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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

WU-NK-101 (W-NK), a Memory-like (ML) NK Cell, Naturally Overcomes Tumor Microenvironment (TME) Metabolic Challenges, Retaining Anti-Tumor Potency

Nupur Bhatnagar[1], *Vincent Petit*[2], *Jayakumar Vadakekolathu, PhD*[3], *Christian Pinset*[1], *Nitin Mahajan, PhD MSc, MS*[4], *John Dean*[4], *Christine Spingola*[5], *Laura Arthur, PhD*[6], *David Boocock, PhD*[7], *Clare Coveney, PhD*[7], *John Muth, MSc*[8], *Jan Baughman, MPH*[9], *Melissa M Berrien-Elliott, PhD*[10], *Marc Sitbon*[11], *Matthew L. Cooper, PhD*[12], *Jan Davidson-Moncada, MDPhDMSc*[13], *Todd A Fehniger, MDPhD*[14], *Sergio Rutella, MDPhDFRCPath*[3]

[1]Metafora Biosystems, Paris, France
[2]Metafora Biosystems, Paris, FRA
[3]John van Geest Cancer Research Centre, Nottingham Trent University, Nottingham, United Kingdom
[4]Wugen, St. Louis
[5]Wugen, St Louis
[6]Wugen, Saint Louis, MO
[7]Nottingham Trent University, Nottingham, United Kingdom
[8]Wugen, St. Louis, MO
[9]Alviso Clinical Research, San Francisco
[10]Washington University at St. Louis, Saint Louis, MO
[11]National Institutes of Health, Bethesda
[12]Wugen Inc., Saint Louis, MO
[13]Wugen, Washington, DC
[14]Washington University School of Medicine in St. Louis, Saint Louis, MO

Cellular engineering revolutionized adoptive cell therapy (ACT) through improved antigen recognition and cellular activation. Cancer cells are metabolically active and extensively consume nutrients from the TME, rendering it nutrient-deprived, acidic, and hypoxic, and resulting in intratumoral metabolic demands which negatively impact ACT antitumor activity. This is central to T cells, however, some of these challenges can be overcome through engineering, an ideal solution would be to have a cellular product that is inherently adept at overcoming these challenges. W-NK is a cytokine-reprogrammed, expanded, cryopreserved, ML-NK cell product derived from healthy human peripheral blood mononuclear cells; it comes with naturally effective potency towards tumor cell killing, and importantly can overcome metabolic challenges present in the TME. We aim at deciphering what metabolic features make W-NK particularly potent in adverse TME.

W-NK characterization of metabolic fitness/adaptability was with transcriptomics (sc-RNA-seq; 10X Genomics) flow cytometry, bioenergetics (Seahorse Real-Time Cell Metabolic Analysis; Agilent), and proteomic (Sciex Zeno TOF 7600 tandem-mass spectrometry). The aforementioned assays evaluated the effect of differing media/TME on W-NK: 1) conventional (N) media (pH 7, glucose 11 mM); 2) TME-aligned media (pH 5.9, glucose 6 mM, immune suppressive agents, *e.g.*, PGE2, TGFb1); and 3) ascites derived from patients with malignancy. Media composition was analyzed biochemically using clinical assays (Core Lab Clinical Studies at Washington University, St. Louis, USA), and by immune secretome profiling (Nomic Bio, Quebec, CA). At baseline, W-NK cells have a unique metabolic phenotype, with higher expression of cell surface nutrient transporters, e.g., GLUT1, CD98, ASCT2, MCT1 and PiT1, compared to conventional NK cells (cNK); additionally, W-NK metabolism was consistent with aerobic ("Warburg") glycolysis, with ~80% of ATP coming from glycolysis *vs.* ~30% for cNK. In TME/ascites media, W-NK surface nutrient receptor expression adapted to meet metabolic demand. For example, transporters for amino acids, lactate and pyruvate are upregulated in these hypoglycemic medias. This coincided with a 50% shift in ATP manufacturing from glycolysis to mitochondria, suggesting that nutrients bypassed glycolysis to enter tricarboxylic acid cycle and oxidative phosphorylation. Proteomic analyses of W-NK cells cultured with either TME-aligned media or ascites supported this hypothesis, with up-regulation of metabolic pathways (ShinyGO) capturing amino-acid and lipid metabolism, and mitochondrial function. Overall, W-NK retained cytotoxic function in TME and ascites, as well as in native TME-aligned 3D assays from

primary surgical tumor samples. Conversely, cNKs did not adapt to TME media, whether in nutrient transporter expression or ATP manufacturing, and demonstrated reduced survival and function, including cytotoxicity.
W-NK has enhanced metabolic fitness, flexibility, and plasticity. This enables W-NK to utilize diverse macronutrients, engage different metabolic pathways, and maintain optimal ATP manufacturing. Overall, W-NK inherently survives and maintains function in the TME, a limiting factor for immune cell-based ACT. These data herald the promise of NK cell therapy; a Phase 1 clinical study of W-NK in acute myeloid leukemia is currently open and enrolling patients (NCT# 05470140).

Disclosures Bhatnagar: *Metafora Biosystems:* Current Employment. **Petit:** *METAFORA biosystems:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees. **Pinset:** *Metafora Biosystems:* Current Employment, Current equity holder in private company. **Dean:** *Wugen:* Current Employment. **Spingola:** *Wugen:* Current Employment. **Arthur:** *Wugen Inc.:* Current Employment, Current holder of *stock options* in a privately-held company. **Muth:** *Wugen:* Current Employment, Current equity holder in private company. **Baughman:** *MacroGenics, Inc.:* Current equity holder in publicly-traded company. **Berrien-Elliott:** *WUGEN:* Consultancy, Current holder of *stock options* in a privately-held company, Patents & Royalties. **Sitbon:** *Metafora Biosystems:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees. **Cooper:** *Wugen:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company, Patents & Royalties. **Davidson-Moncada:** *Wugen:* Current Employment, Current equity holder in private company. **Fehniger:** *HCW Biologics:* Research Funding; *Wugen:* Consultancy, Current holder of *stock options* in a privately-held company, Research Funding; *Smart Immune:* Other: Scientific Advisory Board; *AI Proteins:* Other: Scientific Advisory Board, Research Funding; *Orca Bio:* Current holder of *stock options* in a privately-held company; *Indapta:* Current holder of *stock options* in a privately-held company; *Affimed:* Other: Scientific Advisory Board.

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