Should be directive.. Proteome WILL BE constructed

Assignment 1: Flow chart of figure 3 of:

Autoantigen discovery with a synthetic human peptidome, Larman et al. 2011

Hypothesis: Phage Immunoprecipitation sequencing (PhIP-Seq), using T7-Pep library, can lead to the discovery of novel autoantigens.

Construction of T7 phage display library (T7-Pep)

Synthetic representation of human proteome was constructed using all open reading frames from **build 35.1** of human genome. Peptide coding sequences were cloned in a derivative of T7Select 10-3b phage display vector (so that all peptides would have a C-terminal FLAG-tag)

Techniques used for library optimization:

- Plaque PCR analysis
- Illumina sequencing
- Chao1 analysis (to estimate library complexity)
- Phage immunoprecipitation (anti-FLAG serum Antibodies)

Incubation of unmodified CSF of PND (Paraneoplastic neurological syndrome) patients A, B, and C with T7-Pep

- **SAPK4** commercial antibody was added to Patient A's CSF at the incubation stage as a positive control to check library functionality
- Immuno-precipitation using beads with proteins A and G
- Unbound phages were washed away, and the enriched population was collected for further analysis

Immuno-precipitation

- **PCR** amplification of DNA from enriched phages
- **Sequencing** (to determine predicted autoantigens)

Patient A

63 years old female
Non-Small Cell Lung Cancer and classical cerebellar syndrome
CSF positive for NOVA antibodies

Predicted Autoantigens:

- TGIF2LX
- DBR1
- PCDH1

Hypothetical protein:

- LOC26080

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Patient B

59 years old female
Non-Small Cell Lung Cancer, dysarthria, ataxia, head titubation, and muscle lock
CSF negative to panel of commercial PND autoantigens

Predicted Autoantigens:

- TGIF2LX
- CTAG2
- GAD65

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Patient C

59 years old female
Melanoma, dysarthria, ataxia, and horizontal gaze palsy
CSF negative to panel of commercial PND autoantigens

Predicted Autoantigens:

5 peptides from 2 homologous members of TRIM family:

- TRIM 9
- TRIM 67

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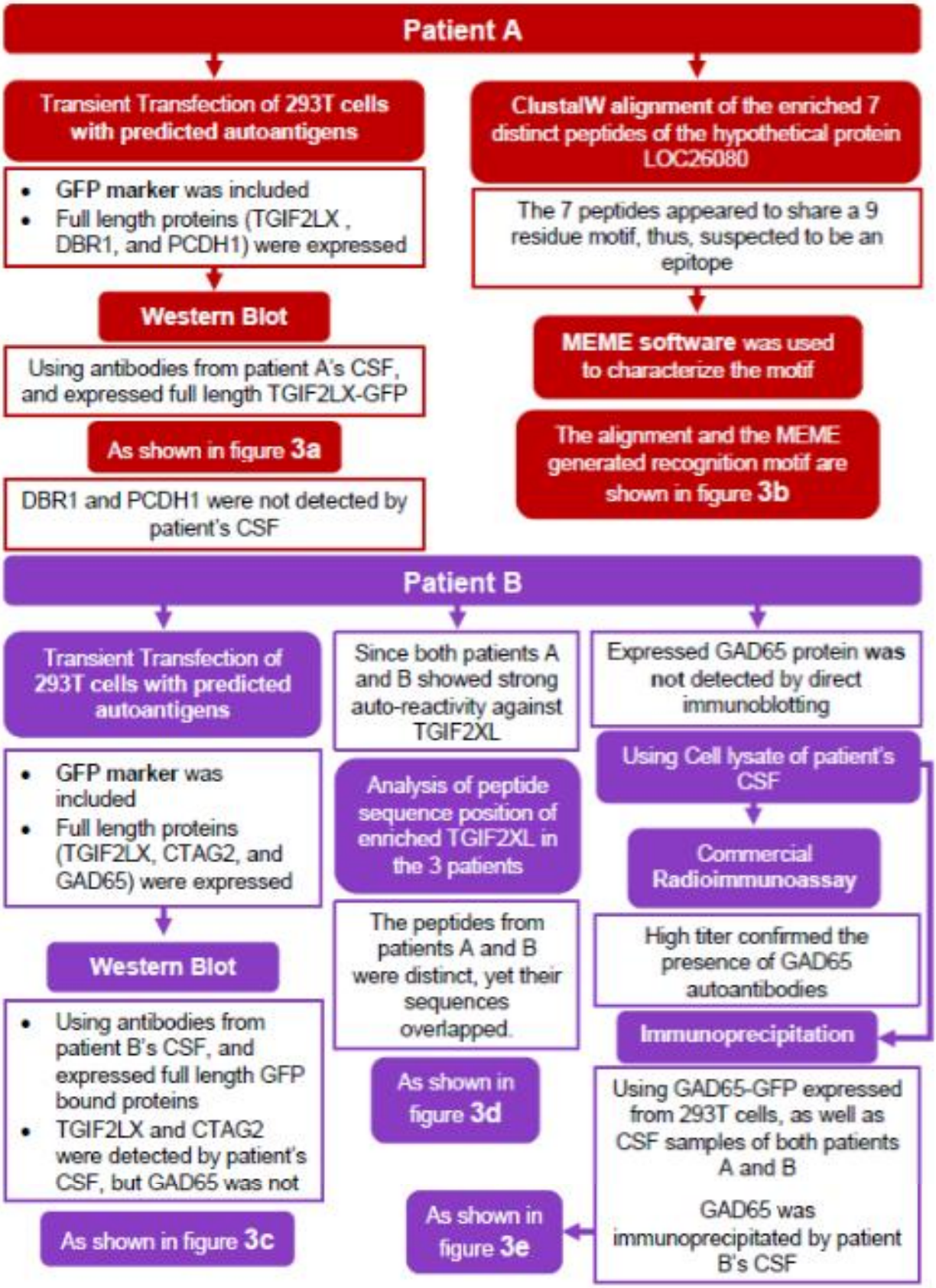
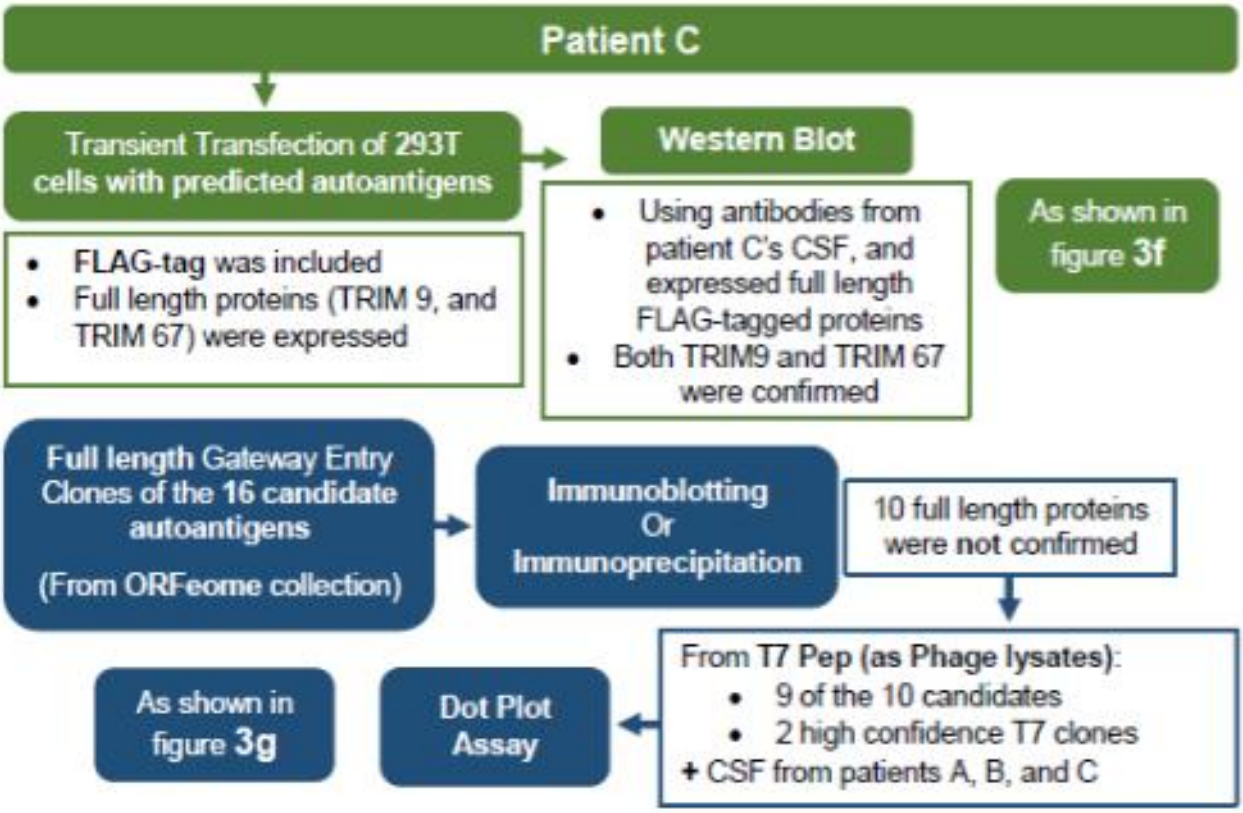

Patient A
Transient Transfection of 293T cells with predicted autoantigens
GFP marker was included
Full length proteins (TGIF2LX , DBR1, and PCDH1) were expressed
Western Blot
Using antibodies from patient A's CSF, and expressed full length TGIF2LX-GFP
As shown in figure 3a
DBR1 and PCDH1 were not detected by patient's CSF
ClustalW alignment of the enriched 7 distinct peptides of the hypothetical protein LOC26080
The 7 peptides appeared to share a 9 residue motif, thus, suspected to be an epitope
MEME software was used to characterize the motif
The alignment and the MEME generated recognition motif are shown in figure 3b
Patient B
Transient Transfection of 293T cells with predicted autoantigens
GFP marker was included
Full length proteins (TGIF2LX, CTAG2, and GAD65) were expressed
Western Blot
Using antibodies from patient B's CSF, and expressed full length GFP bound proteins
TGIF2LX and CTAG2 were detected by patient's CSF, but GAD65 was not
As shown in figure 3c
Since both patients A and B showed strong auto-reactivity against TGIF2XL
Analysis of peptide sequence position of enriched TGIF2XL in the 3 patients
The peptides from patients A and B were distinct, yet their sequences overlapped.
As shown in figure 3d
Expressed GAD65 protein was not detected by direct immunoblotting
Using Cell lysate of patient's CSF
Commercial Radioimmunoassay
High titer confirmed the presence of GAD65 autoantibodies
Immunoprecipitation
Using GAD65-GFP expressed from 293T cells, as well as CSF samples of both patients A and B
GAD65 was immunoprecipitated by patient B's CSF
As shown in figure 3e



Discussion and conclusion:

- Phage Immunoprecipitation sequencing, using T7-Pep library, has led to the discovery of new candidate autoantigens in 3 patients with paraneoplastic neurological syndrome.
- Secondary and tertiary structures of an antigen are sometimes crucial to its immunoreactivity. As demonstrated in patient B, GAD65 epitopes showed no reactivity by direct immunoblotting, since the structure was denatured. Whereas, the immunoprecipitation of the patient's CSF cell lysate against GAD65-GFP was successful.
- Proteins displayed on the T7 phage coat, retain a significant amount of secondary structure. This was demonstrated when phage lysates of 9 candidate autoantigens showed immunoreactivity in a dot plot assay, where the full length Gateway Entry clones of the same candidates showed no immunoreactivity.

References

Larman, HB, Laserson, U, Church, GM, Ciccia, A, Gakidis, MAM, Zhao, Z, et al. 2011. Autoantigen discovery with a synthetic human peptidome. *Nature Biotechnology*. **29**(6):535-41.