Single cell sequencing to identify TCRs that recognize autologous tumor cells after vaccination with allogeinic DRibble vaccine

Hong-Ming Hu
*Earle A. Chiles Research Institute, Providence Portland Medical Center, Portland, OR, USA*, hhu@providence.org

Christopher C. Paustian

Zhifa Wen
*Providence Portland Medical Center, Portland, Oregon, OR, USA

Tarsem L. Moudgil
*Earle A. Chiles Research Institute, Providence Portland Medical Center, Portland, OR, USA,*
Tarsem.Moudgil@providence.org

Traci L. Hilton

*See next page for additional authors*

Follow this and additional works at: [https://digitalcommons.psjhealth.org/sitc2018](https://digitalcommons.psjhealth.org/sitc2018)

Part of the [Oncology Commons](https://digitalcommons.psjhealth.org/sitc2018)

Recommended Citation

Hu, Hong-Ming; Paustian, Christopher C.; Wen, Zhifa; Moudgil, Tarsem L.; Hilton, Traci L.; Bookhardt, Sam; Yu, Guangjie; Tran, Eric; Rajamanickam, Venkatesh; Urba, Walter; Sanborn, Rachel E.; and Fox, Bernard A., "Single cell sequencing to identify TCRs that recognize autologous tumor cells after vaccination with allogeinic DRibble vaccine" (2018). *Society for Immunotherapy of Cancer 2018 Annual Meeting Posters*. 2.

[https://digitalcommons.psjhealth.org/sitc2018/2](https://digitalcommons.psjhealth.org/sitc2018/2)
Authors
Hong-Ming Hu, Christopher C. Paustian, Zhifa Wen, Tarsem L. Moudgil, Traci L. Hilton, Sam Bookhardt, Guangjie Yu, Eric Tran, Venkatesh Rajamanickam, Walter Urba, Rachel E. Sanborn, and Bernard A. Fox
Single cell sequencing to identify TCRs that recognize autologous tumor cells after vaccination with allogeneic DRibble vaccine (DPV-001)

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

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

Background: Adaptive 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 to T-cell surface markers that are upregulated 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 patient with NSCLC (UbiLT-002). A panel of antibodies against T-cell surface antigens was screened to identify markers that are specifically upregulated after stimulation with autologous tumor cells 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 genomics system. The top 10 TCRs were identified by bio-informatics approach and the corresponding alpha and beta chains were synthesized and cloned into a plasmid to expand tumor-specific T cells from PBMNC in vitro stimulation with DPV-001 vaccine-loaded PBMNC.

Results: We identified CD94+ CD137+ (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, CD9, 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 healthy donors. Additionally, ex-vivo culture of vaccine stimulated PBMNC 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.

Table 1. DPV-001 Vaccination Primes/Boosts Polyfunctional T Cells That Recognize Autologous Tumor Cells

Figure 3. CDR of TCR 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 TCR chains with TCR used to transfect Jurkat reporter cells (figure 4).

Figure 4. Transduced Jurkat reporter cells (+/−60% express the TCR) were stimulated with LT101 tumor cells or pancyt48 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 mutan Kras epitope presented by HLA-C0802.

Figure 5. Baseline and week 12 PBMNC from DPV-001 vaccinated NSCLC patient were stimulated ex-vivo with DPV-001 or 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.

Figure 6. Summary of the most numerous TCR identified in the present study. The authors identified a unique TCR clone, TCR101.2 that recognizes an autologous cancer epitope presented by HLA-C0802. The TCR101.2 chain CD3, CDR3L of TCR101.2 has a unique primary sequence that was not found in the public TCR database. The CDR3L sequence was used to identify T cells expressing this TCR in 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 TCR chains with TCR used to transfect Jurkat reporter cells (figure 4).

Figure 7. Schema for ADjuvant Therapy that Patient UbiLT-002 Received

Figure 8. TIL101 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 TIL6 or Met1383. 1383 are TIL autologous to Met1383, 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 4hrs 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 (C) but not other markers associated with activation (E).

Figure 9. Table of the top 10 TCRs identified by bio-informatics approach and the corresponding alpha and beta chains were synthesized and cloned into a plasmid to expand tumor-specific T cells from PBMNC in vitro stimulation with DPV-001 vaccine-loaded PBMNC.

Table 2. 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 used 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 antitumor 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.
- The development of immunotherapy agents on expansion and survival of these cells – An objective put forward by the FDA. (https://www.fda.gov/Drugs/NewsEvents/ucm527466.htm)

Support: This research was supported by NCI R44CA121612, The Harder Family, Robert W Franz, Elise Franz-Finley, Lynn and Jack Loacker, Wes and Nancy Lematta, the Chiles Foundation, Murdock Trust and the Providence Portland Medical Foundation.

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