Results Here we present the successful induction of 4–5 CD8⁺ and 4–7 CD4⁺ T-cell responses per patient, generated using peripheral blood mononuclear cells from multiple melanoma patients during these successful process engineering runs. We then extensively characterized these T-cell responses and demonstrate that these responses are functional, specific and have cytolytic capacity. Moreover, the induced T cells can recognize autologous tumor.

Conclusions NEO-STIM is a novel platform that generates *ex vivo* T-cell responses to high-quality neoantigen targets. NEO-PTC-01, the neoantigen-specific T cell product generated from this process, is a potent adoptive cell therapy targeting multiple immunogenic neoantigens in patients with metastatic melanoma.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0153>

154

MARROW-INFILTRATING LYMPHOCYTES (MILS): A NOVEL ADOPTIVE IMMUNOTHERAPY FOR HEMATOLOGICAL AND SOLID TUMORS

¹Eric Lutz*, ¹Lakshmi Rudraraju, ¹Elizabeth DeOliveira, ¹Amanda Seiz, ¹Monil Shah, ²Celine Colmenares, ²Beverly Dan Fu, ²Daniela Bota, ³Collin Brummel, ³Chad Brenner, ³Paul Swiecicki, ⁴Nicole Fredrich, ⁴David Page, ⁵Eleni Efstathiou, ⁶Ivan Borrello, ¹Kimberly Noonan. ¹WindMIL Therapeutics, Baltimore, MD, USA; ²University of California – Irvine, Irvine, CA, USA; ³University of Michigan, Ann Arbor, MI, USA; ⁴Providence Portland Medical Center, Portland, OR, USA; ⁵The University of Texas MD Anderson Canc, Houston, TX, USA; ⁶Johns Hopkins University School of Med, Baltimore, MD, USA

Background Marrow infiltrating lymphocytes (MILsTM) are the product of activating and expanding bone marrow T cells.¹ The bone marrow is a specialized niche in the immune system enriched for antigen-experienced, memory T cells. In patients with multiple myeloma and other hematological malignancies that relapse post-transplant, MILs have been shown to contain tumor antigen-specific T cells and adoptive cell therapy (ACT) using MILs has demonstrated antitumor activity.^{2–3} The bone marrow has been shown to harbor tumor-antigen specific T cells in patients with melanoma,^{4–5} glioblastoma,⁶ breast,⁷ non-small-cell lung⁸ and pancreatic cancers.⁹ Here, we sought to determine if tumor-specific MILs could be expanded from the bone marrow of patients with a range of different solid tumors.

Methods Bone marrow and blood samples were collected from patients with advanced and metastatic cancers. To date, samples have been collected from a minimum of four patients with non-small cell lung cancer (NSCLC), prostate cancer, head and neck cancer, glioblastoma, and breast cancer. Samples from patients with multiple myeloma were used as a reference control. Utilizing a 10-day proprietary process, MILs and peripheral blood lymphocytes (PBLs) were activated and expanded from patient bone marrow and blood samples, respectively. T cell lineage-specific markers (CD3, CD4 and CD8) were characterized by flow cytometry pre- and post-expansion. Tumor-specific T cells were quantitated in expanded MILs and PBLs using a previously described cytokine-secretion assay [2]. Briefly, autologous antigen-presenting cells (APCs) were pulsed with lysates from allogeneic cancer cell lines and co-cultured with activated MILs or PBLs. APCs pulsed with irrelevant mis-matched cancer cell line lysates or media alone were used as negative controls. Tumor-specific T cells were defined as the IFNγ-producing population by flow cytometry.

Results MILs were successfully expanded from all patient bone marrow samples tested, regardless of tumor type. Cytokine-producing tumor-specific CD4⁺ and CD8⁺ T cells were detected in each of the expanded MILs. In contrast, tumor-specific T cells were not detected in any of the matched activated and expanded PBLs.

Conclusions MILs have been successfully grown for all solid tumor types evaluated, including NSCLC, prostate, head and neck, glioblastoma and breast cancer. Clinical studies have been completed in patients with multiple myeloma and other hematological cancers.^{2–3} A phase IIa trial to evaluate MILs in combination with a checkpoint inhibitor is underway in patients with anti-PD1/PDL1-refractory NSCLC (ClinicalTrials.gov Identifier: NCT04069936). The preclinical data presented herein demonstrate that expanding MILs is feasible. MILs-based therapies hold therapeutic promise across a wide range of tumor indications.

Ethics Approval This study was approved by each participating institution's IRB.

REFERENCES

1. Borrello I and Noonan KA. Marrow-Infiltrating Lymphocytes - Role in Biology and Cancer Therapy. *Front Immunol* 2016 March 30; 7(112)
2. Noonan KA, Huff CA, Davis J, et al. Adoptive transfer of activated marrow-infiltrating lymphocytes induces measurable antitumor immunity in the bone marrow in multiple myeloma. *Sci. Transl. Med* 2015;7:288ra78.
3. Biavati L, Noonan K, Luznik L, Borrello I. Activated allogeneic donor-derived marrow-infiltrating lymphocytes display measurable in vitro antitumor activity. *J Immunother* 2019 Apr;42(3):73–80.
4. Müller-Berghaus J, Ehler K, Ugurel S, et al. Melanoma-reactive T cells in the bone marrow of melanoma patients: association with disease stage and disease duration. *Cancer Res* 2006;66(12):5997–6001.
5. Letsch A, Keilholz U, Assfalg G, et al., Bone marrow contains melanoma-reactive CD8⁺ effector T Cells and, compared with peripheral blood, enriched numbers of melanoma-reactive CD8⁺ memory T cells. *Cancer Res* 2003 Sep 1;63(17):5582–5586.
6. Chongsthakiet P, Jackson C, Koyama S, et al., Sequestration of T cells in bone marrow in the setting of glioblastoma and other intracranial tumors. *Nature Medicine* 2018 Aug 13; 24:1459–1468.
7. Feuerer M, Rocha M, Bai L, et al. Enrichment of memory T cells and other profound immunological changes in the bone marrow from untreated breast cancer patients. *Int J Cancer* 2001; 92(1):96–105.
8. Safi S, Yamauchi Y, Stamova S, et al. Bone marrow expands the repertoire of functional T cells targeting tumor-associated antigens in patients with resectable non-small-cell lung cancer. *Oncoimmunology* 2019;8(12):e1671762.
9. Schmitz-Winnenthal FH, Volk C, Z'Graggen K, et al. High frequencies of functional tumor-reactive T cells in bone marrow and blood of pancreatic cancer patients. *Cancer Res* 2005;65(21):10079–87.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0154>

155

IPSC-DERIVED NK CELLS MEDIATE ROBUST ANTI-TUMOR ACTIVITY AGAINST GLIOBLASTOMA

¹Jeffrey Miller*, ¹Frank Cichocki, ¹Jianfang Ning, ²Ryan Bjordahl, ¹Zachary Davis, ¹Katie Tuininga, ¹Hongbo Wang, ²Paul Rogers, ²Moyar Ge, ²Tom Lee, ²Bob Valamehr, ²Clark Chen. ¹University of Minnesota, Minneapolis, MN, USA; ²Fate Therapeutics, Inc., San Diego, CA, USA

Background Gliomas represent the most common brain tumors within the central nervous system, with glioblastoma being the most aggressive type.¹ Conventional treatment combines several approaches including surgery, chemotherapy, and radiation.² However, the prognosis for glioblastoma remains unfavorable, with only 5% of patients surviving more than 5 years post-diagnosis.³ Thus, new treatment approaches are urgently needed. Natural killer (NK) cells directly lyse malignant transformed or virally infected cells and secrete inflammatory cytokines that polarize cytotoxic immunity. Allogeneic

Abstracts

NK cell adoptive transfer has shown clinical benefit in patients with advanced cancer.^{4–7} However, limitations of this approach include relatively low numbers of donor NK cells that can be isolated during an apheresis and variability in the quality of NK cells between donors. To overcome these limitations, we have developed a GMP manufacturing strategy to mass produce NK cells from induced pluripotent stem cells (iPSCs) as an approach to off-the-shelf cancer immunotherapy. We refer to these cells as ‘iNK’ (iPSC-derived NK) cells. Here, we provide preclinical data demonstrating the efficacy of iNK cells for immunotherapy against glioblastoma.

Abstract 155 Figure 1 Engineered iNK cells exhibit highly effective antitumor function in a xenogeneic model of glioblastoma. (A) Schematic of the experimental design to test iNK cell function against glioblastoma in vivo. (B) Kaplan Meier plots showing survival for groups of mice that received either vehicle alone or iNK cells after tumor engraftment (n=5 mice/group)

Methods We generated iNK cells using previously published methods.^{8–10} iNK cells were used as effectors against an array of patient-derived glioblastoma lines in 2-dimensional live imaging IncuCyte assays where iNK cell-mediated killing was observed over the course of 48 hours. To investigate iNK cell infiltration and cytotoxicity in a more physiological context that accounts for the 3-dimensional architecture of the tumor, we also performed live imaging IncuCyte assays using iNK cells as effectors against glioblastoma spheroids. To test the anti-tumor function of iNK cells in vivo, we implanted patient-derived glioblastoma cells into mice via intracranial injection. Seven days later, 5 mice received intratumoral injections of iNK cells, and 5 mice received vehicle alone (as a control; figure 1A). All mice were monitored for weight and survival over 100 days.

Results iNK cells exhibited strong and sustained cytotoxicity against 6 primary patient-derived mesenchymal glioblastoma lines in 2-dimensional IncuCyte assays and complete infiltration and destruction of glioblastoma spheroids in 3-dimensional IncuCyte assays. In xenogeneic adoptive transfer experiments, all mice receiving intratumoral injections of iNK cells survived out to day 100, while all mice in the vehicle group became moribund and had to be sacrificed by day 60 (figure 1B).

Conclusions iNK cells are highly cytotoxic against glioblastoma cells, and our preclinical in vivo data provides proof-of-concept for future clinical trials.

Ethics Approval This project has been approved by the University of Minnesota IACUC. Approval ID: 1812-36595A

REFERENCES

- Louis D N, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee W K, Ohgaki H, Wiestler O D, Kleihues P, Ellison D W. The 2016 world health organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol* 2016;**131**:803–820.
- Stupp R, Mason W P, van den Bent M J, Weller M, Fisher B, Taphoorn M J B, Belanger K, Brandes A A, Marosi C, Bogdahn U, Curschmann J, Janzer R C, Ludwin S K, Gorlia T, Allgeier A, Lacombe D, Cairncross J G, Eisenhauer E, Mirimanoff R O, European Organization for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;**352**:987–996.
- Thakkar JP, Dolecek TA, Horbinski C, Ostrom QT, Lightner DD, Barnholz-Sloan JS, Villano JL. Epidemiologic and molecular prognostic review of glioblastoma. *Cancer Epidemiol Biomarkers Prev* 2017;**23**:1985–1996.
- Miller J S, Soignier Y, Panoskaltsis-Mortari A, McNearney S A, Yun G H, Fautsch S K, McKenna D, Le C, Defor T E, Burns L J, Orchard P J, Blazar B R, Wagner J E, Slungaard A, Weisdorf D J, Okazaki J, McGlave P B. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood* 2005;**105**:3051–3057.
- Bachanova V, Cooley S, Defor T E, Verneris M R, Zhang B, McKenna D H, Cutsinger J, Panoskaltsis-Mortari A, Lewis D, Hippen K, McGlave P, Weisdorf D J, Blazar B R, Miller J S. Clearance of acute myeloid leukemia by haploidentical natural killer cells is improved using IL-2 diphtheria toxin fusion protein. *Blood* 2014;**123**:3855.
- Ciurea S O, Schafer J R, Bassett R, Denman C J, Cao K, Willis D, Rondon G, Chen J, Soebbing D, Kaur I, Gulbis A, Ahmed S, Rezvani K, Scall E J, Lee D A, Champlin R E. Phase 1 clinical trial using mblL21 ex vivo-expanded donor-derived NK cells after haploidentical transplant. *Blood* 2017;**130**:1857–1868.
- Romee R, Rosario M, Berrien-Elliott M M, Wagner J A, Jewell B A, Schappe T, Leong J W, Abdel-Latif S, Schneider S E, Willey S, Neal C C, Yu L, Oh T, Lee S, Mulder A, Cooper M A, Fehniger T A. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. *Sci Transl Med* 2016;**8**:375ra123.
- Valamehr B, Abujarour R, Robinson M, Le T, Robbins D, Shoemaker D, Flynn P. A novel platform to enable the high-throughput derivation and characterization of feeder-free human iPSCs. *Sci Rep* 2012;**2**:213.
- Valamehr B, Robinson M, Abujarour R, Reznar B, Vranceanu F, Le T, Medcalf A, Lee T T, Fitch M, Robbins D, Flynn P. Platform for induction and maintenance of transgene-free hiPSCs resembling ground state pluripotent stem cells. *Stem Cell Reports* 2014;**2**:366–381.
- Zhu H, Blum R H, Bjordahl R, Gaidarova S, Rogers P, Lee T T, Abujarour R, Bonello G B, Wu J, Tsai P-F, Miller J S, Walcheck B, Valamehr B, Kaufman D S. Pluripotent stem cell-derived NK cells with high-affinity noncleavable CD16a mediate improved antitumor immunity. *Blood* 2020;**135**:399–410.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0155>

156

DISCOVERY OF TSC-100: A NATURAL HA-1-SPECIFIC TCR TO TREAT LEUKEMIA FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANT THERAPY

Ribhu Nayar, Sonal Jangalwe, Mollie Jurewicz, Antoine Boudot, Andrew Basinski, Robert Prenovitz, Elizabeth Olesin, Daniel Pollacksmith, Qikai Xu, Yifan Wang, Amy Virbasius, Jeffery Li, Holly Whitton, Garrett Dunlap, Alexander Cristofaro, Nancy Nabilisi, Ruan Zhang, Candace Perullo, Sida Liao, Kenneth Jahan, Kenneth Olivier, Gavin MacBeath, Gavin MacBeath*. *Tscan Therapeutics, Waltham, MA, USA*

Background Approximately 30–40% of AML patients relapse following allogeneic hematopoietic stem cell transplant therapy, leaving them with very few treatment options.^{1 2} Rare patients that naturally develop an HA-1-specific graft-versus-leukemia T cell response, however, show substantially lower relapse rates.^{3 4} HA-1 (VLHDDLLEA, genotype RS_1801284 A/G or A/A) is an HLA-A*02:01-and hematopoietically restricted minor histocompatibility antigen, making it an ideal candidate for TCR immunotherapy for liquid tumors.⁵