Development of Personalized Therapeutics Using Neo7logix Precision Profiling in Lung Cancer

Anton Yuryev ^A, John Catanzaro ^B, Md Shamsuddin Sultan Khan ^C

Supplementary Information

PBIMASM Lung Cancer Final Selection

<u>#</u>	<u>Protein</u>	Sequence
1	NCAM1	ATGGVSILK
2	PTPN11	YINANIIML
3	SEMA5A	ISYKEIGLW
4	KRT5	FSASSGLGL
5	GRIN2B	ISAQTVTPI
6	DST	LSGKGFHSW
7	PTPRH	MLTNCMEAV
8	CREBBP	ISYLDIIHF
9	CSMD3	CSSVTEPRF
10	CCDC178	ITNTEGVNK
11	FLT4	GTDARTYCK
12	AVPR1B	GLDEELAKV
13	NOTCH1	EPTSESPFY
14	ERBB4	VQIAKGMIY
15	PCDH10	DSVPDTELF
16	SERPINB4	ESYDLKETF
17	MSLN	QPLPQVATL
18	TP53	HMTEVVRRY
19	CYP2E1	RFGPVFTLH

Green is good binding affinity Yellow is average binding affinity Red is based upon criteria 2-6 below HLA epitope prediction and ranking is accomplished by the following criteria:

- 1. Strongest Binding Affinity in one HLA Class
- 2. Strongest / Med Binding Affinity to identical peptide sequence within multiple HLA classes
- 3. Binding Affinity Averaging selection based upon top average
- 4. Structural considerations for HLA-peptide-T-cell receptor complex assembly

Note: Immunopeptides were selected based upon the following criteria and not upon HLA binding affinity alone:

- 1. HLA Affinity Ranking
- 2. Biological Pathway Ranking
- 3. Association with Specified Cancer Risk In Population
- 4. Literature Support
- 5. Pivotal Molecular Protein to Protein Interactions and Cross Talk (PPI-CT) in Immune Augmentation
- 6. Antigen Integrity and Sequence Viability (ThermoFisher Antigen / Synthesis Analyzer)

Clarification on Ranking: Reds were selected based upon criteria 2-6 and will be noted as self-antigens externally synthesized and introduced as foreign epitopes for specified influences on Immuno-molecular controls on receptor signaling, sensitization or blockade.

Peptide Pool Manufacturing Criteria

Peptide QC tests for manufacturer:

- Identity test: MS+HPLC
- Water content test <10%
- Solvent residue test < 0.05%
- Endotoxin test <0.3 IU/mg

Additional Criteria:

Peptide Sequences: On average we will have 10-20 9-amino acid (9 mer) peptides, we will have to run the following QC tests on them as listed above. Some tests should be run on entire mix (pool) of peptides to decrease amount of material needed:

Cost Reduction: Let us know if you can combine solvent residue analysis with MS or HPLC to reduce cost.

Quantity: We will need 100 mg of each sequence per patient.

Logistics: The objective is to reduce cost of final product and also meet requirements. Also, to meet logistic expectations of quick TAT, transit and final packaging with high quality peptide product.

Nano-Adjuvant Requirements: MF59 and CPG-ODN