<table>
<tr><td>PACT pharma</td><td><strong>21-211 patient 0315 imPACT analysis</strong></td><td><strong>Version 1.5</strong></td></tr>
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**Study #:** 21-211

**Patient ID:** 0315

**Study Description:** PACT-0101_0315_imPACT_PACT510C_M01

**Responsible Scientist:** Craig Fett

**Start Date Of Analysis:** 17th March, 2021

**Study Objective:** imPACT analysis of PACT510C_M01

**Study reviewed by:** Reviewed By

## 1.  MATERIALS AND METHODS:

**All experiments were listed in the following table and performed following PD-005 "imPACT process".**

| Start Date | Experiment Id | Description | Initials |
|---|---|---|---|
| 17th March, 2021 | 21000743 | PACT-0101_0315_imPACT_PACT510C_M01 | AS |

## 2.  RESULTS:

**2.1** comPACT input analysis

The information of comPACT received from Protein Science is summarized in **Table 1**.

**Table 1. Summary of the comPACT library information from protein science**

| HLA | #comPACT produced | #comPACT proposed | Hit Rate % | Hit 0.85-2uM | Hit 2-10uM | Hit > 10 uM | #mutation input | produced | (>1.50 uM) |
|---|---|---|---|---|---|---|---|---|---|
| HLA-A*01:01 | 14 | 58 | 24.14 | 7 | 2 | 5 | | | |
| HLA-B*08:01 | 16 | 59 | 27.12 | 10 | 6 | 0 | | | |
| HLA-B*35:01 | 28 | 59 | 47.46 | 5 | 13 | 10 | | | |
| HLA-A*11:01 | 36 | 54 | 66.67 | 0 | 11 | 25 | 58 | 49 | 40 |
| HLA-C*07:01 | 22 | 59 | 37.29 | 12 | 8 | 2 | | | |
| HLA-C*04:01 | 13 | 59 | 22.03 | 6 | 7 | 0 | | | |
| Grand Total | 129 | 348 | 37.07 | 40 | 47 | 42 | | | |

### 2.2  Signal to noise analysis to identify potential neoantigen-specific T cells

In this study, imPACT process was conducted on the PBMC samples for patient 0315 (PACT510C) following the PD-005. The cell information used in the study is summarized below:

- imPACT started with 11.4 M PBMC (97.10% viability).
- CD8 MACS selection yielded 1.84 M cells (98.20% viability).
- 1.81 M CD8 cells were stained for sorting.
- 454 K CD8 cells were analyzed on the cell sorter.
- CD8+ cell percentage 50.30 % on sorter.
- 593 single cells were sorted and analyzed.

After sequencing data analysis, the potential unique neoE-specific TCRs that passed imPACT signal to noise analysis criteria (PD-005) are summarized in **Table 2**. The statistics of the imPACT analysis for neo12 internal positive control and potential neoE-specific T cells are summarized in **Table 3**.

**Table 2. All TCRs passed the PD-005 TCR signal-to-noise analysis filters with paired TCR alpha and beta chain.**

| EXPID | Sample ID | well.position | TRA | TRB | Patient ID | PACT ID | TCR ID | compPACT sense oligo name | comPACT ID | Gene | neoE | HLA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21000743 | M01 | Plate01-F07 | CAVYPRMNYGGATNKLIF | CASSHSLVEPNSGNTIYF | 0315 | PACT510C | TCR1011 | PACT510C_T_PP001679_O85_S_1_HLA-A11:01 | comPACT38550 | PLCB1 | FTMTTKISFKE | HLA-A11:01 |
| 21000743 | M01 | Plate06-H02 | CAVRDMREGFKTIF | CASSPPIQGFKQFF | 0315 | PACT510C | TCR1012 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 |
| 21000743 | M01 | Plate02-A07 | CATVYNAGNNRKLIW | CASSQATTGFSYEQYF | 0315 | PACT510C | TCR1013 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 |
| 21000743 | M01 | Plate02-A07 | CATVYNAGNNRKLIW | CASSQATTGFSYEQYF | 0315 | PACT510C | TCR1013 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 |
| 21000743 | M01 | Plate02-A10 | CAVRDIEGKSTF | CASSSLVQGYEQFF | 0315 | PACT510C | TCR1014 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 |
| 21000743 | M01 | Plate02-A10 | CAVRDIEGKSTF | CASSSLVQGYEQFF | 0315 | PACT510C | TCR1014 | PACT510C_T_PP001679_O55_S_2_HLA-A01:01 | comPACT38732 | STXBP5L | TTEENRENFYN | HLA-A01:01 |
| 21000743 | M01 | Plate02-B04 | CAVRDIVNNNAGNMLTF | CASSSGTGAAYGYTF | 0315 | PACT510C | TCR1015 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 |
| 21000743 | M01 | Plate02-B04 | CAVRDIVNNNAGNMLTF | CASSSGTGAAYGYTF | 0315 | PACT510C | TCR1015 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 |
| 21000743 | M01 | Plate02-B09 | CAYRGSVTGNQFYF | CSALDRGFRSPLHF | 0315 | PACT510C | TCR1016 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 |
| 21000743 | M01 | Plate02-B09 | CAYRGSVTGNQFYF | CSALDRGFRSPLHF | 0315 | PACT510C | TCR1016 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 |
| 21000743 | M01 | Plate07-C07 | CALTTSGTYKYIF | CASSPHGNSPLHF | 0315 | PACT510C | TCR1017 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 |
| 21000743 | M01 | Plate07-C07 | CALTTSGTYKYIF | CASSPHGNSPLHF | 0315 | PACT510C | TCR1017 | PACT510C_T_PP001679_O55_S_2_HLA-A01:01 | comPACT38732 | STXBP5L | TTEENRENFYN | HLA-A01:01 |
| 21000743 | M01 | Plate05-C05 | CAGGGFSGYSTLTF | CASSTPRRQGDTEAFF | 0315 | PACT510C | TCR1018 | PACT510C_T_PP001679_O80_S_1_HLA-A11:01 | comPACT38577 | MCM3 | LIGDPFVAK | HLA-A11:01 |
| 21000743 | M01 | Plate03-E10 | CAVRDENDKIIF | CASSPLTFGYGYTF | 0315 | PACT510C | TCR1019 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 |
| 21000743 | M01 | Plate03-F06 | CAVRDLVNNAGNMLTF | CASSHGGGAGGYTF | 0315 | PACT510C | TCR1020 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 |
| 21000743 | M01 | Plate03-F06 | CAVRDLVNNAGNMLTF | CASSHGGGAGGYTF | 0315 | PACT510C | TCR1020 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 |
| 21000743 | M01 | Plate04-B04 | CAVRDLVTGANNLFF | CASSLGGGADYGYTF | 0315 | PACT510C | TCR1021 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 |
| 21000743 | M01 | Plate04-B04 | CAVRDLVTGANNLFF | CASSLGGGADYGYTF | 0315 | PACT510C | TCR1021 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 |
| 21000743 | M01 | Plate04-B10 | CAVSLTYSTLTF | CASRGTYGYTF | 0315 | PACT510C | TCR1022 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 |
| 21000743 | M01 | Plate04-B10 | CAVSLTYSTLTF | CASRGTYGYTF | 0315 | PACT510C | TCR1022 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 |
| 21000743 | M01 | Plate04-F11 | CAVRDLLAAGNKLTF | CASRLRESAPEAFF | 0315 | PACT510C | TCR1023 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 |
| 21000743 | M01 | Plate04-F11 | CAVRDLLAAGNKLTF | CASRLRESAPEAFF | 0315 | PACT510C | TCR1023 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 |
| 21000743 | M01 | Plate05-A09 | CAMREGSTDKLIF | CASSNTGGLNSPLHF | 0315 | PACT510C | TCR1024 | PACT510C_T_PP001679_O85_S_1_HLA-A11:01 | comPACT38550 | PLCB1 | FTMTTKISFKE | HLA-A11:01 |
| 21000743 | M01 | Plate05-D09 | CAVRDVGPGGGNKLTF | CASSLGAGGYYGYTF | 0315 | PACT510C | TCR1025 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 |

| 21000743 | M01 | Plate05-D09 | CAVRDVGPGGGNKLTF | CASSLGAGGYYGYTF | 0315 | PACT510C | TCR1025 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 |
| 21000743 | M01 | Plate05-G06 | CAFTVSGTYKYIF | CASSFHGSSPLHF | 0315 | PACT510C | TCR1026 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 |
| 21000743 | M01 | Plate06-B03 | CAVRDLTGFGNVLHC | CASSQGGGTYGYTF | 0315 | PACT510C | TCR1027 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 |
| 21000743 | M01 | Plate06-B03 | CAVRDLTGFGNVLHC | CASSQGGGTYGYTF | 0315 | PACT510C | TCR1027 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 |
| 21000743 | M01 | Plate06-C02 | CAVRDIQAGTALIF | CSARRTSGAVGGETQYF | 0315 | PACT510C | TCR1028 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 |
| 21000743 | M01 | Plate06-C02 | CAVRDIQAGTALIF | CSARRTSGAVGGETQYF | 0315 | PACT510C | TCR1028 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 |
| 21000743 | M01 | Plate06-C12 | CAAPSNDYKLSF | CASRGHYNSPLHF | 0315 | PACT510C | TCR1029 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 |
| 21000743 | M01 | Plate06-C12 | CAAPSNDYKLSF | CASRGHYNSPLHF | 0315 | PACT510C | TCR1029 | PACT510C_T_PP001679_O55_S_2_HLA-A01:01 | comPACT38732 | STXBP5L | TTEENRENFYN | HLA-A01:01 |
| 21000743 | M01 | Plate06-D08 | CAGGGFSGYSTLTF | CASSTPRRQGGTEAFF | 0315 | PACT510C | TCR1030 | PACT510C_T_PP001679_O80_S_1_HLA-A11:01 | comPACT38577 | MCM3 | LIGDPFVAK | HLA-A11:01 |
| 21000743 | M01 | Plate01-C11 | CAVRDKGGNNNARLMF | CASSLLGDTGELFF | 0315 | PACT510C | TCR1010 | PACT510C_T_PP001679_O10_S_1_HLA-A01:01 | comPACT38588 | CAND1 | LLDTVLSHL | HLA-A01:01 |

**Table 3. Summary of internal positive-control neo12 and CD8 T cells processed for the imPACT process .**

| Cells | Criteria | Number |
|---|---|---|
| Sorted neo12 control | # neo12 passed SNR analysis | 15 |
| | Average neo12 SNR (passed) | 122.84 |
| | # neo12 not passed SNR analysis | 8 |
| | Average neo12 SNR (not passed) | 5.64 |
| neoE specific T cell analysis | # neoE specific T cells (SNR >= 10) | 50.00 |
| | # neoE specific T cells (SNR >= 10 and SNR < 10) | 0.00 |
| | # Sticky comPACTs (Y/N) | N |

**2.3 Flow analysis for identification of antigen-experienced T cells**

After signal to noise analysis, 21 unique potential neoE-specific TCRs were registered according to PD-005. Based on the indexed flow plot, 50 T cells with these unique TCRs were analyzed on the multimer staining, CD45RA/CD95 and CD39/CD103 plots **(Figures 1 & 2)**. 1 TCRs were identified from naïve T cells. All the rest TCRs were identified as antigen-experienced cells. 0 TCR were excluded from they were from non-specific or non-confident T cells. 0 TCR was excluded from down selection. Refer to section 4 for detailed analysis for exclusion criteria if any.
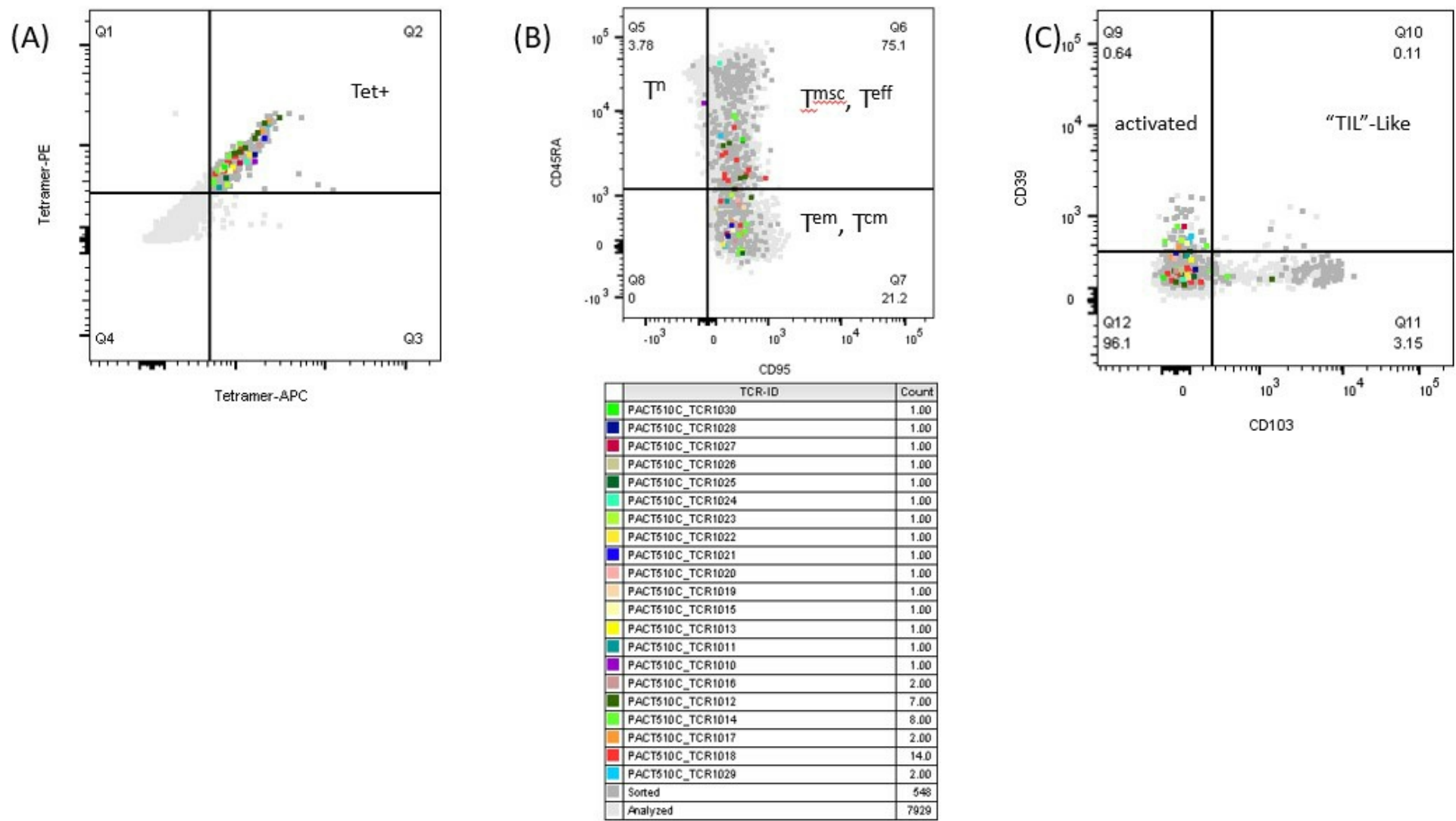


**Figure 1. Flow plot of the neo-E specific TCR candidates.** The gray dots in the flow plot were cells recorded as background to help to draw the gate. The colored dots in the flow plot were single cells sorted. (a) Flow plot of tetramer staining. Q2 gate indicated dual APC-comPACT and PE-comPACT multimer positive cells. (b) Flow plot of CD45RA/CD95 staining. Q5 gate indicated naïve T cells, while Q6 and Q7 gates indicated antigen-experienced cells. (c) Flow plot of CD39/CD103 staining. Q10 gate indicated tumor trafficked T cells. Refer to **Figure 2** for gating strategy for CD45RA/CD95 and CD39/CD103.

| | PACT pharma | **21-211 patient 0315 imPACT analysis** | **Version 1.5** |
|---|---|---|---|
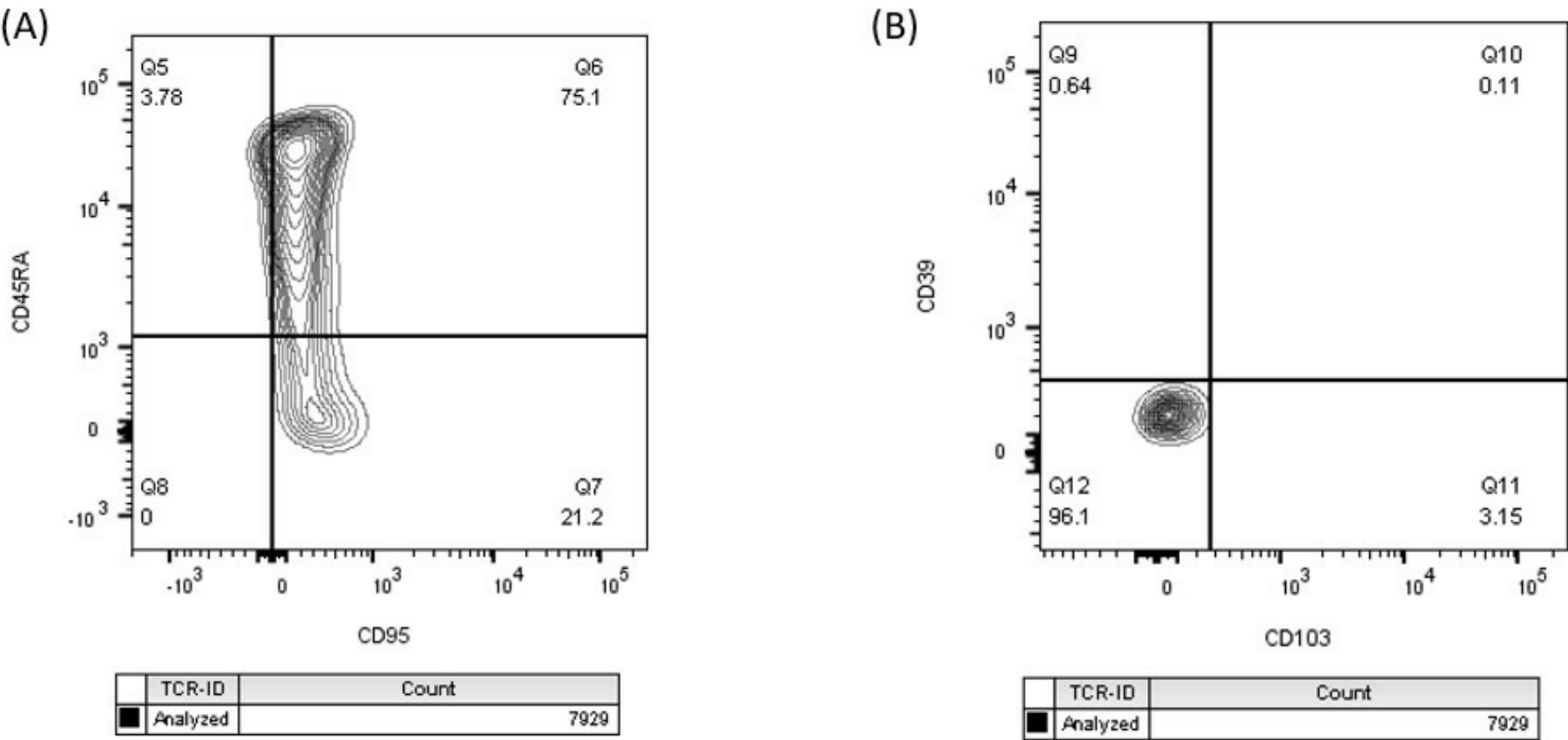


**Figure 2. Gating strategy of the CD45RA/CD95 (contour plot) and CD39/CD103 (contour plot).** (a) Gating strategy of CD45RA/CD95. The horizontal CD45RA line was first drawn at the contour boundary of the CD45RA positive/negative population. Then the vertical CD95 line was drawn at the cross point of the CD45RA line and the outside contour boundary. (b) Gating strategy of CD39/CD103. CD39 and CD103 gate was defined based on the contour plot.

### 2.4 TCR candidates for PS and GE

There were 20 neoE-specific TCRs selected to pass to gene editing and protein science teams. The TCR candidate information and flow analysis plot were summarized in **Table 4** and **Figure 3** . These neoE-specific T cells recognize mutations from 4 genes (KLC2, MCM3, PLCB1, STXBP5L) presented by 5 different comPACTs on HLA-B35:01, HLA-A11:01, HLA-A01:01, which were illustrated in the imPACT plot **(Figure 4)** .

**Table 4. Summary of selected TCR candidates for PS and GE.**

| EXPID | Sample ID | well.position | TRA | TRB | Patient ID | PACT ID | TCR ID | compPACT sense oligo name | comPACT ID | Gene | neoE | HLA | Predicted sticky comPACT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21000743 | M01 | Plate01-F07 | CAVYPRMNYGGATNKLIF | CASSHSLVEPNSGNTIYF | 0315 | PACT510C | TCR1011 | PACT510C_T_PP001679_O85_S_1_HLA-A11:01 | comPACT38550 | PLCB1 | FTMTTKISFKE | HLA-A11:01 | N |
| 21000743 | M01 | Plate06-H02 | CAVRDMREGFKTIF | CASSPPIQGFKQFF | 0315 | PACT510C | TCR1012 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 | N |
| 21000743 | M01 | Plate02-A07 | CATVYNAGNNRKLIW | CASSQATTGFSYEQYF | 0315 | PACT510C | TCR1013 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 | N |
| 21000743 | M01 | Plate02-A07 | CATVYNAGNNRKLIW | CASSQATTGFSYEQYF | 0315 | PACT510C | TCR1013 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | N |
| 21000743 | M01 | Plate02-A10 | CAVRDIEGKSTF | CASSSLVQGYEQFF | 0315 | PACT510C | TCR1014 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 | N |
| 21000743 | M01 | Plate02-A10 | CAVRDIEGKSTF | CASSSLVQGYEQFF | 0315 | PACT510C | TCR1014 | PACT510C_T_PP001679_O55_S_2_HLA-A01:01 | comPACT38732 | STXBP5L | TTEENRENFYN | HLA-A01:01 | N |
| 21000743 | M01 | Plate02-B04 | CAVRDIVNNNAGNMLTF | CASSSGTGAAYGYTF | 0315 | PACT510C | TCR1015 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | N |
| 21000743 | M01 | Plate02-B04 | CAVRDIVNNNAGNMLTF | CASSSGTGAAYGYTF | 0315 | PACT510C | TCR1015 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 | N |
| 21000743 | M01 | Plate02-B09 | CAYRGSVTGNQFYF | CSALDRGFRSPLHF | 0315 | PACT510C | TCR1016 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | N |
| 21000743 | M01 | Plate02-B09 | CAYRGSVTGNQFYF | CSALDRGFRSPLHF | 0315 | PACT510C | TCR1016 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 | N |
| 21000743 | M01 | Plate07-C07 | CALTTSGTYKYIF | CASSPHGNSPLHF | 0315 | PACT510C | TCR1017 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 | N |
| 21000743 | M01 | Plate07-C07 | CALTTSGTYKYIF | CASSPHGNSPLHF | 0315 | PACT510C | TCR1017 | PACT510C_T_PP001679_O55_S_2_HLA-A01:01 | comPACT38732 | STXBP5L | TTEENRENFYN | HLA-A01:01 | N |
| 21000743 | M01 | Plate05-C05 | CAGGGFSGYSTLTF | CASSTPRRQGDTEAFF | 0315 | PACT510C | TCR1018 | PACT510C_T_PP001679_O80_S_1_HLA-A11:01 | comPACT38577 | MCM3 | LIGDPFVAK | HLA-A11:01 | N |
| 21000743 | M01 | Plate03-E10 | CAVRDENDKIIF | CASSPLTFGYGYTF | 0315 | PACT510C | TCR1019 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 | N |
| 21000743 | M01 | Plate03-F06 | CAVRDLVNNAGNMLTF | CASSHGGGAGGYTF | 0315 | PACT510C | TCR1020 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | N |
| 21000743 | M01 | Plate03-F06 | CAVRDLVNNAGNMLTF | CASSHGGGAGGYTF | 0315 | PACT510C | TCR1020 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 | N |
| 21000743 | M01 | Plate04-B04 | CAVRDLVTGANNLFF | CASSLGGGADYGYTF | 0315 | PACT510C | TCR1021 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | N |
| 21000743 | M01 | Plate04-B04 | CAVRDLVTGANNLFF | CASSLGGGADYGYTF | 0315 | PACT510C | TCR1021 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 | N |
| 21000743 | M01 | Plate04-B10 | CAVSLTYSTLTF | CASRGTYGYTF | 0315 | PACT510C | TCR1022 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | N |
| 21000743 | M01 | Plate04-B10 | CAVSLTYSTLTF | CASRGTYGYTF | 0315 | PACT510C | TCR1022 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 | N |
| 21000743 | M01 | Plate04-F11 | CAVRDLLAAGNKLTF | CASRLRESAPEAFF | 0315 | PACT510C | TCR1023 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | N |
| 21000743 | M01 | Plate04-F11 | CAVRDLLAAGNKLTF | CASRLRESAPEAFF | 0315 | PACT510C | TCR1023 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 | N |
| 21000743 | M01 | Plate05-A09 | CAMREGSTDKLIF | CASSNTGGLNSPLHF | 0315 | PACT510C | TCR1024 | PACT510C_T_PP001679_O85_S_1_HLA-A11:01 | comPACT38550 | PLCB1 | FTMTTKISFKE | HLA-A11:01 | N |
| 21000743 | M01 | Plate05-D09 | CAVRDVGPGGGNKLTF | CASSLGAGGYYGYTF | 0315 | PACT510C | TCR1025 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | N |
| 21000743 | M01 | Plate05-D09 | CAVRDVGPGGGNKLTF | CASSLGAGGYYGYTF | 0315 | PACT510C | TCR1025 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 | N |
| 21000743 | M01 | Plate05-G06 | CAFTVSGTYKYIF | CASSFHGSSPLHF | 0315 | PACT510C | TCR1026 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 | N |
| 21000743 | M01 | Plate06-B03 | CAVRDLTGFGNVLHC | CASSQGGGTYGYTF | 0315 | PACT510C | TCR1027 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | N |
| 21000743 | M01 | Plate06-B03 | CAVRDLTGFGNVLHC | CASSQGGGTYGYTF | 0315 | PACT510C | TCR1027 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 | N |
| 21000743 | M01 | Plate06-C02 | CAVRDIQAGTALIF | CSARRTSGAVGGETQYF | 0315 | PACT510C | TCR1028 | PACT510C_T_PP001679_O202_S_4_HLA-B35:01 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | N |
| 21000743 | M01 | Plate06-C02 | CAVRDIQAGTALIF | CSARRTSGAVGGETQYF | 0315 | PACT510C | TCR1028 | PACT510C_T_PP001679_O203_S_1_HLA-B35:01 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 | N |
| 21000743 | M01 | Plate06-C12 | CAAPSNDYKLSF | CASRGHYNSPLHF | 0315 | PACT510C | TCR1029 | PACT510C_T_PP001679_O54_S_1_HLA-A01:01 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 | N |

| 21000743 | M01 | Plate06-C12 | CAAPSNDYKLSF | CASRGHYNSPLHF | 0315 | PACT510C | TCR1029 | PACT510C_T_PP001679_O55_S_2_HLA-A01:01 | comPACT38732 | STXBP5L | TTEENRENFYN | HLA-A01:01 | N |
| 21000743 | M01 | Plate06-D08 | CAGGGFSGYSTLTF | CASSTPRRQGGTEAFF | 0315 | PACT510C | TCR1030 | PACT510C_T_PP001679_O80_S_1_HLA-A11:01 | comPACT38577 | MCM3 | LIGDPFVAK | HLA-A11:01 | N |



| TCR-ID | Count |
|--------|-------|
| PACT510C_TCR1030 | 1.00 |
| PACT510C_TCR1028 | 1.00 |
| PACT510C_TCR1027 | 1.00 |
| PACT510C_TCR1026 | 1.00 |
| PACT510C_TCR1025 | 1.00 |
| PACT510C_TCR1024 | 1.00 |
| PACT510C_TCR1023 | 1.00 |
| PACT510C_TCR1022 | 1.00 |
| PACT510C_TCR1021 | 1.00 |
| PACT510C_TCR1020 | 1.00 |
| PACT510C_TCR1019 | 1.00 |
| PACT510C_TCR1015 | 1.00 |
| PACT510C_TCR1013 | 1.00 |
| PACT510C_TCR1011 | 1.00 |
| PACT510C_TCR1016 | 2.00 |
| PACT510C_TCR1012 | 7.00 |
| PACT510C_TCR1014 | 8.00 |
| PACT510C_TCR1017 | 2.00 |
| PACT510C_TCR1018 | 14.0 |
| PACT510C_TCR1029 | 2.00 |
| Sorted | 548 |
| Analyzed | 7929 |

**Figure 3. Flow plot of the neo-E specific TCR candidates.** The gray dots in the flow plot were cells recorded as background to help to draw the gate. The colored dots in the flow plot were single cells sorted. (a) Flow plot of tetramer staining. Q2 gate indicated dual APC-comPACT and PE-comPACT tetramer positive cells. (b) Flow plot of CD45RA/CD95 staining. Q5 gate indicated naïve T cells, while Q6 and Q7 gates indicated antigen-experienced cells. (c) Flow plot of CD39/CD103 staining. Q10 gate indicated tumor trafficked T cells. Refer to **Figure 2** for gating strategy for CD45RA/CD95 and CD39/CD103.



| TCR ID | comPACT | Gene | neoE | HLA | T cell count |
|--------|---------|------|------|-----|--------------|
| PACT510C_TCR1015 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | 1 |
| PACT510C_TCR1016 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | 2 |
| PACT510C_TCR1020 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | 1 |
| PACT510C_TCR1021 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | 1 |
| PACT510C_TCR1022 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | 1 |
| PACT510C_TCR1023 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | 1 |
| PACT510C_TCR1025 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | 1 |
| PACT510C_TCR1027 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | 1 |
| PACT510C_TCR1028 | comPACT38484 | KLC2 | FPNEDEQSL | HLA-B35:01 | 1 |
| PACT510C_TCR1013 | comPACT38487 | KLC2 | FPNEDEQSLA | HLA-B35:01 | 1 |
| PACT510C_TCR1011 | comPACT38550 | PLCB1 | ITHGFTMTTK | HLA-A11:01 | 1 |
| PACT510C_TCR1024 | comPACT38550 | PLCB1 | ITHGFTMTTK | HLA-A11:01 | 1 |
| PACT510C_TCR1018 | comPACT38577 | MCM3 | LIGDPFVAK | HLA-A11:01 | 14 |
| PACT510C_TCR1030 | comPACT38577 | MCM3 | LIGDPFVAK | HLA-A11:01 | 1 |
| PACT510C_TCR1010 | comPACT38588 | CAND1 | LLDTVLSHLYN | HLA-A01:01 | 1 |
| PACT510C_TCR1012 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 | 7 |
| PACT510C_TCR1014 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 | 8 |
| PACT510C_TCR1017 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 | 2 |
| PACT510C_TCR1019 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 | 1 |
| PACT510C_TCR1026 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 | 1 |
| PACT510C_TCR1029 | comPACT38731 | STXBP5L | TTEENRENFY | HLA-A01:01 | 2 |

**Figure 4. imPACT Plot for the TCR candidates passed to gene editing and protein science.** (Top) 33 different TCRs recognize mutations from 4 different genes presented by 5 different comPACTs on HLA-B35:01, HLA-A11:01, HLA-A01:01 HLAs. (bottom) Neoepitopes are displayed by highest to lowest predicted HLA binding affinity (x-axis). The left y axis displays each neoepitopes cellular prevalence (CP, surrogate measure of clonal/truncal mutations). Green circle indicates a clonal/truncal mutation, while red circle indicates a sub-clonal mutation. The right y axis displays each neoepitope mRNA read from tumor RNA sequencing.

## 3. CONCLUSIONS:
In this study, 11.4 M PBMC from 0315(PACT510C) were analyzed by the imPACT process. The key findings are summarized as following:

| | 21-211 patient 0315 imPACT analysis | Version 1.5 |
|---|---|---|

- 593 single cells were sorted and sequenced.
- 21 TCR ( 50 T cells) were predicted to be potential neoE-specific TCRs based on signal to noise analysis.
- 1 TCR were identified to come from naïve T cell.
- 0 TCR were excluded as non-specific or non-confident.
- 0 TCR was excluded from down selection.
- 20 unique TCR sequences were selected for GE and PS, recognizing mutations from 4 different genes presented by 5 different comPACT on 3 HLA. These TCR sequences were passed to GE and the cognate comPACTs were ordered for large scale production from PS.
- Based on the acceptance criteria in PD-005, this analysis was considered successful.

**ADDITIONAL COMMENTS:**

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