Single cell sequencing to identify TCRs that recognize autologous tumor cells after vaccination with allogeneic DRibble vaccine (DPV-001)

Hong Ming Hu[1], Christopher Paustian[2], Zhifa Wen[1], Tarsem Moudgil[1], Traci Hilton[2], Sam Bookhardt[2], Guangjie Yu[1], Eric Tran[1], Venkatesh Rajamanickam[1], Walter Urba[1], Rachel Sanborn[1], Bernard A. Fox[1]

[1]Earle A. Chiles Research Institute, Robert W. Franz Cancer Center, Portland, OR; [2]UbiVac, Portland, OR;

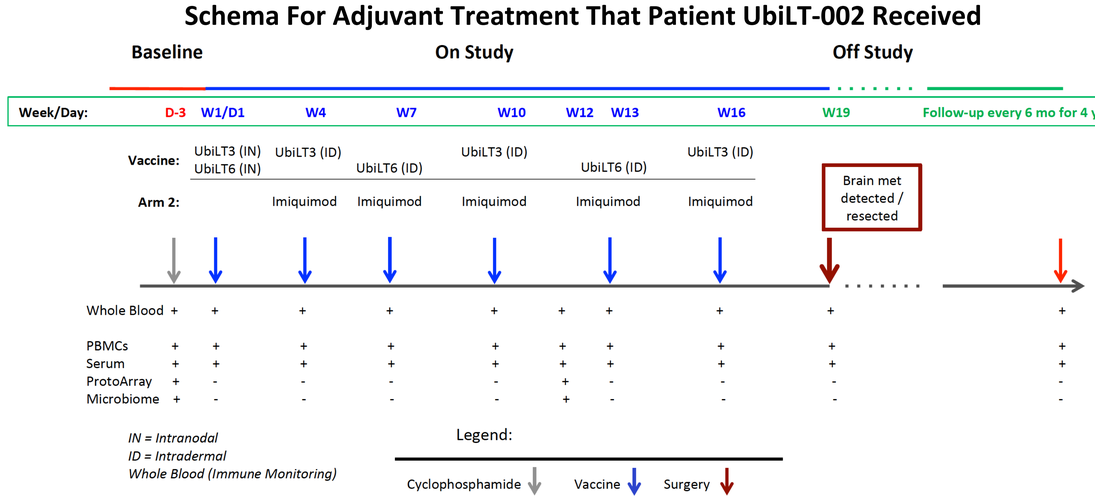
Background Adoptive immunotherapy with tumor-specific TCR gene-modified T cells has the potential to eradicate bulky disease. Traditional methods of TCR identification require lengthy in vitro culture to generate clonal T-cell populations, which adds time and complexity to this promising therapy. Here we described a simplified and reliable method to identify TCRs by single cell TCR sequencing of cells sorted with antibodies against T-cell surface markers that are up-regulated only when they are stimulated with specific tumor cell antigens.

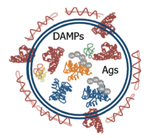
Materials and Methods A tumor-infiltrating lymphocyte (TIL) culture with T cells reactive against autologous tumor was generated from a brain metastasis of a patients with NSCLC (UbiLT-002). A panel of antibodies against T-cell surface antigens was screened to identify markers that are specifically up-regulated after stimulation with autologous tumors but not with related allogeneic tumor cells. Tumor-reactive T cells were sorted from TIL with three suitable antibodies and expanded by a rapid expansion protocol. Expanded T cells were examined for their tumor-specificity and subjected to single cell TCR sequencing using the 10X genomic system. The top 10 TCRs were identified by bio- informatics approach and the corresponding alpha and beta chains were synthesized and cloned into a retroviral vector based on MSG backbone. PBMC from healthy donors were transduced with the retrovirus supernatant after activation. Tumor-reactivity of transduced T cells was determined after expansion in media supplemented with IL-2, IL-7, and IL-15. To identify tumor-reactive TCRs in PBMC from the same patient after vaccination with an off-the-shelf allogeneic proteasome-blocked autophagosome vaccine (DPV-001, UbiVac), we also developed a protocol to expand tumor-specific T cells from PBMC with in vitro stimulation with DPV-001 vaccine-loaded PBMC.

Results We identity CD94, CD137(4-1BB), CD355 (CRTAM) as specific markers for antigen-specific activation of T-cells by autologous tumor cells, whereas other "check point" markers such as CTLA-4, PD-1, Tim3, CD39, CD103 were up-regulated by stimulation with unrelated tumor cells. These antibodies were successfully used to sort and enrich tumor-specific T cells. The top 10 TCRs from each sorting were different but with overlapping clones. Five TCR clones were tumor-specific and capable to recognize the autologous tumor cells when they were expressed on T-cells from health donors. Additionally, ex-vivo culture of vaccine stimulated PBMC from a post-vaccine timepoint generated T cells enriched for activity against autologous tumor.

Conclusions We developed a simplified work flow to identify tumor-specific TCRs. This flow will be further improved with antibody with DNA bar codes and used to identify tumor-reactive TCRs in a streamlined fashion. **Trial Registration** NCT01909752

Schema For Adjuvant Treatment That Patient UbiLT-002 Received





Top TCRs From Each Sorting Were Different But With Overlapping Clones

Figure 3. CDR of TCRβ chains for LT101 and associated Ag-specific sorted populations were assessed using Adaptive Biotechnologies immunoSEQ. The graph sorts clones by the most numerous within the original TIL101. Heat mapping of the TIL101 and sorted populations illustrate the most numerous (red) and least numerous (green) clones within each column. The top CRTAM sorted clones were assessed for single cell gene expression of immune receptors using 10X Genomics Chromium. The last column associates the CDR of TCRβ chains with TCR used to transfect Jurkat reporter cells (figure 4).

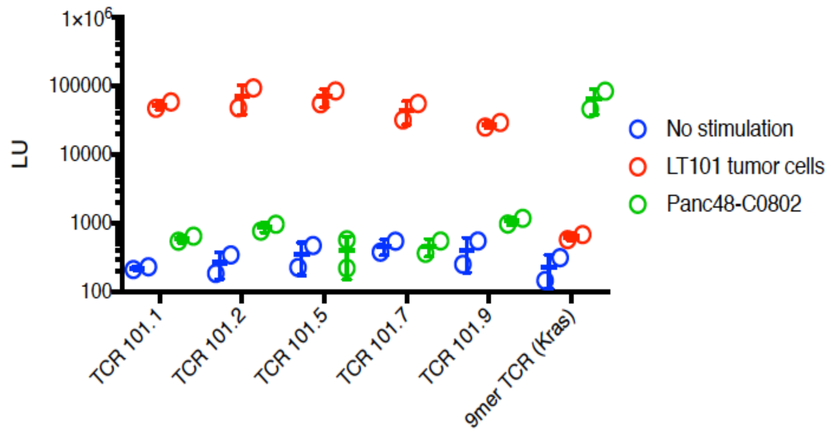
TCR101.1, TCR101.2 and TCR101.5 share the same β chain CDR. TCR101.1 has a unique α chain while TCR101.2 and TCR101.5 share the same α chain CDR.

Amino Acid	CD137	CD94	CRTAM	TIL101	TCR 101.
CASSQGTEAFF	0.155606	0.007985	0.136015	19.605097	
CASTPGWNTEAFF	0.068467	0.071869	0.075855	8.19213	
CASSSLAGGVYEQYF	0	0	0	4.740232	
CSAWGAREASYEQYF	0	0	0	3.248845	
CASGIRASQETQYF	0	0	0	2.835737	
CASNPAPTANYGYTF	0	0	0.007847	2.814732	
CASSLLAGGSDTQYF	6.940036	33.201573	36.922916	2.57667	1, 2, 5
CASSYVGANVLTF	14.328217	0.027949	0.496979	2.401624	
CASSLSTGLHYNEQFF	0	0	0	2.128553	
CASSQGRTIYF	0.069712	0.127767	0.214486	2.107548	
CASRDFSSYEQYF	0	0	0	1.981515	
CASSEGTVVSGANVLTF	0	0	0	1.981515	
CASSIAGPSYNEQFF	0	0	0.023541	1.855482	
CASSSGTPSGTYSNQPQHF	31.280577	0.007985	8.5585	1.638426	9
CASSSRGANYGYTF	0	0	0	1.358353	
CASRDSGIAYGYTF	0	0	0	1.30934	
CASSLRGGETQYF	0	0	0	1.225319	
CASSSSYGEQYF	0	0	0	1.141297	
CASSVAGAGDTQYF	2.031594	2.10417	2.963564	1.134295	
CASSLSVEYEQYF	0	0	0	1.127293	
CASSLDGGDTQYF	0	0	0	1.127293	
CAISEPQRGVYEQYF	0	20.147332	0.347885	1.092284	
CASSPLSGYEQYF	0.017428	0	0	1.022266	
CASSFLGGHTGELFF	0.001245	0	0	0.861224	
CASRTGTAYNEQFF	3.138265	0	0.627763	0.784204	
CSVLVAARAYEQYF	0	0.221597	0	0.7702	
CSARGLAGIINSYEQYF	0	0	0	0.735191	
CASSEDRSYGYTF	0	0	0.196176	0.721188	
CASSLTGTNYNEQFF	0.03859	0	0.873637	0.714186	
CASSTRDSPTDTQYF	0.001245	0.301451	0.002616	0.69318	
CASSLTGLYYNEQFF	0	0.151724	2.696764	0.61616	
CASSLTSGSYNEQFF	0	0	0	0.609158	
CASSQNRQMNTGELFF	0	0	7.875808	0.46212	7

Four* TCR Clones Were Tumor-reactive And Capable To Recognize The Autologous Tumor Cells When They Were Expressed By Jurkat T Cells

Figure 4. Transduced Jurkat reporter cells (>60% express the TCR) were stimulated with LT101 tumor cells or panc48 cells for 18 hours. Jurkat cell alone were used as the negative control. The luciferase activity in the supernatant was determined by luciferase assay. The 9 mer TCR (from Dr. Eric Tran) recognizes the mutant Kras epitope presented by HLA-C0802.

* Two TCRs were identical – Only 4 unique TCRs



CD94, CD137(4-1BB), CD355 (CRTAM) as Markers for Antigen-Specific Activation of T-cells by Autologous Tumor Cells

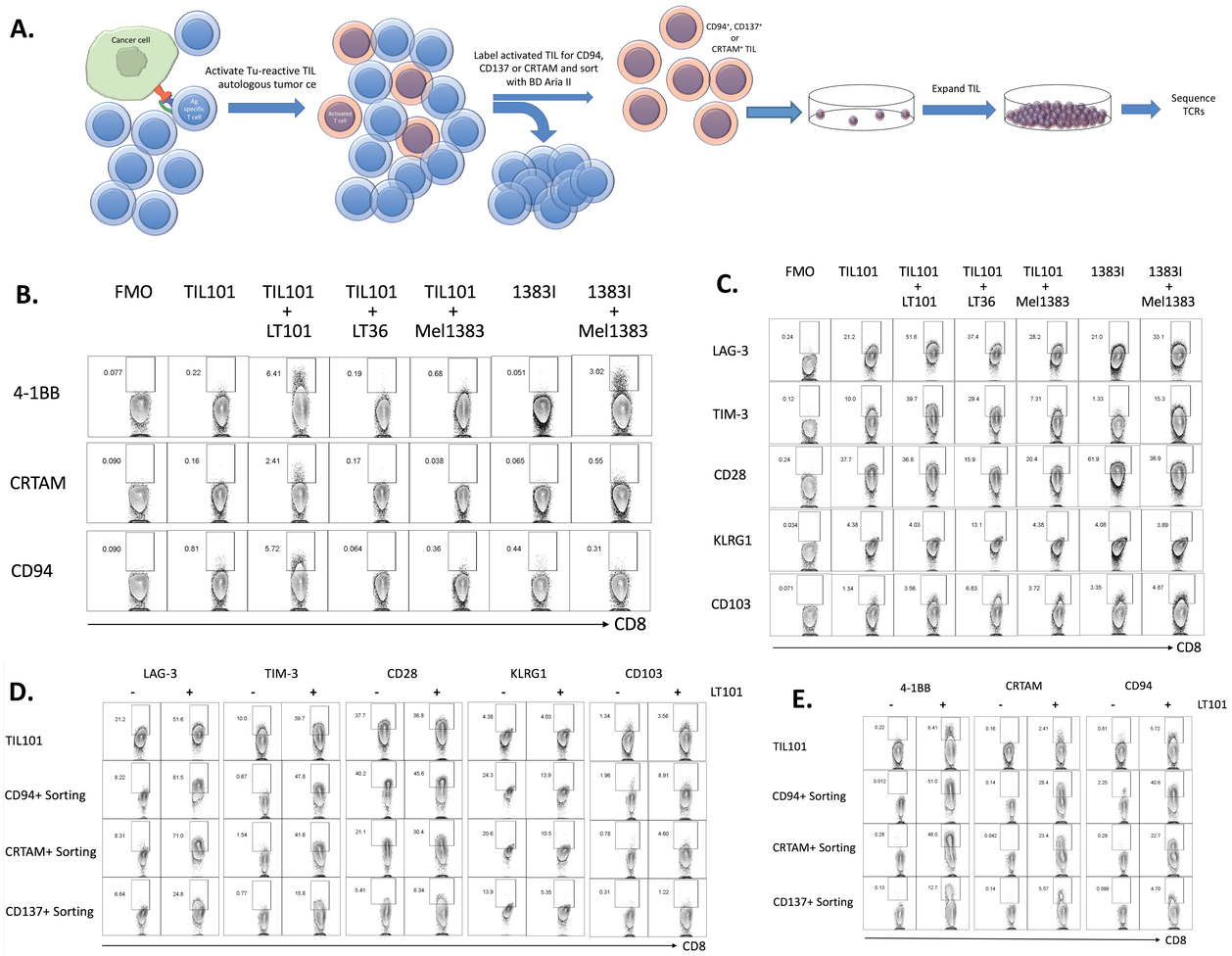
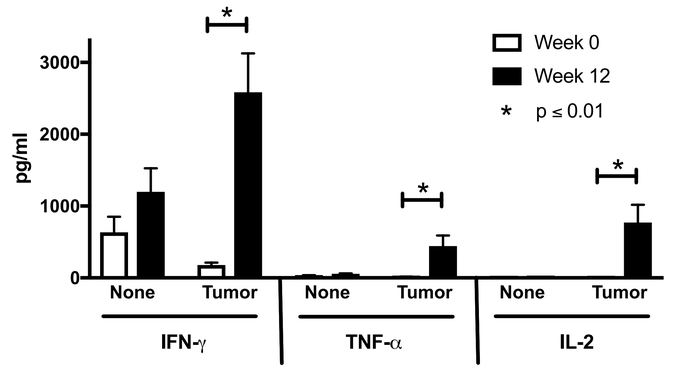


Figure 2. TIL from UBLT002 were assessed and sorted for antigen specificity towards autologous tumor. A) Schema for cell isolation. B) 4-1BB, CRTAM and CD94 are markers for antigen-specific activation of TIL101 by LT101 autologous tumor cells but not allo tumors LT36 or Mel1383. 1383I are TIL autologous to Mel1383, showing that this tumor is capable of stimulating an Ag-specific response. C) Other markers of antigen activation did not confer the specificity seen in A. D and E) LT101 cells were co-cultured with LT101 for 24hrs prior to sorting for 4-1BB, CRTAM and CD94 CD8 T cells. After expansion, rest and restimulation, these populations were enriched for Ag-specific expression of those same markers (D) but not other markers associated with activation (E).

DPV-001 Vaccination Primes/Boosts Polyfunctional T Cells That Recognize Autologous Tumor Cells

Figure 5. Baseline and week 12 PBMC from DPV-001-vaccinated NSCLC patient were stimulated ex-vivo with DPV-001 (or not – neg control) for 24 hours prior to expansion for 11 days on IL2 and IL15. Cells were harvested and restimulated with autologous tumor or no stimulus (neg) and anti-CD3 (pos) controls. 24 hour cytokine secretion assessed with cytokine bead array. Mean and SEM of experimental nonuplets (primary stim triplicates divided into secondary stim triplicates) shown.



Conclusions, Future Plans And Comments On Development Of Combination Immunotherapy

- We have developed a simplified work flow to identify tumor-reactive TCRs and are applying this to study vaccine-induced autologous tumor-reactive T cells in the peripheral blood.
- These TCRs, particularly those induced to common shared cancer antigens, may be useful for TCR gene therapy of NSCLC.
- This approach may be improved by incorporating DNA bar-coded mAbs to identify tumor-reactive T cells and corresponding TCRs.
- While clinical trials of T cell agonists have been largely disappointing as single agents or combined with checkpoint blockade, preclinical studies suggest that T cell agonists, like anti-OX40 and anti-GITR, can augment and sustain vaccine-induced therapeutic anticancer responses (Yu, G. et al., Sci Rep 2016).
- Even without fully characterizing the functional activity of a specific TCR, the identification of TCRs of T cells that upregulate expression of CRTAM, 4-1BB or CD94 following exposure to autologous tumor, provides a novel approach to monitor persistence and expansion of tumor-reactive T cells using TCR analysis.
- This strategy will be useful to assess the impact of immunotherapy agents on expansion and survival of these cells − An objective put forward by the FDA (https://www.fda.gov/Drugs/NewsEvents/ucm562746.htm)

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Conflict of Interest: Drs. Hong-Ming Hu and Bernard A. Fox are founders of and have stock in UbiVac.