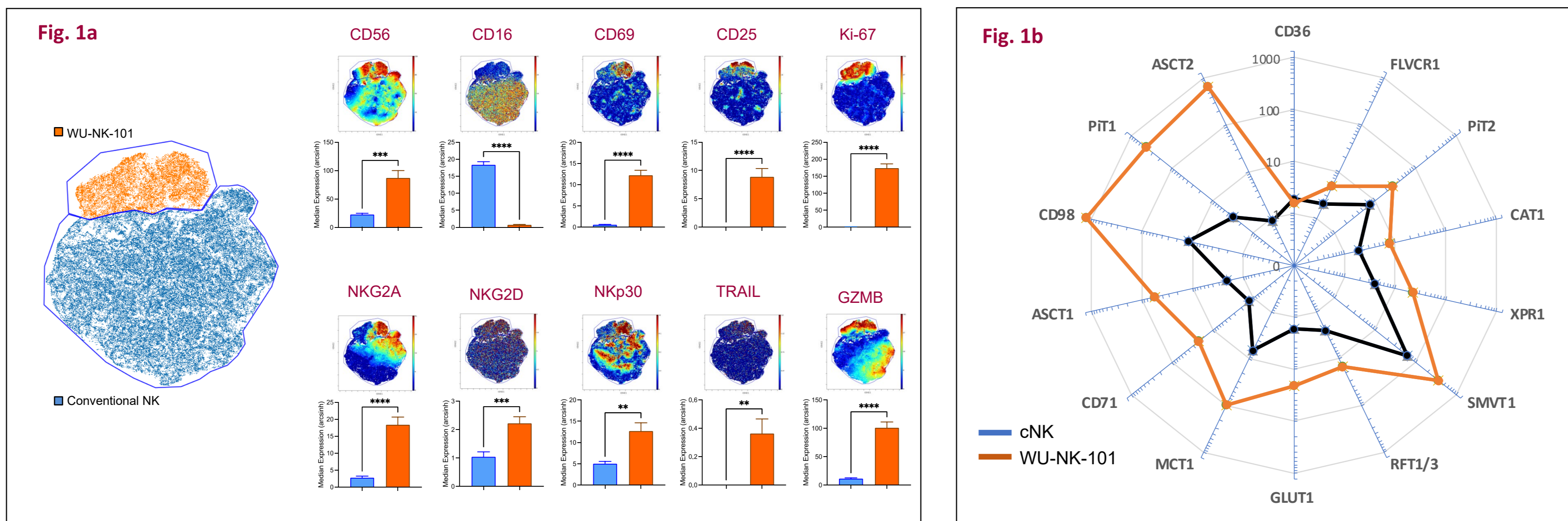
- Cell phenotypes were evaluated using flow and mass cytometry.
- Proteomic analysis was performed using tandem-mass spectrometry.
- Cell trafficking/penetration to the TME was measured by tracking fluorescently-labeled WU-NK-101 cells  $\pm$  monoclonal antibody (mAb) in NSG mouse bearing subcutaneous xenografts tumors.
- WU-NK-101  $\pm$  cetuximab (Ctx) was evaluated in vitro in 2D cytotoxicity assays in conventional (N; 20% IMDM) or TME-aligned media.
- In vivo efficacy of WU-NK-101  $\pm$  Ctx was evaluated in NSG mice bearing LoVo xenograft CRC tumors.
- WU-NK-101 cytotoxicity was further assessed against primary CRC surgical samples in native-TME-aligned 3D assays.

## REFERENCES

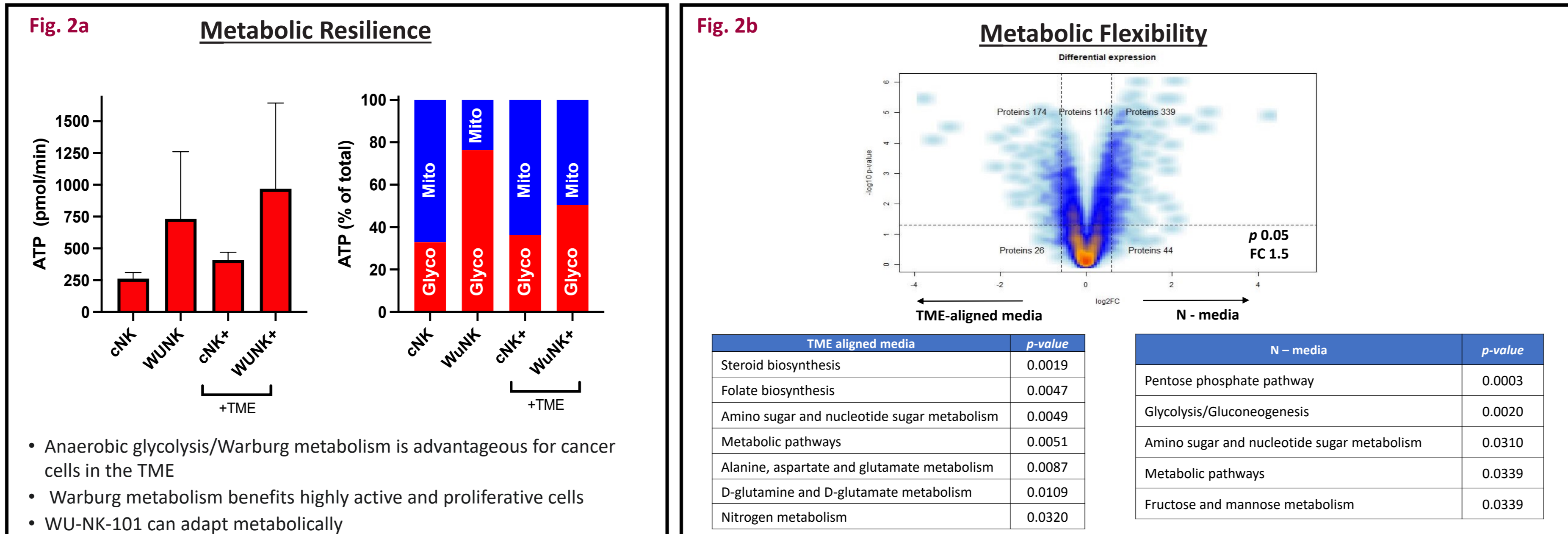
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## RESULTS

### WU-NK-101 has a unique phenotype optimized for robust cytotoxicity, metabolic flexibility and resistance to adverse and immune suppressive TME

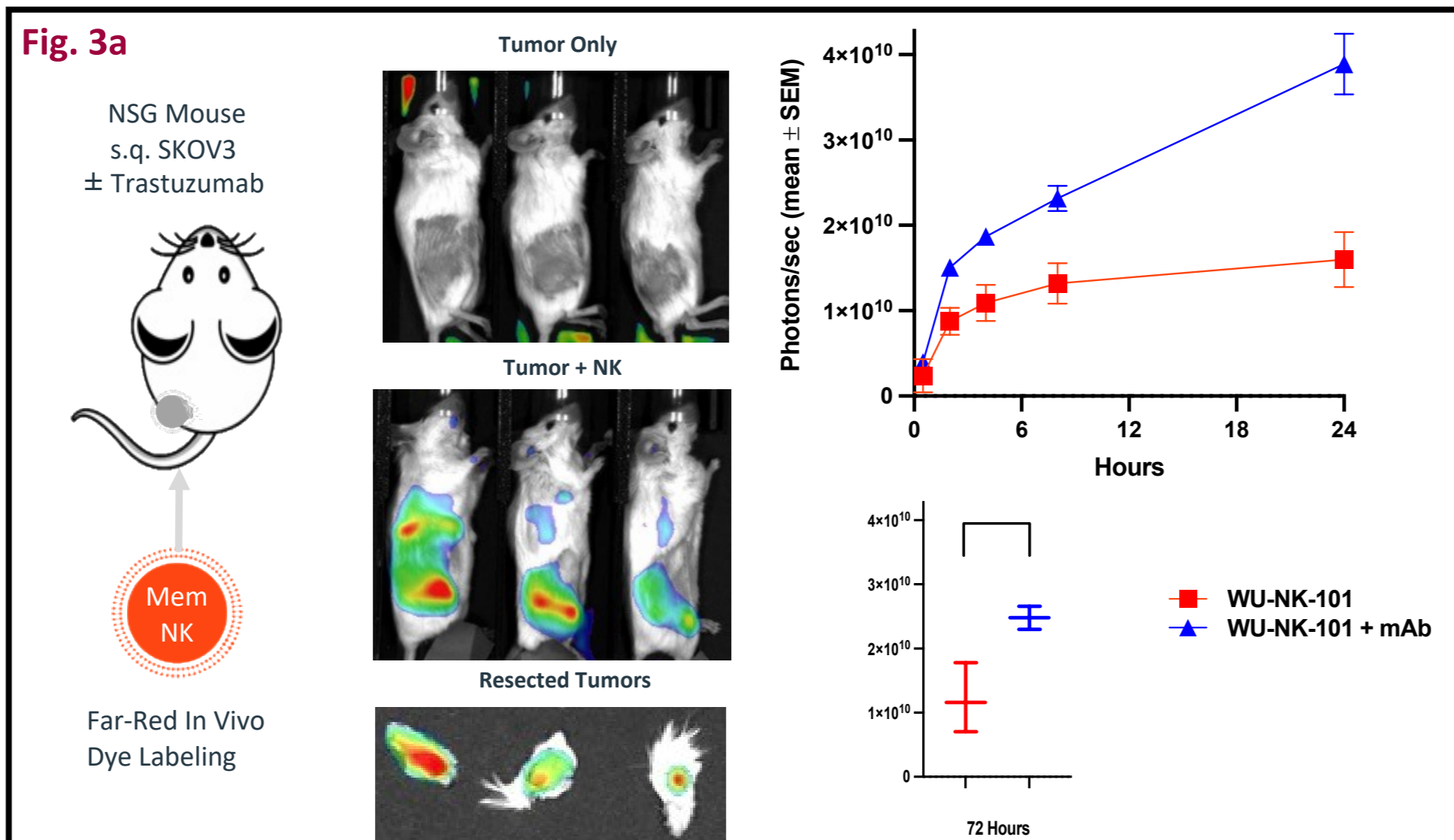


WU-NK-101 expressed higher levels of activation markers (Fig. 1a), high levels of cell surface nutrient transporters as summarized in this radar plot, (Fig. 1b), compared to conventional (c)NK cells. This phenotype is consistent with the ML NK phenotype that has been associated with persistence and enhanced functionality in vivo. Furthermore, this phenotype exhibits improved nutrient utilization in nutrient-poor environments, suggesting metabolic flexibility.

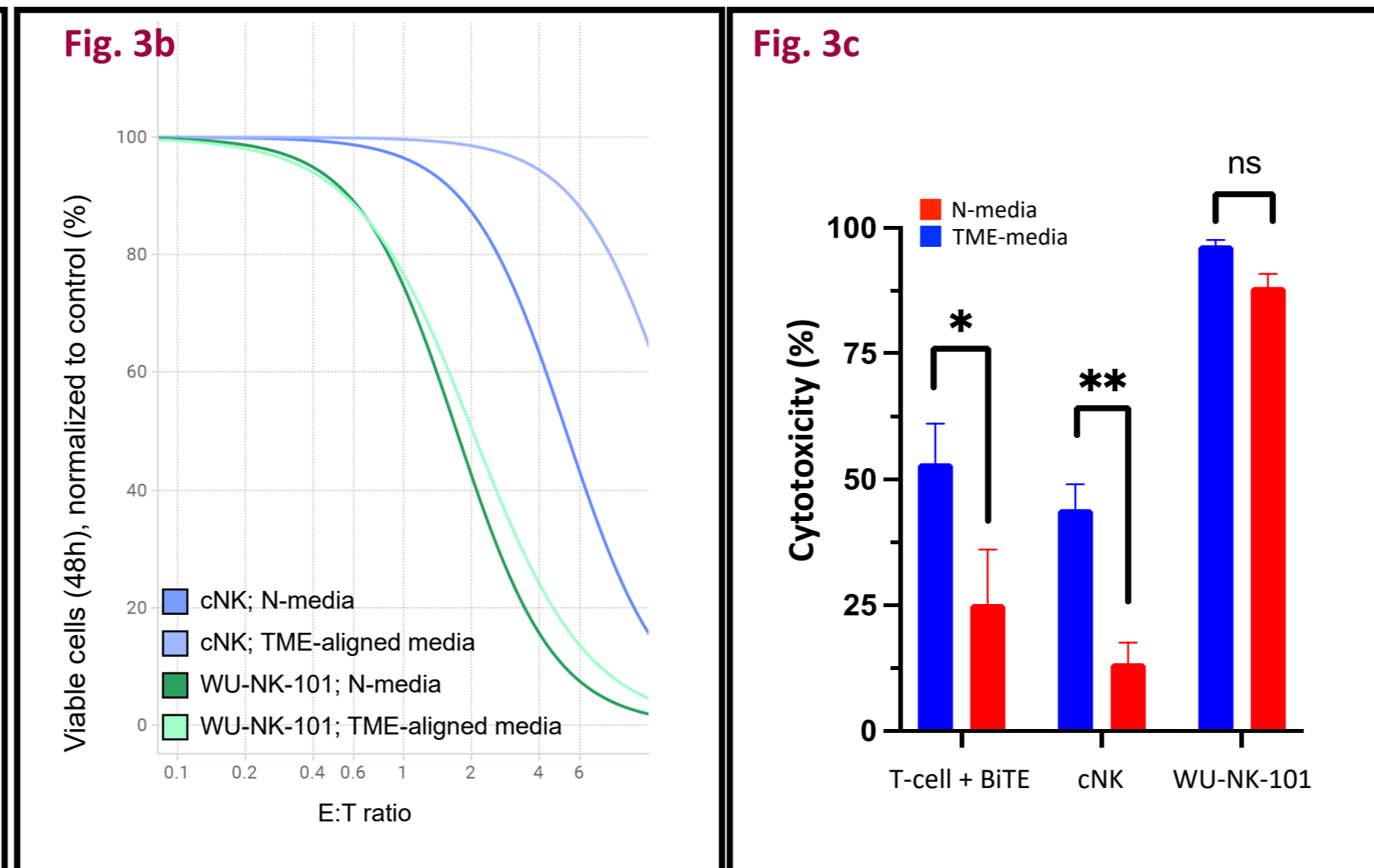


WU-NK-101 showed robust ATP production, mainly glycolytic, compared to cNK cells (Fig. 2a; left). ATP production increased in hypoglycemic TME-aligned media with a shift from glycolytic to mitochondrial ATP production suggesting **metabolic resilience** (Fig. 2a; right). This was further highlighted by metabolic pathway enrichment changing from glucose to amino-acid catabolism suggesting **metabolic flexibility** (Fig. 2b).

### Improved TME homing with mAb combination

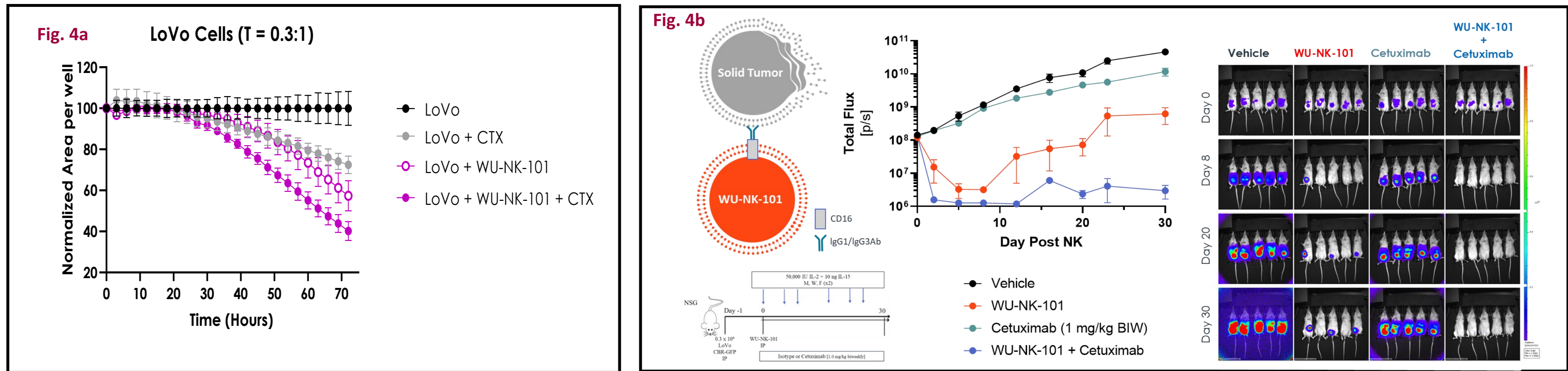


### WU-NK-101 function is not hampered in TME



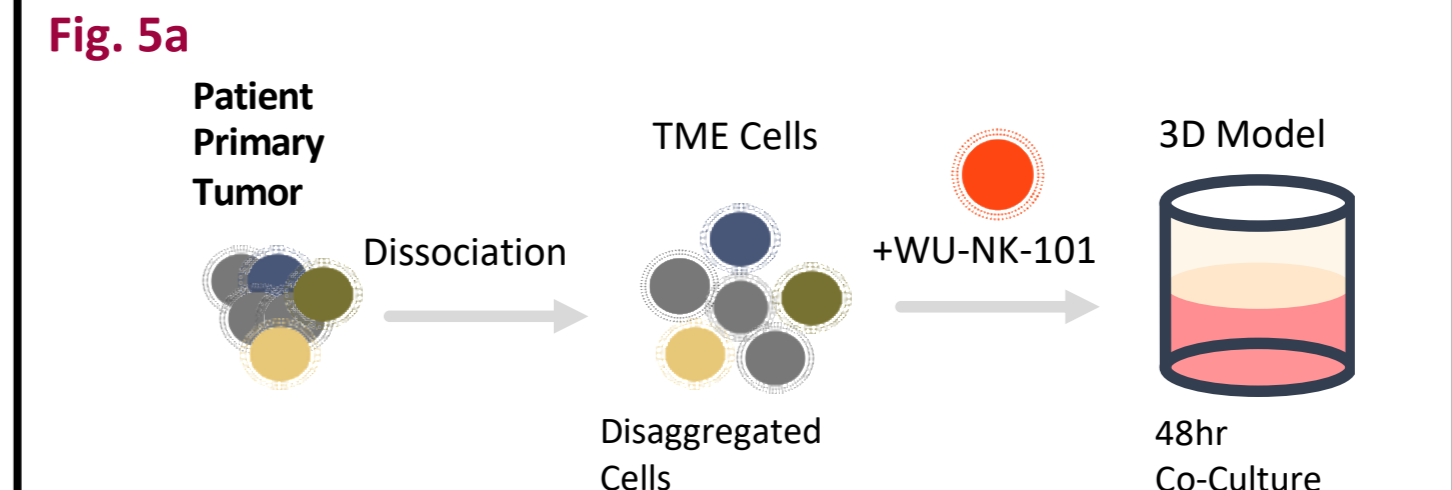
WU-NK-101 intra-tumor penetration and persistence was increased when combined with mAb in an NSG mouse model bearing SKOV3 tumors (n=10; Fig. 3a). WU-NK-101 is more potent,  $\sim$ 3-fold, than cNK (CD3<sup>neg</sup>/CD56<sup>pos</sup>) paired samples generated from healthy donors (n=5), at lysing tumor cells (HL-60) in vitro; mEC50 1.7 and 5.2 ( $p=0.032$ ), respectively. Cytotoxicity is not hampered by adverse TME-aligned media (an acidic and hypoglycemic-conditioned media containing high levels of nitric oxide, PGE2, IDO, IL-10, TGF $\beta$ 1, and other immune suppressive agents<sup>1,2,3</sup>) as compared to cNK cells; mEC50 2.0 vs 16.3 ( $p=0.018$ ), respectively (Fig. 3b), and T-cells (Fig. 3c).

### WU-NK-101 in combination with Ctx shows superior efficacy against CRC tumor cell lines in vitro and in vivo vs. monotherapy alone and against primary CRC samples in 3D “native” TME

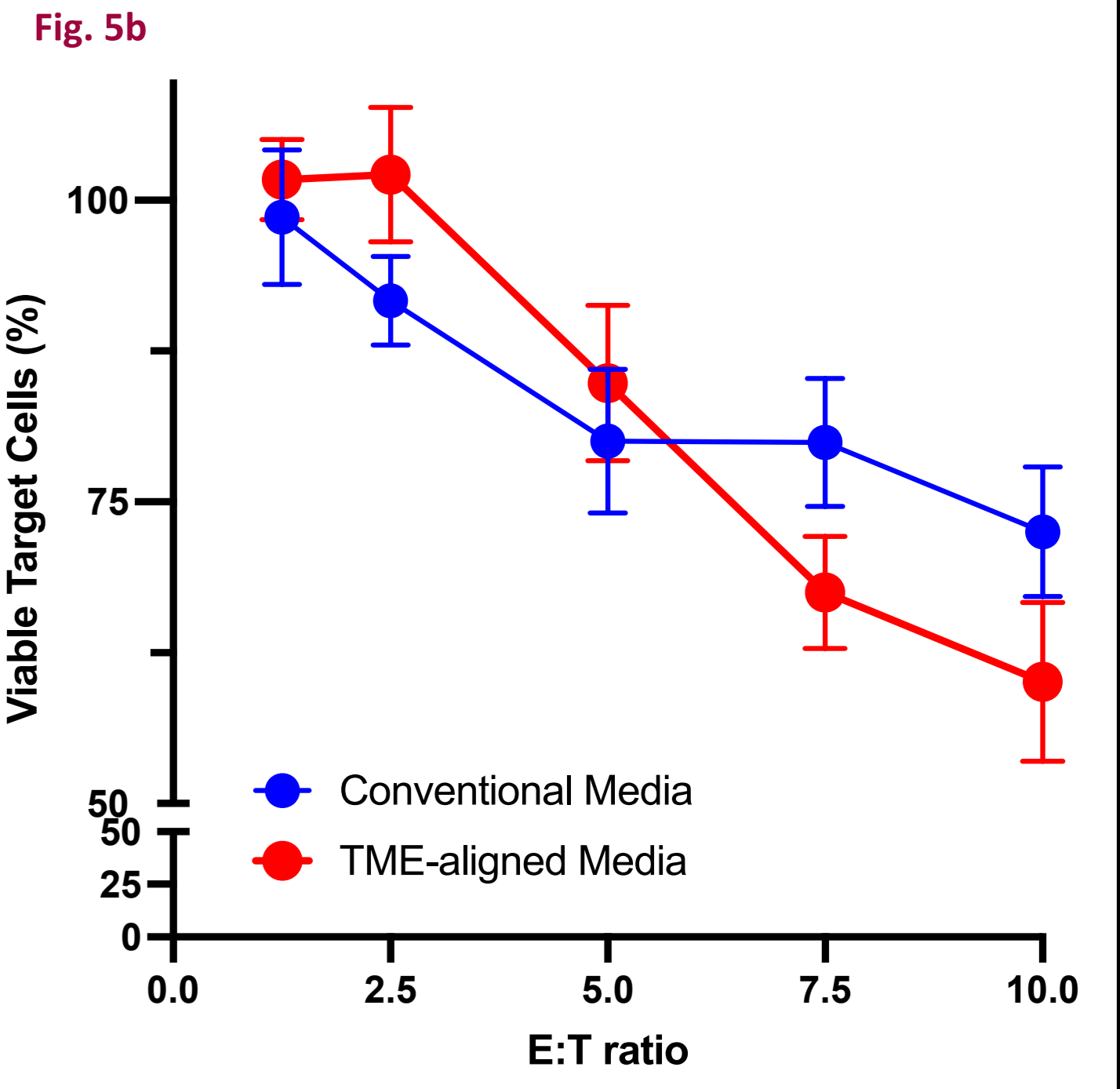


WU-NK-101 potency against EGFR+ LoVo cells was enhanced when combined with Ctx in vitro and in vivo confirming an ADCC MOA. In vitro, combination of WU-NK-101 at a low (0.3:1) E:T ratio with Ctx provided superior LoVo cell killing compared to WU-NK-101 and Ctx monotherapy treatment (Fig. 4a). In vivo, NSG mice were inoculated with LoVo cells by intraperitoneal injection on Day -1 followed by D0 intraperitoneal injection of WU-NK-101. Tumor progression was monitored by luminescent imaging BIW. WU-NK-101 combination with Ctx resulted in significant and durable tumor control compared to WU-NK-101 and Ctx monotherapy treatments (Fig. 4b).

Pt #	Pathology	Gender	Age	Surgical Sample
1	CRC	Female	41	Newly Diagnosed
2	CRC	Female	69	Newly Diagnosed
3	CRC	Male	57	Newly Diagnosed



Fresh tumor samples were collected from CRC patients undergoing tumor resection. Available clinical information is summarized (Table). Tumor samples were digested mechanically using scalpels, 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<sup>®</sup> Basement Membrane Extract - laminin, collagen IV, entactin, and heparan sulfate proteoglycans) that mimics in vivo tumor microenvironment (including low glucose, low pH, for TME-aligned media) (Fig. 5a). Cytotoxicity in 3D TME model with native TME cellular component and N- or TME-aligned media (Fig. 5b).



## 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

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

### Inclusion Criteria

- 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...
- 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

- We show that WU-NK-101 exerted potent activity against CRC, and in combination with Ctx showed improved intra-tumor infiltration/persistence and anti-tumor activity. Also, WU-NK-101 cells had enhanced metabolic fitness/flexibility and decreased susceptibility to immunosuppression, overcoming limitations encountered by ACT for solid tumors.
- A Phase 1b clinical trial is in development, which may reshape ACT in CRC and other EGFR-expressing tumors.

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