A simple blood base test for predicting clinical benefit of cancer immunotherapy

Luhui Shen[1], Jianfeng Chen[3], Stephen Albert Johnston[1,2], David Hong[3], Jianjun Gao[3], Aung Naing[3],
[1]Center for Innovations in Medicine, The Biodesign Institute, Arizona State University, Tempe, AZ 85287 ; [2]Calviri, Inc., Tempe, AZ 85287
[3]UT MD Anderson Cancer Center, Houston, TX 77030

Abstract # 2231

Abstract

Immunotherapy with immune check point inhibitors (CPI) has dramatically transformed cancer therapy. However, only ~25% cancer patients have a positive clinical response to CPI. Moreover, the cost of the treatment and the risk of severe side effects makes it necessary to develop biomarkers to predict the benefit of the treatment.

It has been shown that the tumor neoantigen load correlates with a positive response to treatment. This indicates that pre-existing anti-tumor immune responses to neoantigens can be used for the CPI response prediction. We have discovered a new source of frameshift (FS) neoantigens created by errors in RNA production in tumor cells, including the insertion and deletion (INDEL) of microsatellite regions during the RNA transcription and the mis-splicing of exons. These errors can generate FS neoantigens, which are highly immunogenic and can elicit both T cell and B cell immune response in cancer patients. We have shown that, although most antibody reactivity to FS peptides (FSPs) are personal, there are common antibodies reactive in different cancer patients, even across different cancer types. The FSPs with positive reactive antibodies can offer protection in mouse tumor models as vaccines.

We thus hypothesize that antibodies reactive to FSPs in cancer patients can be used for predicting the clinical benefit of cancer immunotherapy. There is a total of~ 220,000 potential FS neoantigens that can be generated by INDELs of transcription and mis-splicing of genes. These neoantigens can be represented by ~400,000 FSPs, 15-amino acids peptides. We have created arrays of by in-situ synthesis of these FSPs. We used these array to test our hypothesis with pre-treatment serum of 40 cancer patients, from 18 different cancer types, who received a treatment regimen that contained CPI. A total of 14 patients had a clinical response to CPI treatment. Similar to ELISA, diluted serum were applied to the FSP array, and total IgG were detected by fluorescent labeled antibody. IgG reactive to each FSP was measured by the fluorescent intensity and then median normalized within each array for the analysis. As predicted, there are common IgG antibodies reactive to FSPs in the response patients. By selecting 100 to 500 most significantly different reactive FSPs between two groups of patients, and trained with prediction models, such as SVM, our FSP array can reach up to 96% accuracy in the prediction of clinical response with leave-one-out validation. We hypothesize that the FSPs with positive IgG reactive in response patients may be related to anti-tumor immune response, which is need to be further investigated. We also showed that the FSP array can potentially predict the patients who may have high grade immune related adverse events with the CPI treatment.

Background

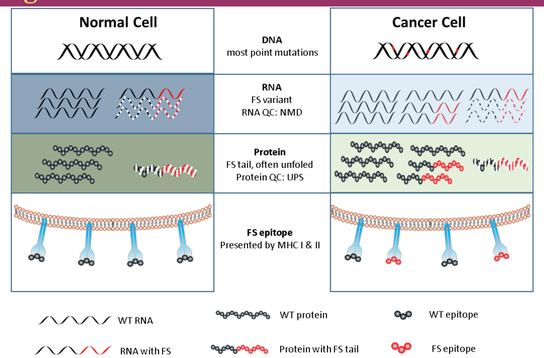

Figure 1. The model for broad, frame-shift peptide production in tumor cells. Normal Cell: Errors in DNA replication are very low and repaired. Transcription error rates are higher but also rare as are mis-splicing during intron excision. Additionally, the cellular QC system will eliminate FS transcripts and aberrant proteins. The net result is that very few FS peptides are presented on MHC or escape the cell to be presented to the immune system. **Cancer Cell**: All levels of information transfer become more error prone, which overwhelms cellular QC system. More errors are made in DNA replication, but only when cells divide. Most DNA mutations are point mutations and encode low or non-immunogenic peptides. Transcription is generally less accurate and even more so through MSs producing Indels. RNA splicing is also far less accurate, creating more FS transcripts from each out-of-frame splicing between exons from the same gene and different genes. Consequently, more truncated proteins with the FS peptide will be translated leading to more FS peptides being presented on MHC I and II and mis-secreted or released from the cancer cell which the immune system can respond to.

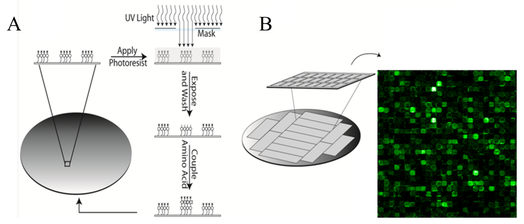

Figure 2. Mask-based synthesis of peptides was performed on silicon wafers with thermal oxide coating, starting with an aminosilane–glycine monolayer and building peptides through cycles of patterned acid formation in a photoresist removing Boc groups from the N-terminal amines of nascent peptides and coupling of the next amino acid B. The wafer is diced into standard slide-sized, each of which contains 16 arrays of 392,000, 8-µm features. Samples are individually applied to each array and scanned on a laser scanner. On the far right is an image of the array (at 800x magnification) of serum applied to the array and antibody binding detected with a fluorescent secondary antibody

Predicting the response to immunotherapy

Table 1. Patient summary. The pre-treatment serum of total 40 cancer patients were collected. It was mixed by total 18 different cancers, including breast cancer, lung cancer, cervical cancer, skin cancer and colorectal cancer, etc.

Response	Age range/Median age(yr)	Male/Female	irAE ≥2
Yes (N=16)	42-75/56	8/8	N=1
No (N=24)	30-83/60	8/16	N=5

Table 2. Evaluation the performance of different significant FSPs. The most significant FSPs were selected based on the two-tailed student t-test. The prediction performance (%) were evaluated by leave-one-out validation with SVM. The top 250 FSPs showed the best performance on the accuracy.

# of FSP selection	Sensitivity	Specificity	NPV	PPV	Accuracy
TOP50	62.5	100	80	100	85
TOP100	75	91.7	84.6	85.7	85
TOP200	87.5	91.7	91.7	87.5	90
TOP250	93.8	95.8	95.8	93.8	95
TOP300	75	95.8	85.2	92.3	87.5
TOP400	75	100	85.7	100	90
TOP500	75	100	85.7	100	90

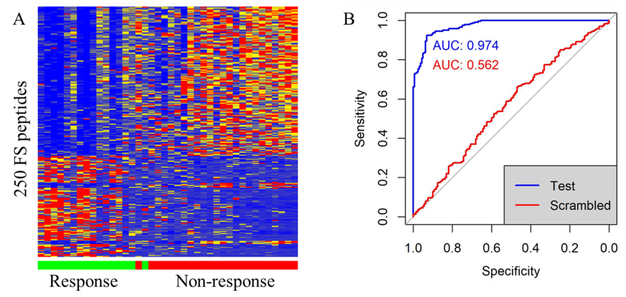

Figure 3. Evaluation the best 250 FS peptides. A. Heatmap of the best 250 FSPs with hierarchical cluster. B. ROC analysis of the best 250 FSPs in the outcome prediction with SVM. Blue curve: the actual 40 samples. Red curve: Randomly assign of these 40 samples of response and non-response.

Predicting irAE

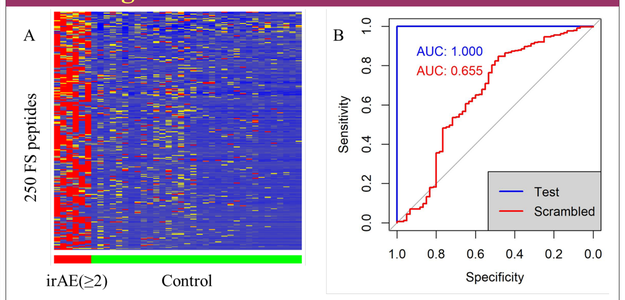

Figure 4. Predicting of high grade (≥ 2) immune related adverse events (irAE) There are total 6 out of 40 patients developed high grade irAE. Total 250 most significant FSPs were selected based on the two tailed student t-test. A. Heatmap of 250 FSPs with hierarchical cluster. B. B. ROC analysis of the best 250 FSPs in the outcome prediction with SVM. Blue curve: the actual 40 samples. Red curve: Randomly assign of 6 patients with high irAE.

Discussion and Future Plan

This preliminary experiment represents the most complex clinical setting with multiple cancer types and different cancer immunotherapy. The results of the experiment showed the potential of our high density frameshift peptide array in predicting clinical benefit of immunotherapy. This also indicates that the predicted FSPs are potential cancer neoantigens involving anti-cancer response.

We plan to expand the experiment and test our hypothesis in a larger sample size. Also we will analysis the significant FSPs in both response/non-response and irAE patients and investigate their roles in anti-cancer immune response and irAE.

Key Reference

1. Zhang, J., L. Shen, and S.A. Johnston, *Using Frameshift Peptide Arrays for Cancer Neo-Antigens Screening.* Sci Rep, 2018. **8**(1): p. 17366
2. Legutki, J.B., et al., *Scalable high-density peptide arrays for comprehensive health monitoring.* Nature communications, 2014. **5**.

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