Use of Whole Exome and RNAseq Analysis to Identify Mutated Tumor Neo-Antigens

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Presenter Disclosure Information

Elaine Mardis

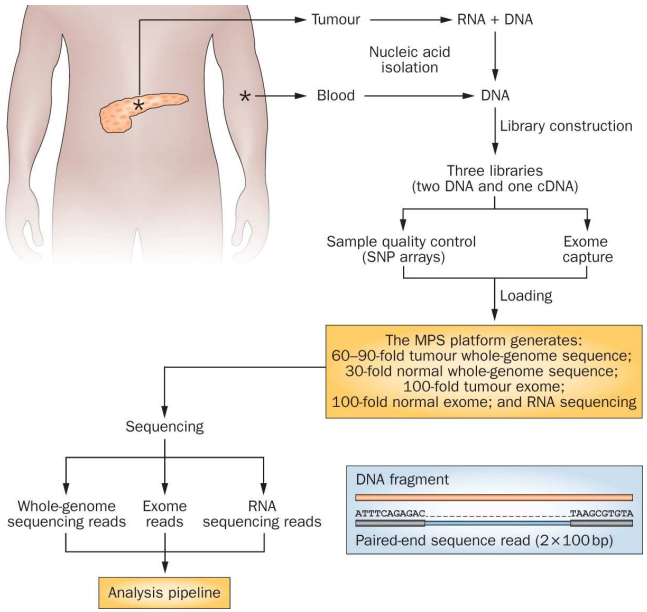
The following relationships exist related to this presentation:

No Relationships to Disclose

Integrating DNA and RNA data in N of 1 Studies

Identification of druggable targets and cancer drivers

A comprehensive clinical sequencing strategy for precision cancer medicine



Expected output

- SNVs (single nucleotide variants)
- Indels (small insertions and deletions)
- CNVs (amplification/deletion)
- Gene/Isoform expression
- SVs (structural variants)
- Gene fusions

Timeline is ~4 weeks

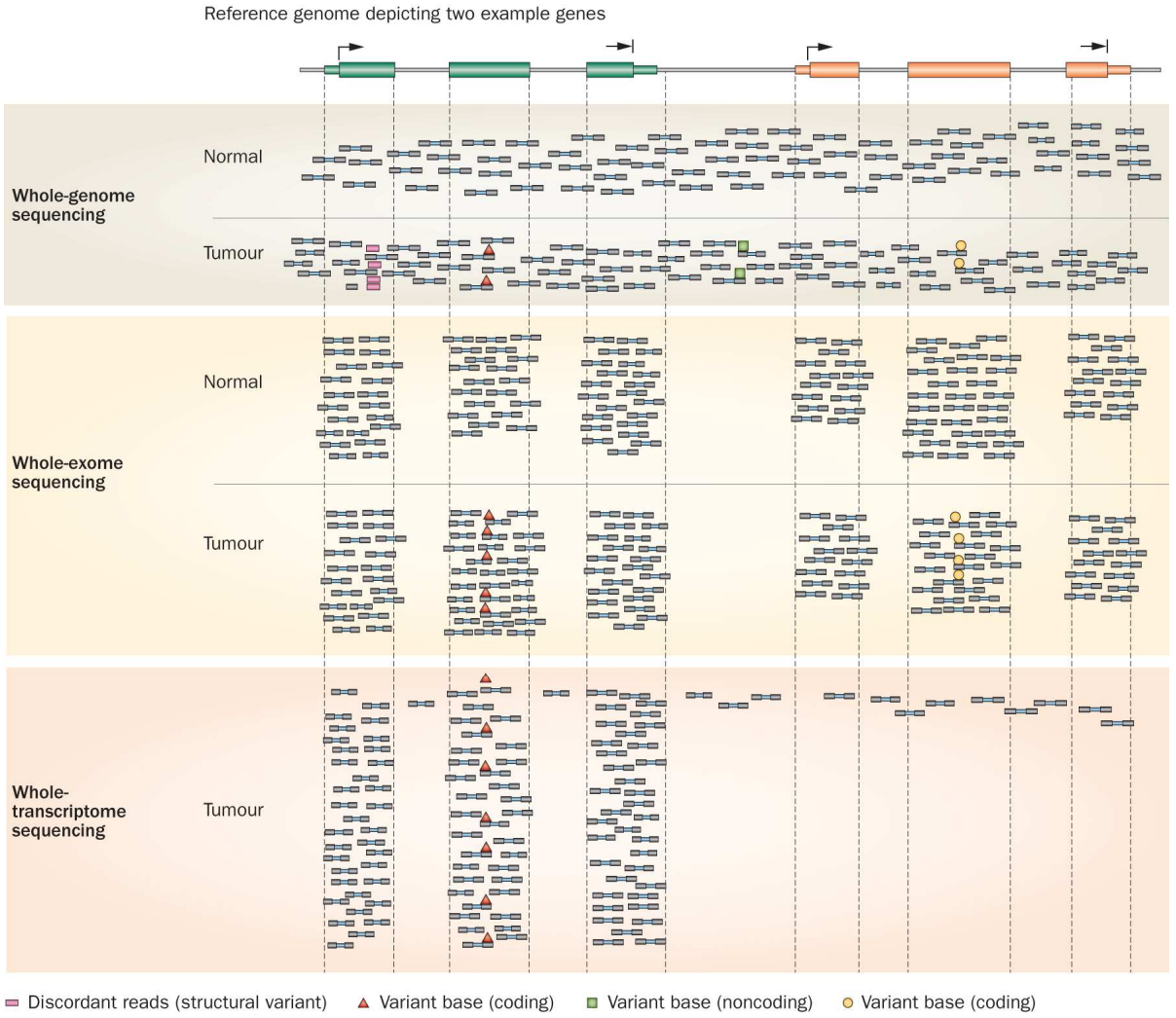
Griffith, M. et al., PLoS Comp. Biol 2015

Combining data types maximizes our power to detect and confirm different event types

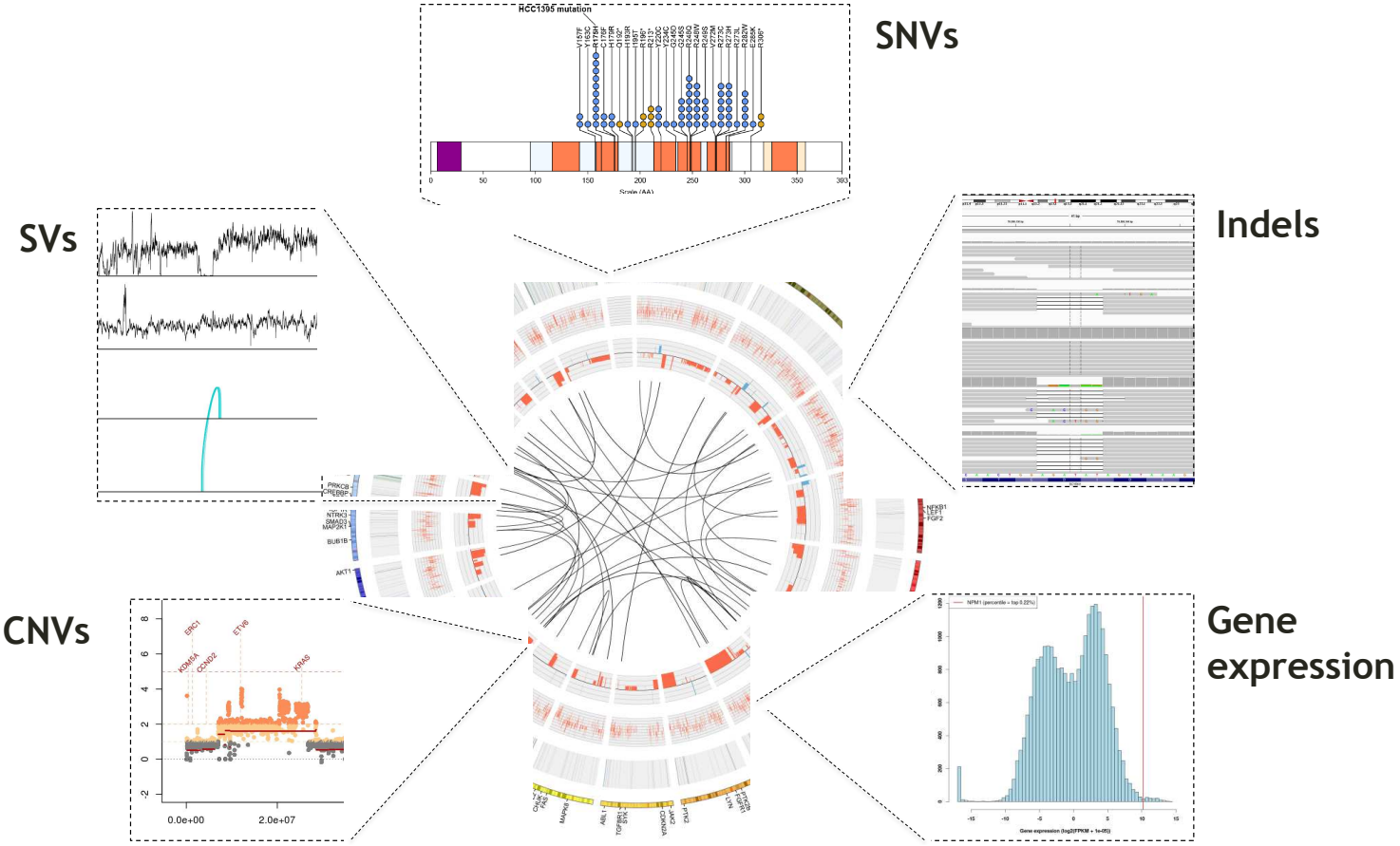
‘WGS’

‘Exome’

‘RNA-seq’



Events of various types discovered in each tumor

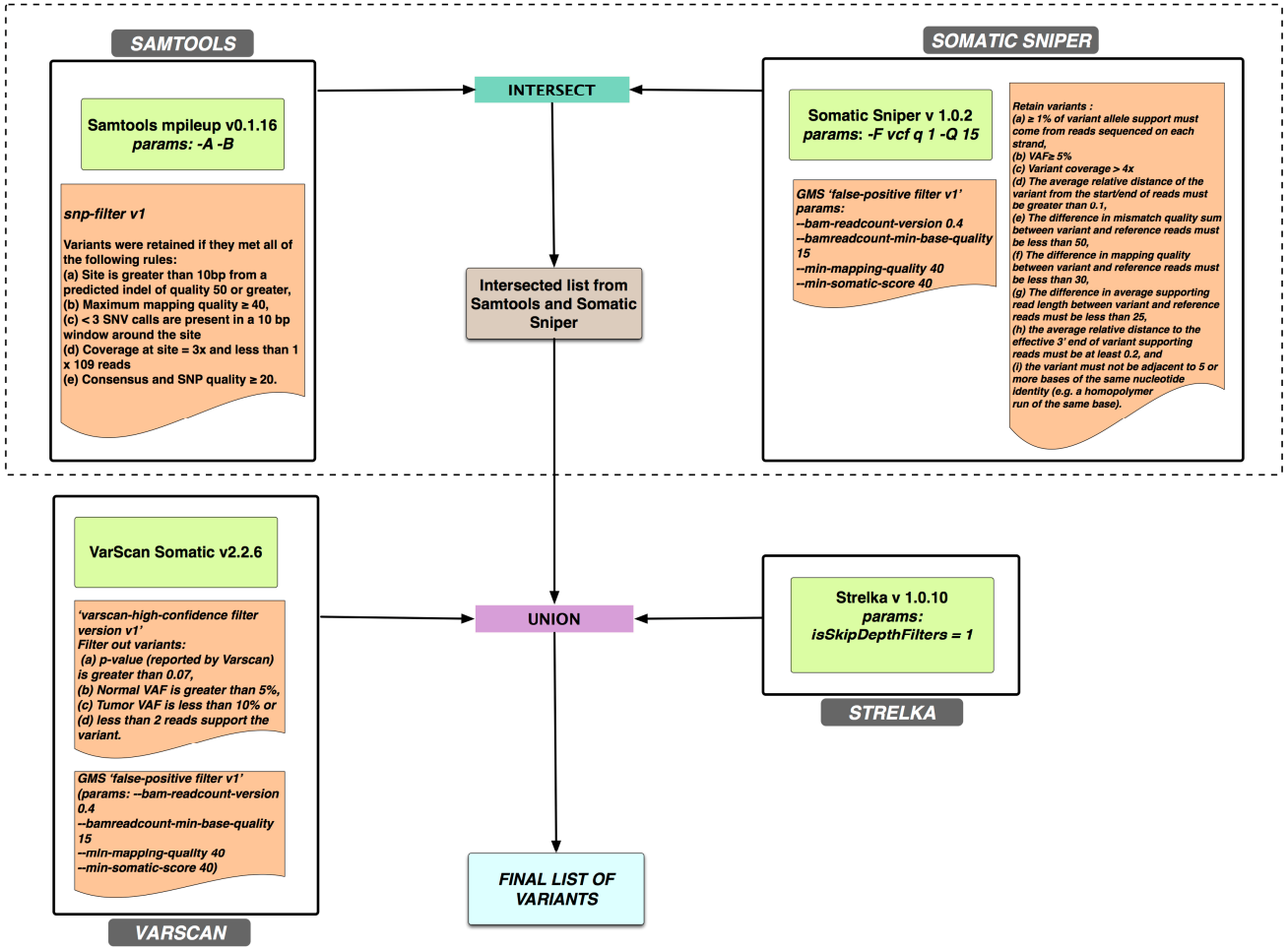


Integrating DNA and RNA in N of 1 Personalized Vaccine Design

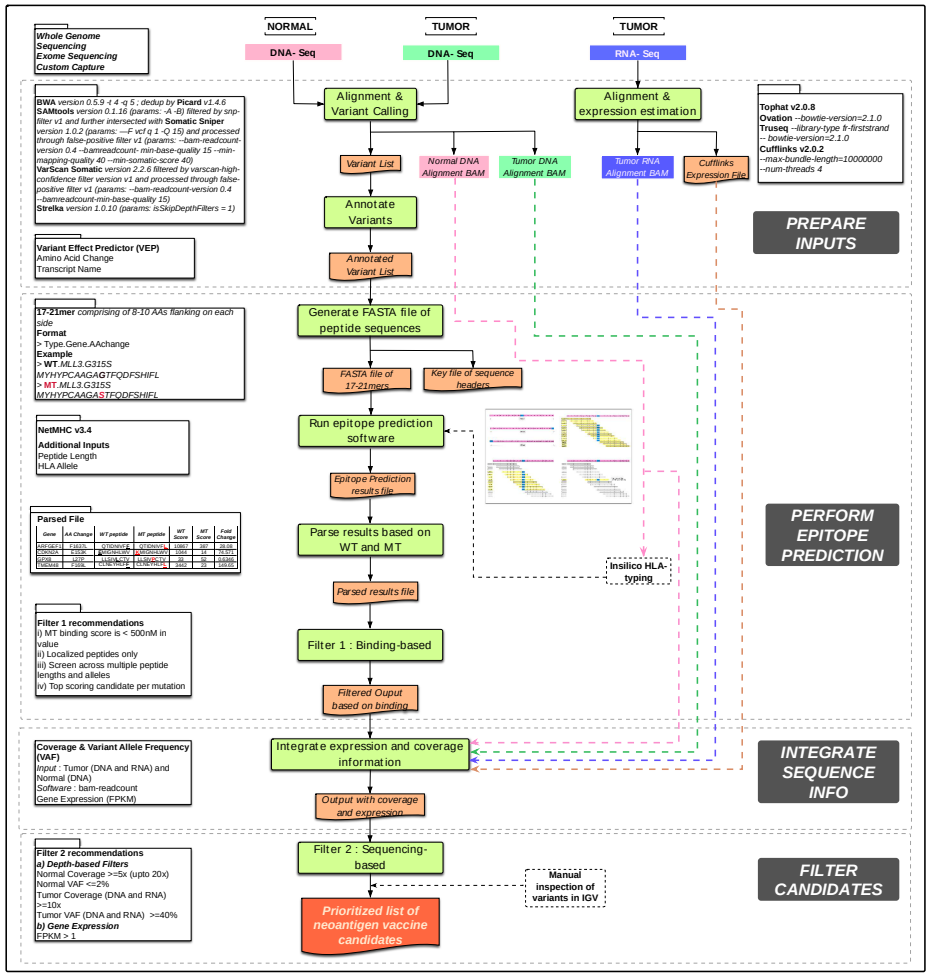
Genome-guided Immunotherapy

- In using genomic data to design vaccines or predict neoantigen load for a specific tumor, we utilize:
  - the power of massively parallel sequencing and analysis to compare cancer and normal genomes (or exomes) and identify cancer-unique peptides
  - the HLA haplotypes of the individual patient
  - RNA sequencing data from the cancer cells to identify genes that are mutated **and** expressed
- These input data are considered by algorithms that model the binding of peptides to the MHC and calculate binding energies, producing a list of tumor mutated specific antigens (TMSA) or neoantigens
- This information can describe the cancer's neoantigen load or can be used to design a vaccine meant to elicit an immune response to the TMSA

Somatic Variant Identification



pVac-Seq Pipeline: Current Iteration



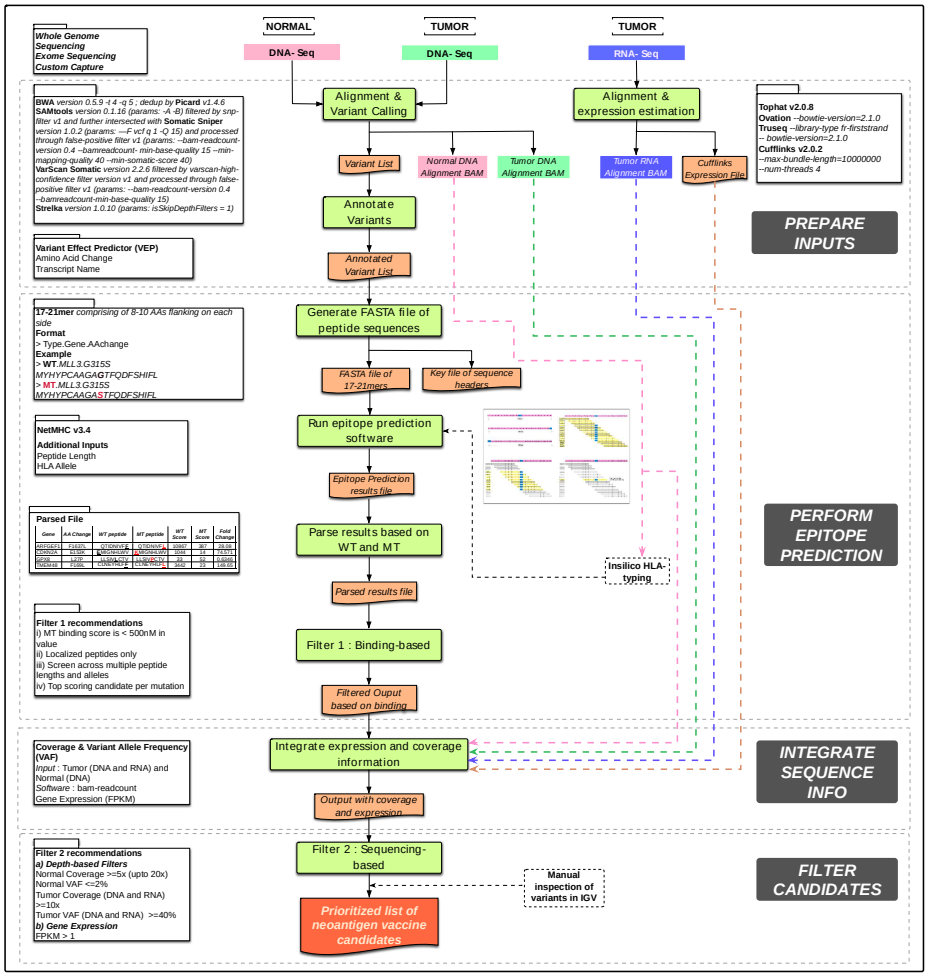
Prepare Inputs:

Exome sequencing data from tumor and normal are analyzed to identify somatic SNVs

Each nonsynonymous SNV is converted to a mutant peptide sequence

RNAseq data from tumor consists of FPKM values for each gene expressed and information regarding allele-specific expression

pVac-Seq Pipeline: Current Iteration



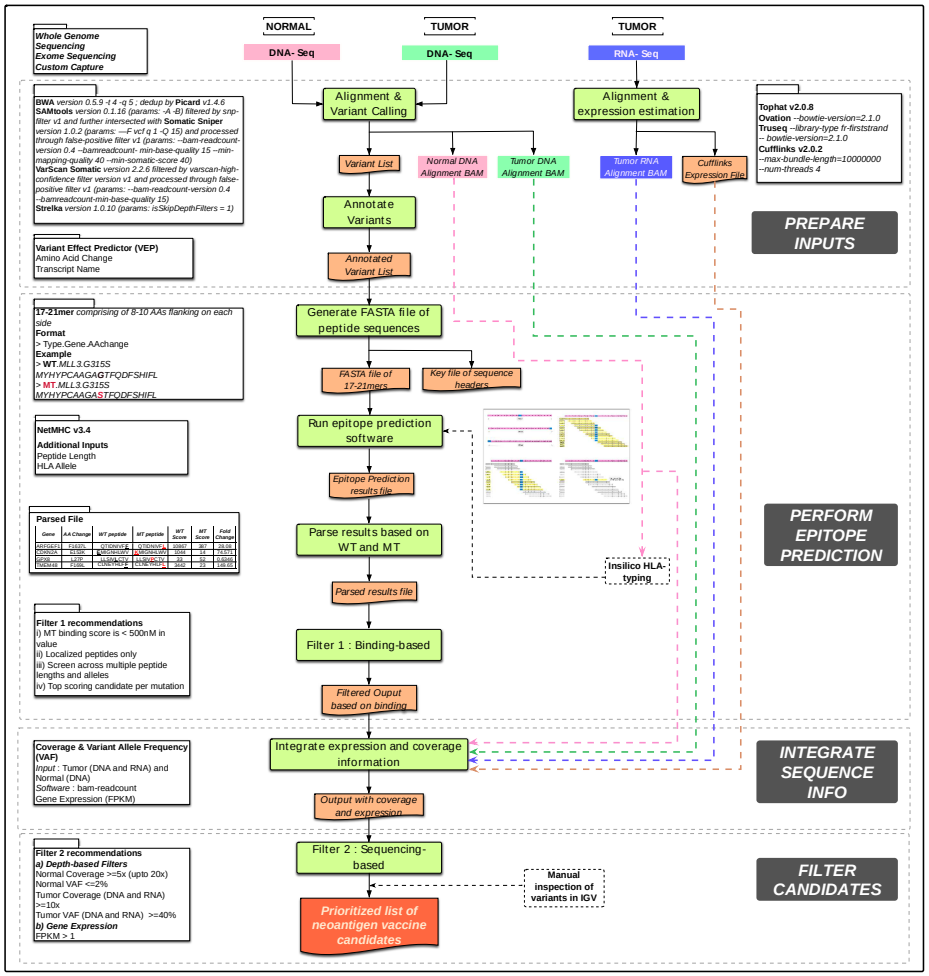
Perform Epitope Prediction:

netMHC is used to evaluate each mutant and wildtype peptide relative to the patient MHC haplotypes

MHC haplotypes are derived from clinical assay or from WES data of the normal match using Athlates software

A list of peptides, predicted binding affinities and peptide identities is parsed to the next step in the pipeline

pVac-Seq Pipeline: Current Iteration



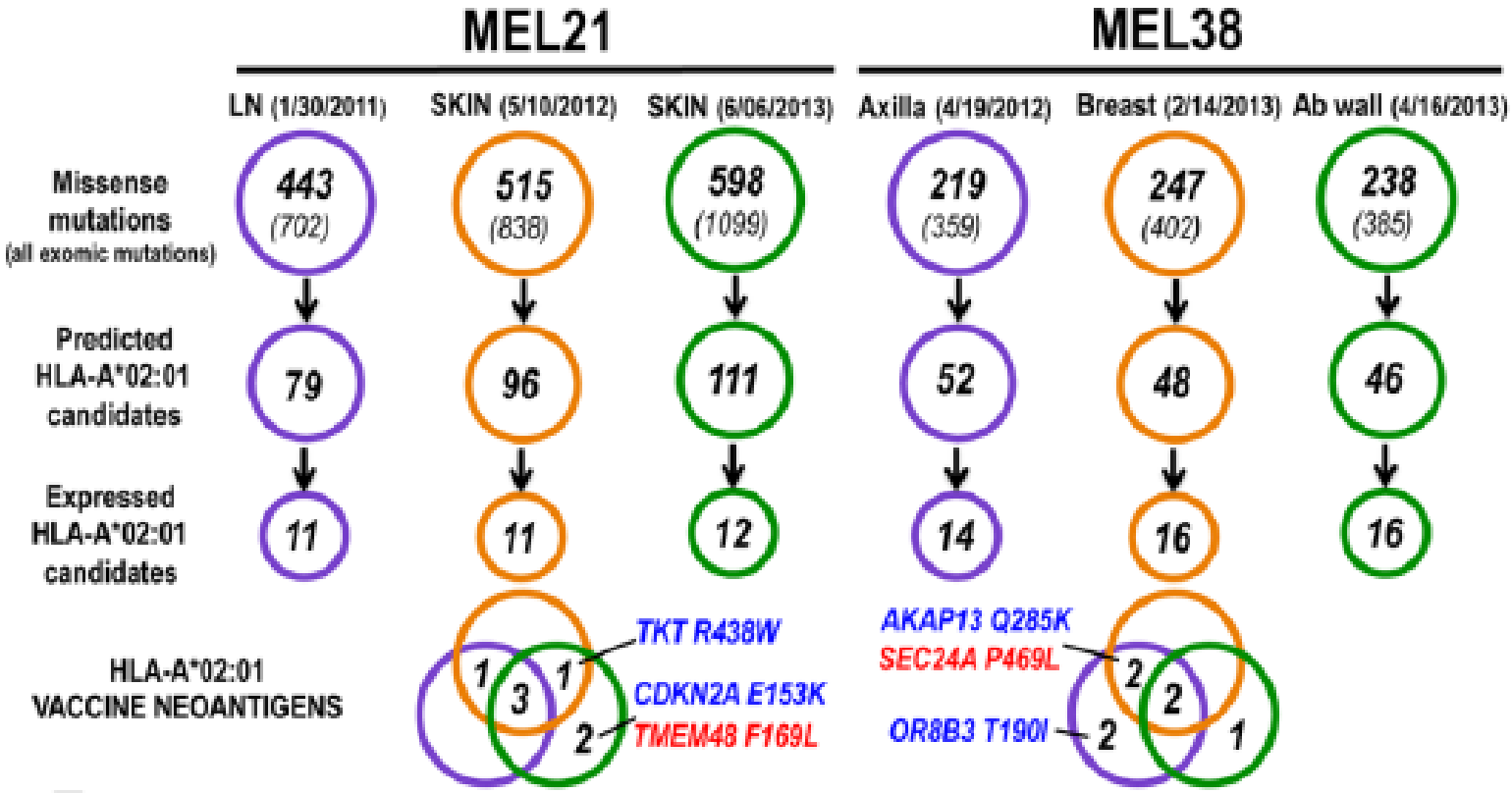
Integrate MPS Data Filters:

We filter out mutant peptides based on known sources of false positivity in MPS data
- normal and tumor read data coverage levels
- variant allele fractions that indicate subclonal variants

We filter based on RNA data using two criteria

- FPKM >1
- Evidence for variant allele expression

Temporal and clonal analysis of candidate neo-antigens



Carreno et al., Science 2015

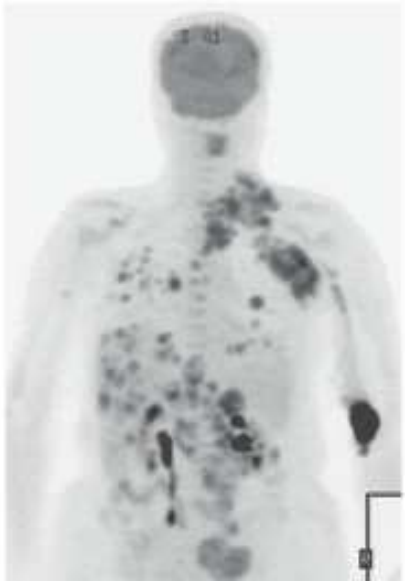
Improving our Neoantigen Prediction Pipeline

- Added functionality to predict from indel/frameshift and nonsense/nonstop mutation events (rarer but likely highly immunogenic)
- Fusion proteins also should provide strong neoantigens
- Automate the integration of DNA and RNA data
  - Filtering out false positives due to lack of coverage, focus on founder clone mutations
  - Expressed mutations and expression levels
- Improved prediction algorithms for neoantigens
  - Class II HLA predictors
  - Machine learning “feedback” to improve prediction accuracy
- Screen selected peptides vs. proteome
- Evaluate HLA mutations

Genome-guided Personalized Vaccine Design

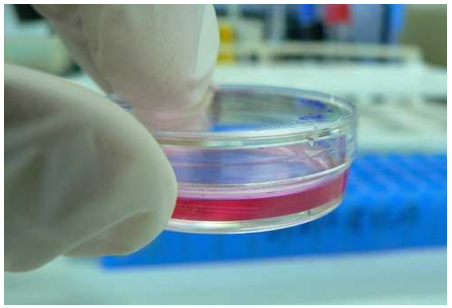
An initial clinical trial in melanoma

Genome-guided cancer immunotherapy in melanoma



Patient biopsied metastatic melanoma lesions

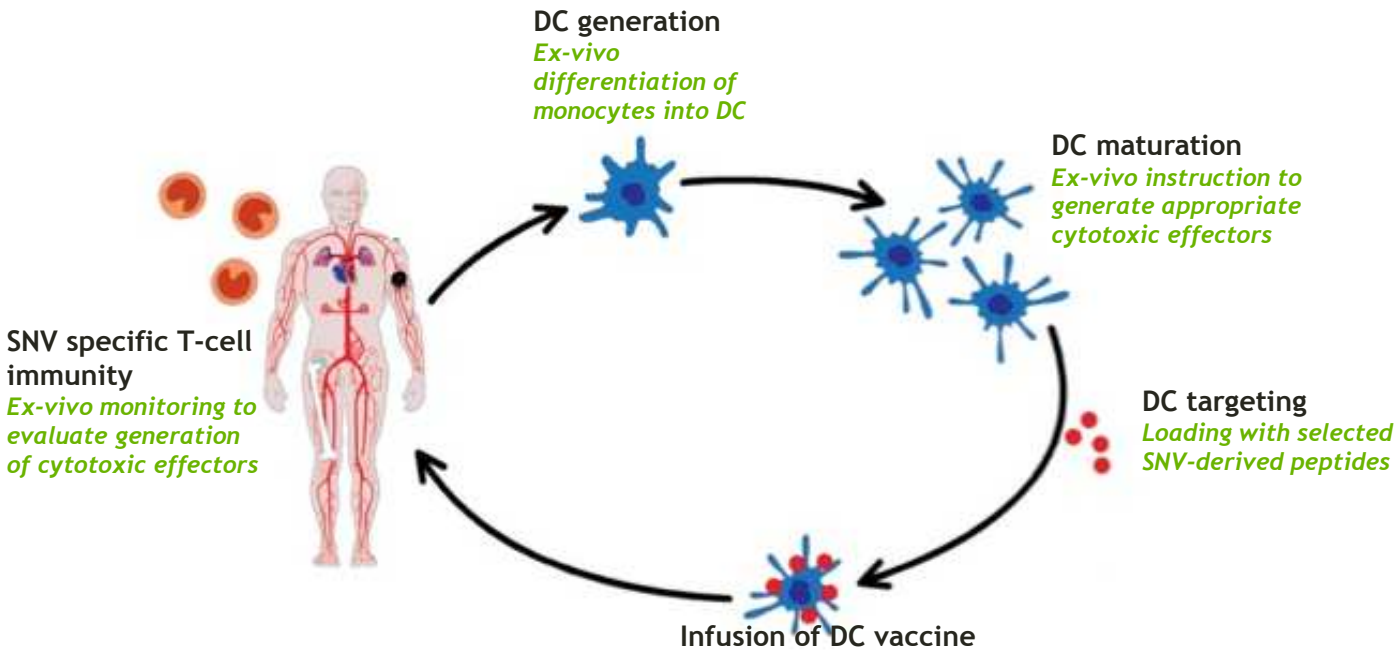
Tumor and germline DNA sequenced, somatic mutations identified; RNA capture verifies expressed mutations and expression level; netMHC algorithm + filtering identifies putative immunoepitopes



Apheresis samples from patient used for *in vitro* assays to re-order the algorithmically-identified immunoepitopes (EliSpot and IFNγ)

Carreno et al., Science 2015

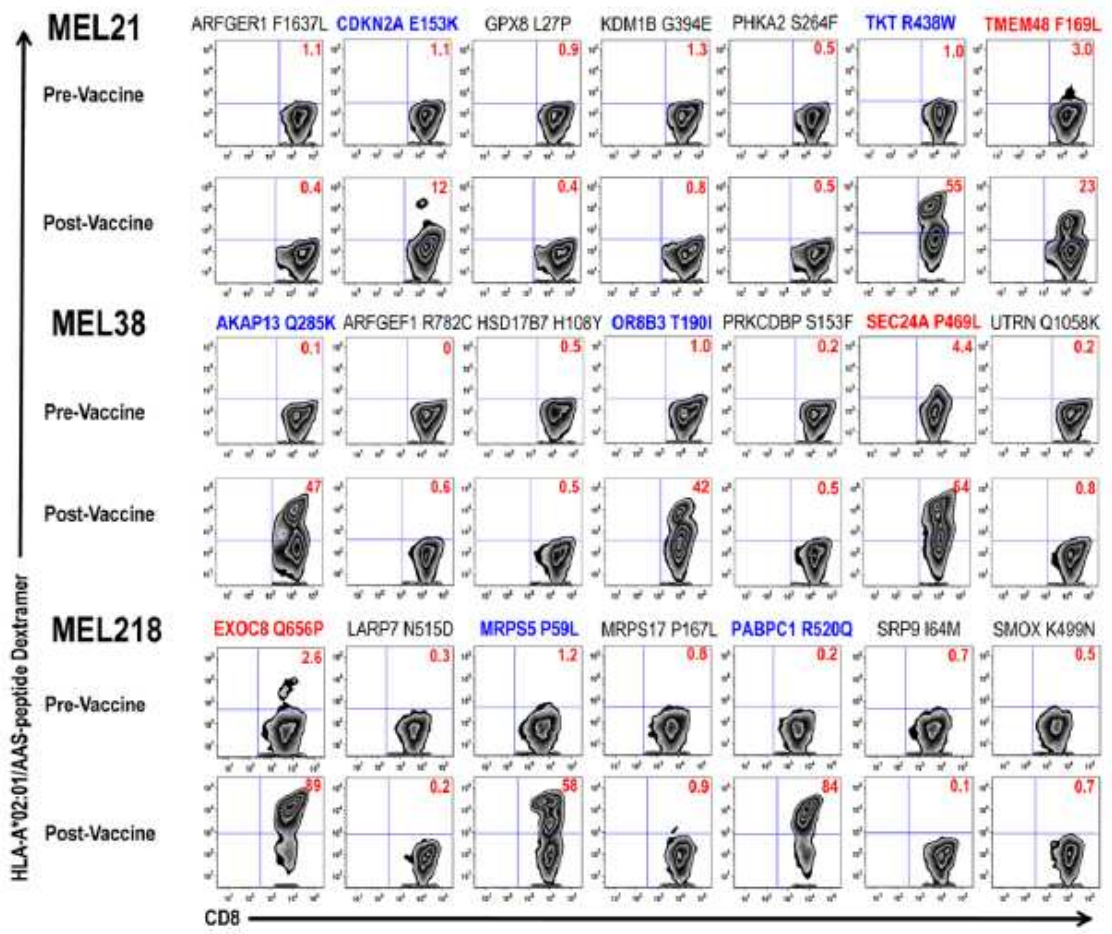
Dendritic Cell Vaccine Platform



A dendritic cell-based approach was tested in an FDA approved IND protocol for **metastatic melanoma** patients:

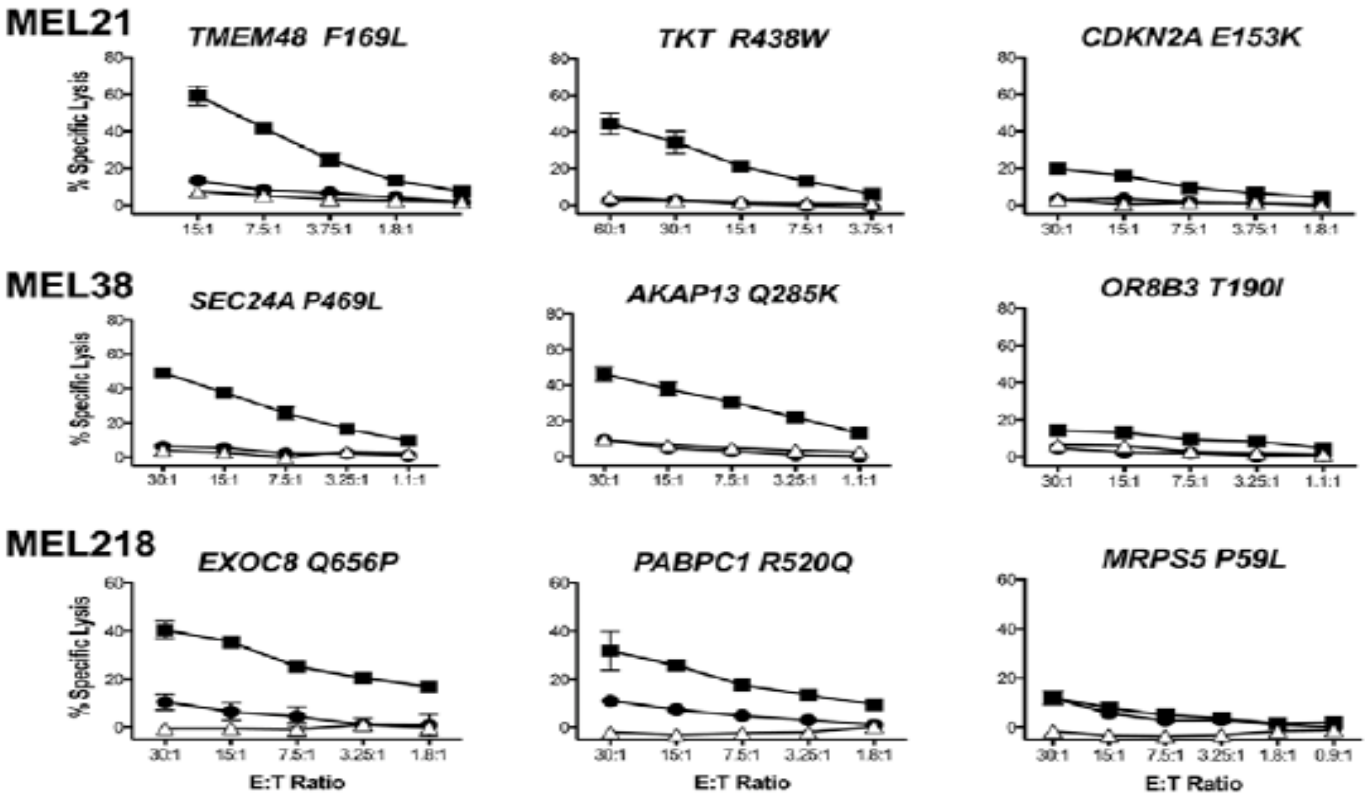
- Patients 1-5 have received all three doses of vaccine, and are being monitored

Monitoring CD8+ T cell response to mutation-containing peptides



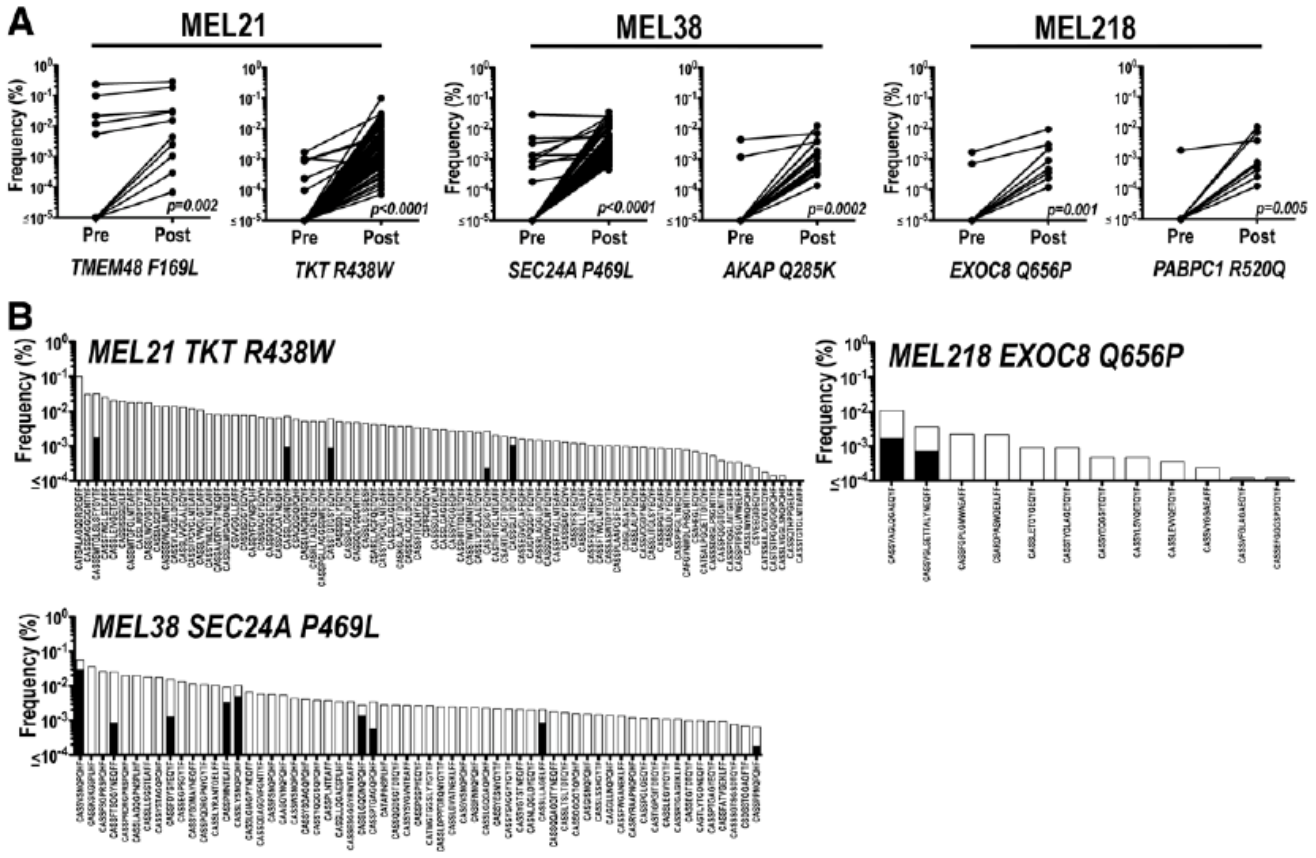
Dextramer assays compare PBMC before vaccination and at peak post-vaccine administration

Neoantigen Processing and Presentation



Neoantigen-specific T cells co-cultured with vector expressing mutant or wildtype TMC in a 4h ^{51}Cr-release assay to monitor lysis.

Vaccination Promotes a Diverse Neoantigen-specific T Cell Repertoire



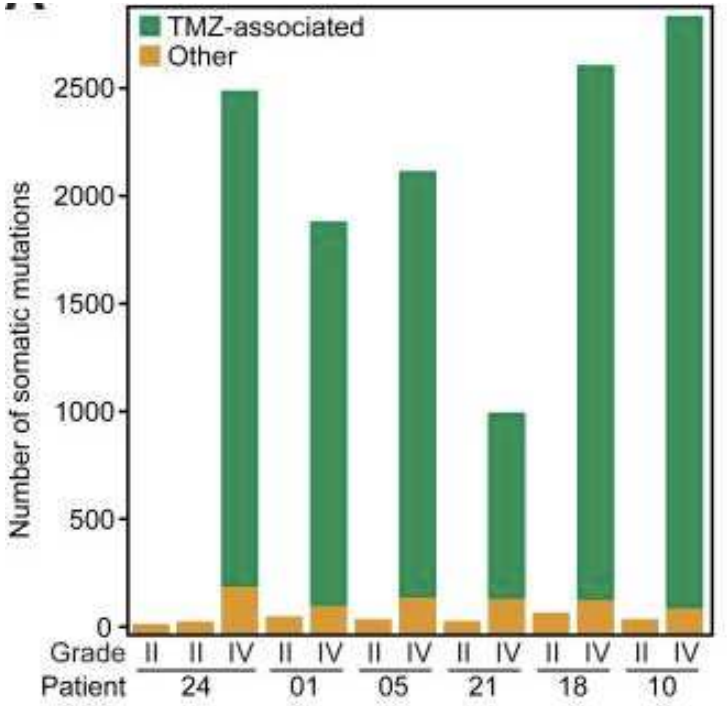
TCRβ clonotypes in CD8+ T cell populations isolated from PBMC before and after vaccination

Melanoma Dendritic Cell Vaccines: Conclusions

- Our first-in-human trial has demonstrated safety and a partial response of eliciting CD8+ T-cell memory for the tumor-unique neoantigens in three patients
- We used HPLC fractionation and mass spectrometry to demonstrate predicted peptides were bound by HLA A*02:01 in patient bloods.
- The T cell repertoire in each patient was shown to be composed of diverse clonotypes post-vaccination

Next Steps

- Melanoma vaccines: Next trial combining anti-PD1 drug with DC vaccine
- Triple Negative Breast Cancer trial
  - DNA vector-based vaccination
  - Long peptide cocktail vaccination
  - Both approved by FDA
- Pediatric glioma vaccines



Lessons and Take Home Messages

- In addition to identifying targeted therapy dependencies, MPS-based assays can characterize the neoantigen landscape of a cancer genome

- This approach is potentially impactful in cancer patient care as it may predict the likelihood of response to checkpoint blockade immunotherapy and/or may be utilized to design personalized vaccines for individual patients

- Considering the heterogeneity of cancer cell genomes is important, as are the patient-specific assays that refine vaccine candidate lists

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