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

Study #: 21-211

Patient ID: 0315

Study Description: PACT-0101_0315_imPACT_PACT510C_M01

Responsible Scientist: Craig Fett

Start Date Of Analysis: 18th 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	58	49	40
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			
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-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_O202_S_4_HLA-B35:01	comPACT38484	KLC2	FPNEDEQSL	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-B10	CAVSLTYSTLTF	CASRGTYGYTF	0315	PACT510C	TCR1022	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_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-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-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

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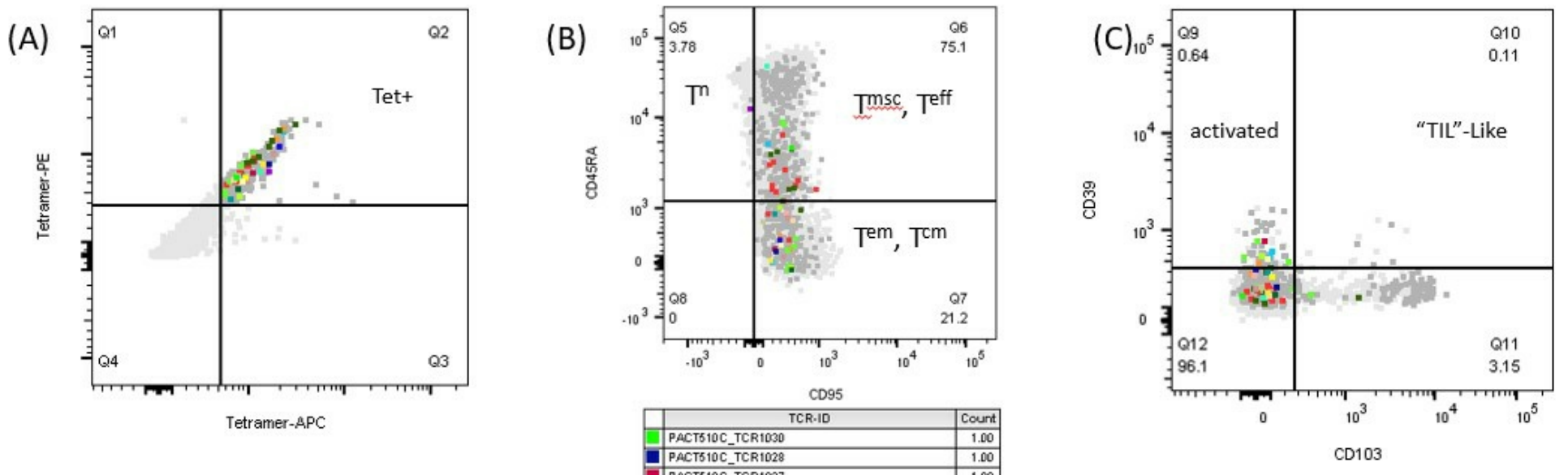
21000743	M01	Plate06-C12	CAAPSNDYKLSF	CASRGHYNSPLHF	0315	PACT510C	TCR1029	PACT510C_T_PP001679_O54_S_1_HLA-A01:01	comPACT38731	STXBP5L	TTEENRENFY	HLA-A01:01
21000743	M01	Plate06-C12	CAAPSNDYKLSF	CASRGHYNSPLHF	0315	PACT510C	TCR1029	PACT510C_T_PP001679_O55_S_2_HLA-A01:01	comPACT38732	STXBP5L	TTEENRENFYN	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.



	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_TCR1010	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 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.

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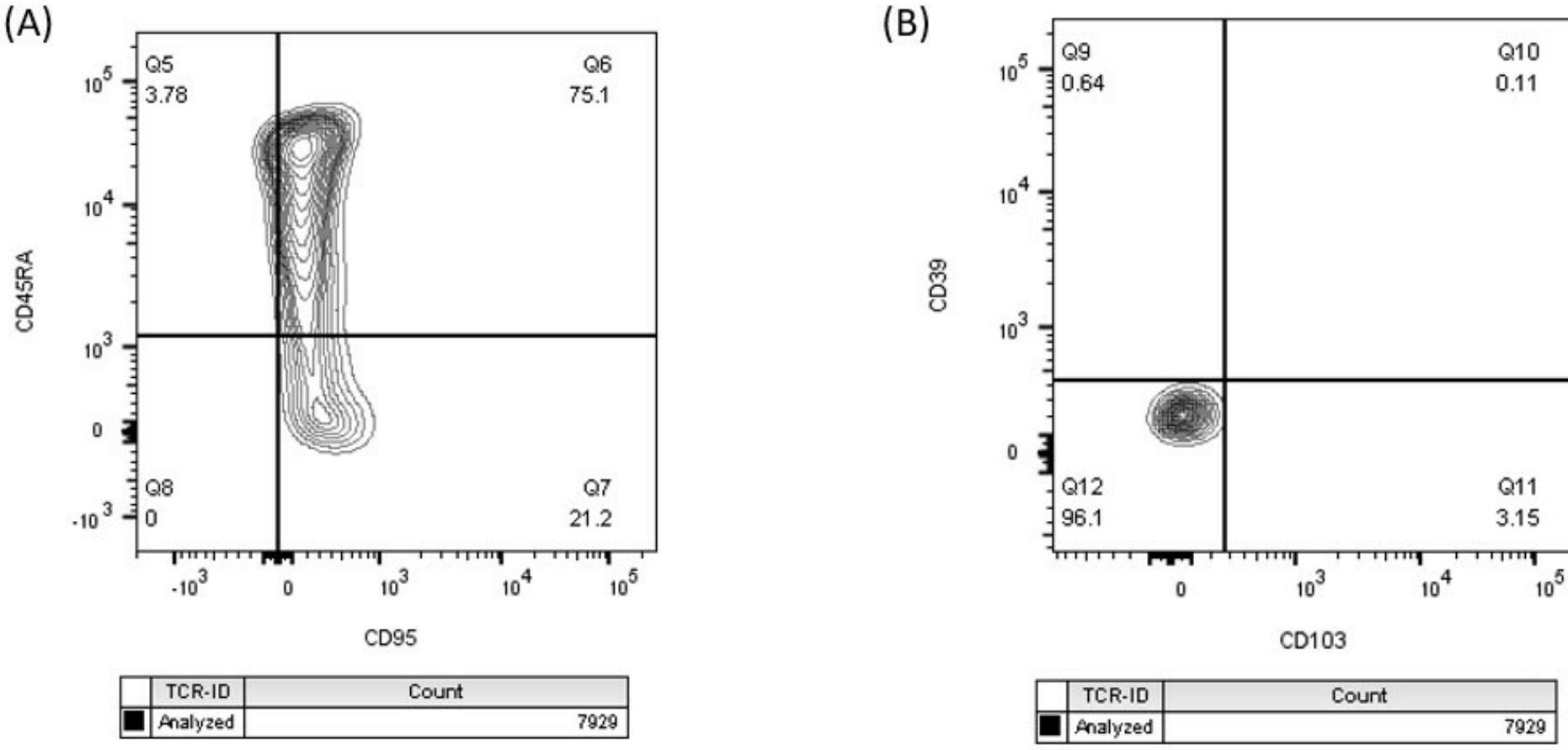

Figure 2. Gating strategy of the CD45RA/CD95 (contour plot) and CD39/CD103 (contour plot). (a) Gating strategy of CD45RA/CD95. The horizontal CD45RA line was first drawn at the contour boundary of the CD45RA positive/negative population. Then the vertical CD95 line was drawn at the cross point of the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

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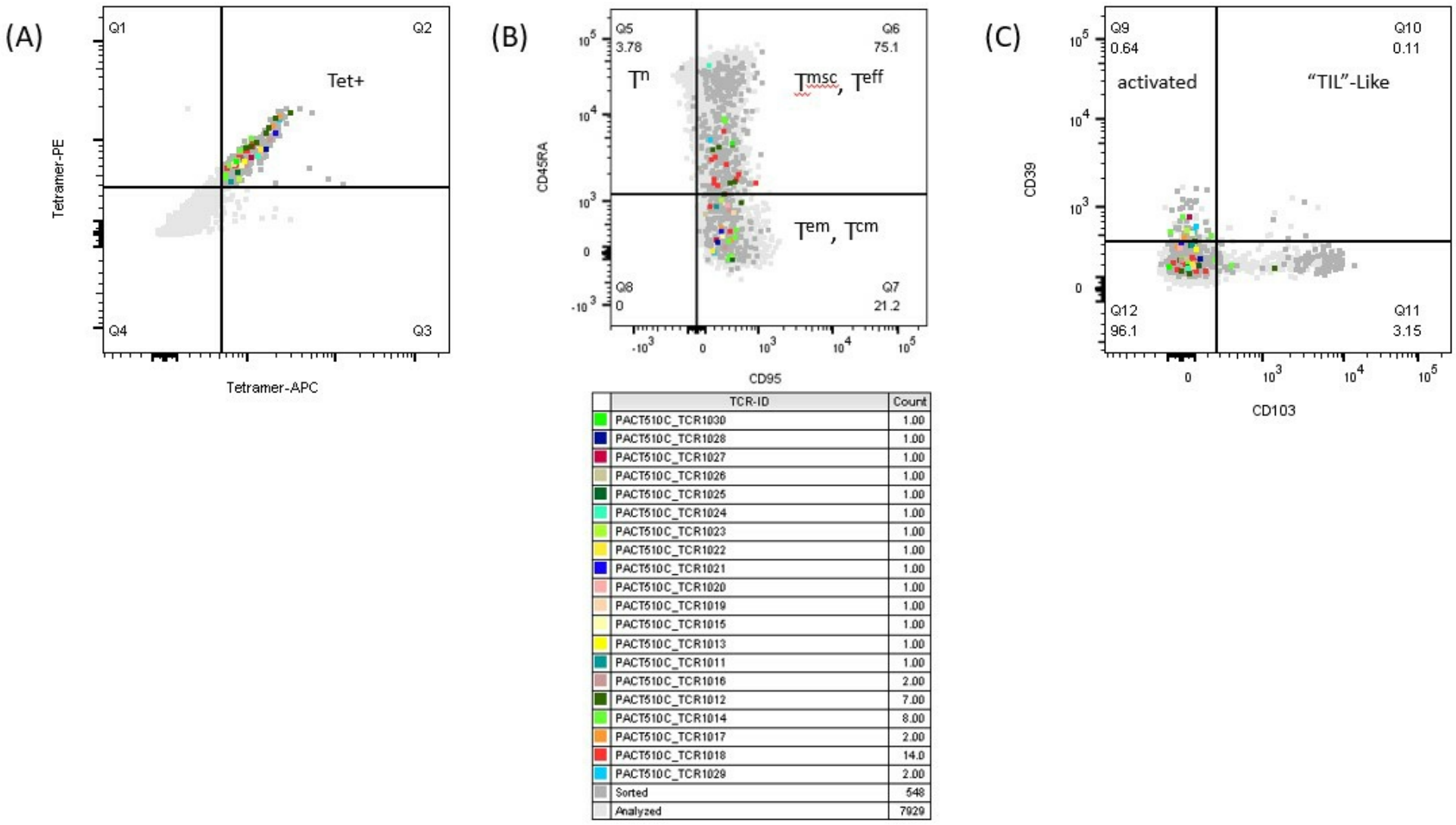
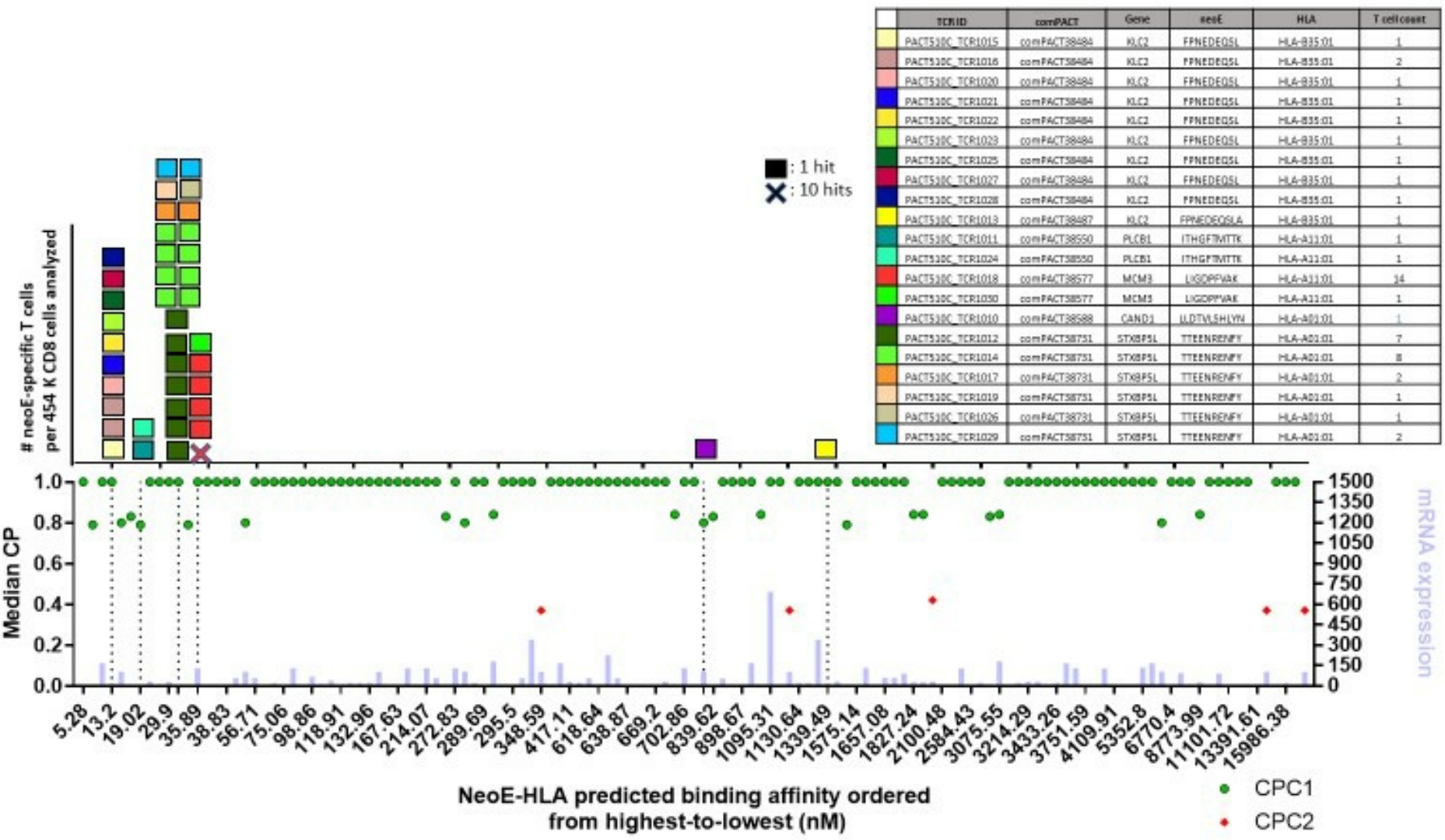


	TCR-ID	Count
	PACT510C_TCR1030	1.00
	PACT510C_TCR1028	1.00
	PACT510C_TCR1027	1.00
	PACT510C_TCR1026	1.00
	PACT510C_TCR1025	1.00
	PACT510C_TCR1024	1.00
	PACT510C_TCR1023	1.00
	PACT510C_TCR1022	1.00
	PACT510C_TCR1021	1.00
	PACT510C_TCR1020	1.00
	PACT510C_TCR1019	1.00
	PACT510C_TCR1015	1.00
	PACT510C_TCR1013	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	STX8P5L	TTEENRENFY	HLA-A01:01	7
PACT510C_TCR1014	comPACT38731	STX8P5L	TTEENRENFY	HLA-A01:01	8
PACT510C_TCR1017	comPACT38731	STX8P5L	TTEENRENFY	HLA-A01:01	2
PACT510C_TCR1019	comPACT38731	STX8P5L	TTEENRENFY	HLA-A01:01	1
PACT510C_TCR1026	comPACT38731	STX8P5L	TTEENRENFY	HLA-A01:01	1
PACT510C_TCR1029	comPACT38731	STX8P5L	TTEENRENFY	HLA-A01:01	2

Figure 4. imPACT Plot for the TCR candidates passed to gene editing and protein science. (Top) 27 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:

- 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.

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- 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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