Supplementary Methods

Study Ethics

The study was conducted in accordance with local regulations, the guidelines for Good Clinical Practice, and the principles of the current version of the Declaration of Helsinki, and the study protocol and its amendments were approved by the University of California, Los Angeles, Institutional Review Board (UCLA IRB #15-001433).

Trial Eligibility

The patient was had to be HLA-A*0201 by high-resolution molecular phenotyping, with locally advanced or metastatic solid tumor, and with no available standard therapeutic options. The patient's tumor was screened for NY-ESO-1 positivity by IHC. Other important inclusion criteria included the fact the patient had to be >16 years of age, have an ECOG performance status of 0 or 1, have adequate organ function required to receive systemic IL2, be seronegative for Hepatitis B/C and HIV, and the patient must have had at least 1 lesion amenable for outpatient biopsies.

Exclusion criteria included but were not limited to patients with active brain metastases, bone marrow involvement on CT or PET at time of screening, the patient could not have received systemic treatment for cancer within one month prior to initiation of dosing within protocol nor could they have received 3 or more prior myelotoxic treatment regimens, and they could not have a history of, or significant evidence of risk for, chronic inflammatory or autoimmune disease.

Manufacture of NY-ESO-1 TCR transgenic lymphocytes

Transgenic lymphocytes were manufactured as described previously.[1] Briefly, the clinical grade retroviral vector expressing NY-ESO-1 TCR was manufactured at the Indiana University Viral Production Facility based on a master cell bank that was provided by Drs. Steven A. Rosenberg and Paul F. Robbins from the Surgery Branch, National Cancer Institute (NCI). This vector was previously used in a clinical trial run by the NCI[2] and there have been multiple clinical trials using adoptively transferred T cells specific for NY-ESO-1(HLA-A*0201) in the setting of advanced malignancies (e.g. NCT01343043[3], NCT00871481[4]).

A non-mobilized leukapheresis processing two plasma volumes from the study patient was processed in the UCLA Human Gene and Cell Therapy Facility. PBMCs were isolated by Ficoll gradient centrifugation and cultured in AIM V media (Gibco, Invitrogen, Chicago, IL) supplemented with 5% human AB serum (Omega Scientific, Tarzana, CA) in the presence of 50 ng/ml anti-CD3 (OKT3,

Miltenyi Biotec, Auburn, CA) and 300 IU/mL IL-2 to stimulate T-cell growth to prepare for viral vector transduction. Activated PBMC were then infected by the clinical grade MSGV1-A2aB-1G4A-LY3H10 retroviral vector supernatant using retronectin-coated plates (Retronectin, Takara, Otsu, Shiga, Japan) for two consecutive days, maintained in culture for 4 days from the start of transduction in IL-2, and then infused fresh into the trial subject. In-process and final product testing included Gram stain, fungal stain, sterility culture for bacteria and fungus, mycoplasma assay (MycoAlert assay, Lonza, Walkersville, MD), and endotoxin assay (Endosafe PTS system, Charles River, Charleston, SC). Transduction efficiency was tested with MHC dextramer analysis for NY-ESO-1 (Immudex, Copenhagen, Denmark) gated in CD3 (BD Bioscience, Franklin Lakes, NJ) positive lymphocytes. Finally, the potency of the NY-ESO-1 TCR transgenic cells was assessed using a co-culture system to detect NY-ESO-1- specific IFN-γ production by ELISA.

Manufacture of NY-ESO-1 peptide-pulsed dendritic cells

DC vaccines were manufactured as described previously[1]. Briefly, autologous DC were differentiated from adherent peripheral blood monocytes in a one-week *in vitro* culture in media containing 5% heat-inactivated autologous plasma supplemented with GM-CSF and IL-4 as previously[1,5,6]. Phosphor activation of STAT-6 and STAT-5 was used to measure the functionality of IL-4 and GM-CSF respectively[7]. DC were pulsed with the NY-ESO-$1_{157\text{-}165}$ anchor-modified immunodominant peptide (Biosynthesis Lewisville, TX) in the HLA-A2*0201 haplotype and administered intradermally. In-process and final product testing included Gram stain, fungal stain, sterility culture for bacteria and fungus, mycoplasma assay and endotoxin assay.

Flow Cytometry Phenotyping Analysis:

Antibodies against CD3, CD8, CD4, IgG4, CD279(PD-1), CCR7 and CD45RA (Biolegend, San Diego), CD45RA, as well as 7-Aminoactinomycin D (Beckman Coulter) or SYTOXX®AADvanced (Thermo Fisher Scientific, Waltham, MA). NY-ESO-1 HLA-A*0201(SLLMWITQV) dextramers and negative controls were purchased from Immudex.

For T-cell phenotypic classification, we defined naïve T-cells as $CCR7^+/CD45RA^+$, central memory T-cells as $CCR7^+/CD45RA^-$, effector memory T-cells as $CCR7^-/CD45RA^-$, and effector memory RA/effector T-cells as $CCD7^-/CD45RA^+$. Coexpression of CD39+ PD1+ was used to phenotype exhausted T cells.[8] All flow data analyses were done with FlowJo (Tree Star Inc., Asland, OR).

Immunofluorescence Experiment:

Multiplex immunofluorescence analysis was conducted on baseline and on-treatment biopsies. Serial sections from patient tumor samples were deparaffinized and rehydrated with a series of graded ethanols to deionized water on a BOND RX platform (Leica Biosystems). Full details of antibodies used, antigen retrieval techniques, and antibody dilutions and incubation times are summarized in Supplemental Table 1. Briefly, antigen retrieval was performed in either an EDTA based pH 9 buffer (ER2) or a citrate-based pH 6 buffer (ER1). Then, slides were then serially stained with anti-CD8 clone C8/144B (1:100, DAKO), anti–PD-1 clone NAT105 (1:50; Cell Marque), anti–PD-L1 clone SP142 (1:100; SpringBio), anti-IgG4 clone HP6205 (1:200, Millipore), which served as a marker of nivolumab, or anti-TLE1 clone ab183742 (1:2,000; Abcam), which served as a specific nuclear marker for undifferentiated pleomorphic sarcoma tissue. TSA-based Opal method was used in this study for multiplex immunofluorescence (mIF) staining (Opal Polaris 7-Color Automation IHC Kit; Akoya Biosciences). Since TSA and DAB oxidation are both peroxidase-mediated reactions, the primary antibody conditions and order of staining determined using DAB detection were directly applied to the fluorescent assays, unlike conventional IHC wherein a chromogenic peroxidase substrate is used for antigen detection, each antibody is paired with an individual Opal fluorophore for visualisation. The Opal fluorophores were used at a 1:150 dilution. As such, a fluorescent singleplex was performed for each biomarker and compared to the appropriate chromogenic singleplex to assess staining performance.

Once each target was optimized in uniplex slides, the Opal multiplexed assay was used to generate multiple staining slides. Primary antibodies were applied to normal human tonsil specimens as controls at optimized concentrations previously determined on the uniplex control tissues. Slides Staining was performed consecutively Leica BOND RX by using the same steps as those used in uniplex IF, and the detection for each marker was completed before application of the next antibody. The sequence of antibodies for multiplex staining was determined for panel combination is: CD8 (opal 480), PDL1 (opal 520), PD1 (opal570), IgG4 (opal 620), TLE1 (opal 690), and spectral DAPI (Invitrogen).

All fluorescently labelled slides were scanned on the Vectra Polaris (Akoya Biosciences) at 40× magnification using appropriate exposure times. Sections that did not have any antibodies or fluorescent labeling were used to capture the background tissue autofluorescence. Prior to analysis, all images were assessed for quality control. Criteria for rejection included poor tissue quality, (e.g. folded tissue, missing sections) or staining artifacts (e.g. air bubbles, signal dropout, or inadequate washing). The data from the multispectral camera were analyzed by the imaging InForm software (Akoya Biosciences) and Halo (Indica Labs).

Immune Repertoire Analysis:

Immune repertoire analysis was performed using the TRUST4 algorithm.[9] The frequency, or proportion of read count, was used to rank abundancy of the TCRα and TCRβ chains. Clonality (1 - Shannon entropy/log(N), where N is the number of unique clones) and Shannon entropy calculations were performed on the reconstructed repertoires.

Promoter Methylation:

The following primers were used to amplify the NY-ESO-1 promoter region from -119 to +84:
Fwd: 5'-ACGTGTTTCGGGGTTTATTCGG
Rev: 5'-CCCCGACCTTCGACCTACATAAC

References

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