Abstracts

included T cells reactive to a p53 mutation in an autologous manner for the treatment of patients with metastatic epithelial cancers (n=12). Except for the two patients who exhibited an objective response (RECIST), most of the patients did not

Abstract 152 Table 1 Anti-mutant p53 TCR library
1 N=51,782 solid tumors (<http://p53.fr/>)
2 Phenotype frequency (<http://www.allelefrequencies.net/>)
3 Malekzadeh *et al.* J Clin Invest, 2019. 129(3): p. 1109–1114.
4 Lo *et al.*, Cancer Immunol Res, 2019. 7(4): p. 534–543

TCR source (patient ID)	p53 mutation	p53 mutation frequency (%) ¹	HLA restriction	HLA frequency (%) ²	Potentially treatable patient with solid cancer (%)
4141 ³ , 4196 ⁴	R175H	4.406	A*02:01	47.4	2.088
4259	Y220C	1.521	A*02:01	47.4	0.721
4273 ³	R248W	2.527	DPB1*02:01	27.3	0.69
4386	R273C	2.754	DPB1*04:02	24.2 (60–80% in hispanic populations, ~20% in African American and Asian populations)	0.666
4149 ³ , 4343	Y220C	1.521	DRB3*02:02	32.8	0.499
4127 ³	G245S	1.494	DRB3*02:02	32.8	0.49
4285 ³	R175H	4.406	DRB1*13:01	10	0.441
4259 ³	Y220C	1.521	DRB1*04:01	17.3	0.263
4304	M237I	0.411	DRB1*01:01	20	0.206
4316	C135Y	0.29	DRB1*07:01	26	0.075
4266 ³	R248W	2.527	A*68:01	2.8	0.071
4316	C135Y	0.29	A*29:02	6	0.017
4350	L111R	0.02	A*11:01	60% in Chinese populations	0.012
4324	T211I	0.076	C*06:02	11.2	0.009
4356	Q331H	0.04	B*40:01	11	0.004
4350	L111R	0.02	DRB1*08:03	7–20% in Chinese populations	0.004
4293	Y236S	0.018	DRB3*02:01	0.33	0.001
4114	C135R	0.08	Class II	na	na
Sum					6.258

respond to the therapy, possibly due to low frequencies of anti-mutant p53 cells in the infusion product, exhausted phenotype, and/or poor persistence (table 2). To overcome these barriers to TIL treatment, we retrovirally transduced autologous peripheral blood T cells to express an allogeneic anti-mutant p53 TCR. We engineered the HLA-A*02:01-restricted anti-p53 R175H TCR into patient 4349's lymphocytes (transduction efficiency of 64%) and saw less expression of exhaustion markers relative to the TIL infusion products (table 2). This patient with metastatic breast cancer was refractory to the six prior chemotherapy regimens. After the transfer of 5.3e10 cells, the patient experienced an objective partial response, showing regression by 55% of skin and mediastinal lesions for 7 months. The persistence of the infused T cells was higher than the other patients who received the TIL treatment (table 2).

Conclusions The library of anti-mutant p53 TCRs we have generated can potentially be used to treat ~6% of all cancer patients. We are pursuing the adoptive transfer of TILs against mutant p53 naturally occurring in the tumor or TCR-engineered cells using 'off-the-shelf' receptors against mutant p53. **Ethics Approval** This study was approved by the Institutional Review Board (IRB) of the NCI, and the approval numbers are as follows: Protocol 10-C-0166 (TIL treatment); Protocol 18-C-0049 (allogeneic TCR engineered T cell therapy)

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- Lo, W., *et al.*, Immunologic Recognition of a Shared p53 Mutated Neoantigen in a Patient with Metastatic Colorectal Cancer. Cancer Immunol Res, 2019. 7(4): p. 534–543.

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NEO-PTC-01 (BNT-221), AN AUTOLOGOUS NEOANTIGEN-SPECIFIC T-CELL PRODUCT FOR ADOPTIVE CELL THERAPY OF METASTATIC MELANOMA

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Abstract 152 Table 2 Patients who received mutant p53-reactive cell products

1 TCRB sequencing (Adaptive Biotechnology)
2 Flow cytometry against the murine TCR β constant region
3 Lo *et al.*, Cancer Immunol Res, 2019. 7(4): p. 534–543.
NA not available, NR no response, PR partial response, PD progressive disease, SD stable disease, TX treatment

Patient ID	Tumor type	p53 mutation	Protocol	HLA restriction	# of cells	# of Pem brixi unab	%mut-p53 reactive cells in infusion product ¹	Total # of cells given to patient	% persistence at 6 weeks post-TX ²	%PD1+ in infusion product	%TIM3+ in infusion product	%CD38+ in infusion product	Response (months)
4114	Pancreatic	C136R	Selected TIL (NCI-10-C-0166)	Class II	2	0	18.9	2.14E+10	Not detected	43.1	20.6	16.83	NR
4127	Ovarian	G245S	Selected TIL (NCI-10-C-0166)	DRB3*02:02	6	2	2.8	1.43E+11	Not detected	16.66	33.83	71.8	PR (4)
4141	Colon	R175H	Selected TIL (NCI-10-C-0166)	A*02:01	4	4	2.2	6.90E+10	0.06	46.2	32	87.4	NR
4149	Ovarian	Y220C	Selected TIL (NCI-10-C-0166)	DRB3*02:02	6	4	11.1	3.71E+10	Not detected	68.7	15.27	93.2	SD (5) - PD
4196 ³	Colon	R175H	Selected TIL (NCI-10-C-0166)	A*02:01	0	4	3.3	9.18E+10	0.02	NA	NA	NA	SD (3) - PD
4266	Colon	R248W	Selected TIL (NCI-10-C-0166)	A*68:01	6	0	50.9	1.01E+11	0.19	64	89.5	99.8	NR
4273	Rectal	R248W	Selected TIL (NCI-10-C-0166)	DPB1*02:01	2	2	6.78	1.17E+11	NA	38.4	42.7	86.3	NR
4285	Colon	R175H	Selected TIL (NCI-10-C-0166)	DRB1*13:01	6	0	2.43	6.96E+10	Not detected	66.9	66.5	76.11	NR
4304	Colorectal	M237I	Selected TIL (NCI-10-C-0166)	DRB1*01:01	4	4	13 (3 TCRs)	8.97E+10	0.01	30.6	19.32	97.4	NR
4324	Colorectal	T211I	Selected TIL (NCI-10-C-0166)	C*06:02	2	4	45 (2 TCRs)	8.48E+10	1.46	28.7	75.7	29.8	SD (3) - PD
4343	Breast	Y220C	Selected TIL (NCI-10-C-0166)	DRB3*02:02	4	4	1 (2 TCRs against p53 Y220C) 90.2 against FAM105A Y220C, 0.1 against TTF2 F120L	7.88E+10	0.002	63.2	pending	97.1	PR (7)
4350	Colon	L111R	Selected TIL (NCI-10-C-0166)	A*11:01, DRB1*08:03	6	2	11	6.69E+10	pending	30.9	2	94.2	NR
4349	Breast	R175H	Allogeneic TCR (NCI-18-C-0049)	A*02:01	0	1	64 ²	6.30E+10	14.4	13	21.7	52	PR (7)

Background Neoantigens are tumor-specific antigens that are important in the anti-tumor immune response. These antigens are not subject to central immune tolerance and are therefore potentially more immunogenic than tumor-associated antigens. NEO-STIM[®], our propriety *ex vivo* induction process, was developed to generate T-cell products specific to these neoantigens from the peripheral blood of patient. Here, we present the results of a proof of concept, pre-clinical study with multiple successful process engineering runs generating a neoantigen-specific T-cell product (NEO-PTC-01) using leukaphereses from metastatic melanoma patients. These products contain specific T-cell responses targeting multiple neoantigens from each individual patient's tumor.

Methods Patient-specific neoantigens were predicted using our RECON[®] bioinformatics platform. Predicted high-quality neoantigens were utilized in our *ex vivo* stimulation protocol, NEO-STIM, in the process engineering runs of NEO-PTC-01. NEO-STIM is used to prime, activate and expand memory and de novo T-cell responses from both the CD4⁺ and the CD8⁺ compartment. High throughput flow cytometric analysis was performed to characterize the specificity and functionality (cytokine production and cytolytic capacity) of the induced T-cell responses.

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.

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

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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.

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

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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