Discovery of Antigenic peptides for immunotherapy

Jagannath Swaminathan, Cofounder of Erisyon Inc. – jagannath@erisyon.com

- **Erisyon introduces the platform technology, fluorosequencing, for single molecule peptide sequencing.**
- **Tumor cells display mutated forms of peptides (antigenic) on their MHC-I protein complex. Identifying them is critical for developing a T-cell immunotherapy or cancer vaccines.**
- **Currently, DNA and RNA sequencing is performed to indirectly predict these antigenic peptides. Mass spectrometry is not sensitive enough to identify these peptides from the tissue biopsy.**
- **In combination with genomic prediction, fluorosequencing is capable to directly sequence and confirm the antigenic peptides, owing to its high sensitivity and low sample requirements.**

Erisyon (a development stage startup) introduces the needed technology for identification of multitude of peptides and proteins present in complex biological samples at the unprecedented sensitivity of a single molecule. We, scientists at the University of Texas at Austin, are developing this patented platform technology (*fluorosequencing*) to first isolate and identify the antigenic peptides presented by tumor cells. Identifying these target antigens is a critical

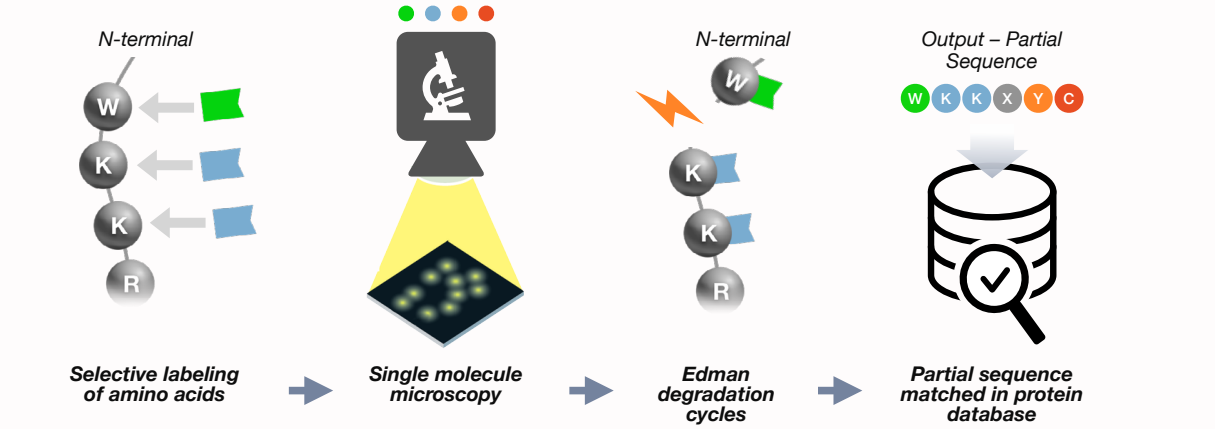

Figure 1. Principle of fluorosequencing *– A) Every bright spot shown in the image from the microscope is a single protein molecule. The change in the intensity of a spot after a cycle of chemistry correlates to a specific protein signature. By tracking intensity changes for many spots across multiple chemistry cycles, we can accurately identify billions of different proteins in a sample. B) Using samples supplied by customers, peptides are isolated and labeled for fluorosequencing. Using our patented technology, we sequence them at the single molecule level to identify and digitally quantify the proteins in the sample.*

unmet need for the development of a successful *adoptive cell immunotherapy*.

Promise of endogenous T-cell therapy

Protein complex on the cell surface, called the *Major Histocompatibility Complex* (MHC) or Human Leukocyte Antigen complex (HLA), present a diverse set of peptides (9-11 amino acid long) called MHC class-I peptides. These peptides can be considered as a display signal, indicating the health of the cell. The body's immune system, comprising a class of cells called *T-cells*, has been trained to identify the naturally occurring displayed peptides (that correspond to healthy cells) and not engage. However, with viral infection or cancer, there are new or mutated proteins produced by the cells that results in the display of new set of MHC peptides, called neoantigens. Under normal and healthy conditions, the T-cells typically recognize these neoantigens, engages with these aberrant cells and trigger the cells to undergo apoptosis. However, in many tumors, the T-cell fails to either recognize the displayed antigenic peptides or engage effectively with the cells. But with the knowledge of the patient's cancer specific neoantigen, the patient's T-cells can be harvested, externally expanded with enhanced functionality to target only cells with the neoantigens and introduce them back into the patient. This treatment modality is termed *endogenous T-cell therapy*, a class within a generalized adoptive cell therapy; its critical component being the identification of the neoantigens[1].

Since these neoantigens are present is extremely low amounts in the tumor sample (approximately 10-50,000 different peptides are displayed in a cell), directly identifying these neoantigen peptides by proteomics method such as mass spectrometry has been found to be extremely difficult (owing to the lack of sensitivity and dynamic range of the mass spectrometers)[2]. Indirect methods, combining the use of genome sequencing, RNA sequencing, antigenic peptide prediction algorithms and invitro testing of the synthesized peptides is the current state of art for neoantigen discovery.

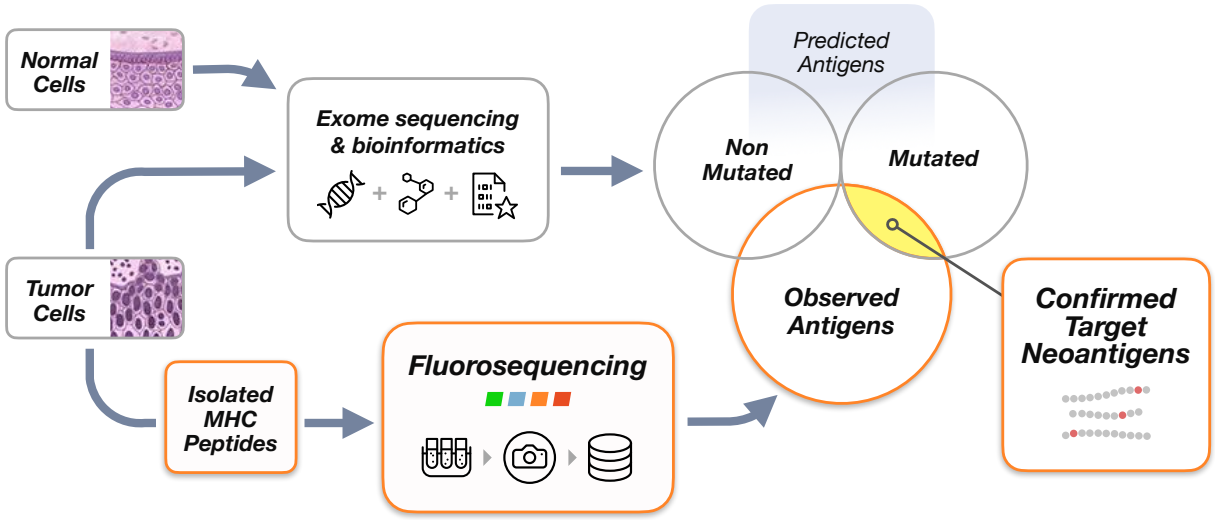

Figure 2. Proposed neoantigen discovery pipeline – *Exome sequencing of tumor and normal cell samples, coupled with bioinformatics tool for antigen prediction would generate a predicted set of mutated peptide and non-mutated peptides. Fluorosequencing results from antigens isolated by tumor samples will provide confirmation of peptide sequences existing in the mutated antigen set. Direct confirmation of some of these antigenic peptides indicates lesser risk in the downstream testing and treatment modalities.*

But this strategy is less than ideal as information of the peptides (such as abundances, post-translational modifications) are lost from nucleic acid translations and many algorithms still predicting only 10-30% of reactive antigenic peptides[3]. Thus a highly sensitive method for directly identifying the MHC peptides displayed by the cancerous cells would open the door for personalized adoptive cell therapies.

Fluorosequencing as a platform technology for antigen discovery

By being able to directly identify single peptide molecules, Erisyon offers the promise for cataloguing the peptides displayed by the cancerous cell. Briefly, the concept for single molecule protein sequencing is based on the principle that the positional information of a small number of amino acid types in a peptide (such as x-C-x-x-C-; x = any amino acid; C = Cysteine) may be sufficiently reflective of the peptides' identity, to allow its identification in a known protein sequence database. To enable the experimental implementation, the following strategy, termed fluorosequencing has been implemented - selectively labeling one or more amino acids with fluorophores, sequentially degrading the immobilized peptides on the slide by Edman chemistry and monitoring the change in fluorescence intensity for each peptide, in parallel, as it loses one amino acid per cycle.

For the purpose of antigen discovery (see **Figure 2** for proposed pipeline), fluorosequencing can obtain partial peptide sequences of the antigens isolated by the tumor biopsy/expanded cells.

Utilizing the patient's HLA information in the prediction algorithms along with the mutated exome sequences found in the tumor cells, the partial peptide sequences will be mapped to identify the antigenic peptides arising from the mutated proteins in the tumor cells. These directly confirmed neoantigens can then be synthesized and used as the target required for endogenous T-cell therapy.

References

1. Dudley, M. E. & Rosenberg, S. A. Nat. Rev. Cancer 3, 666–675 (2003).
2. Yadav, M. et al. Nature 515, 572–576 (2014).
3. Vitiello, A. & Zanetti, M.. Nat. Biotechnol. 35, 815–817 (2017).