



# Neoantigen Vaccination

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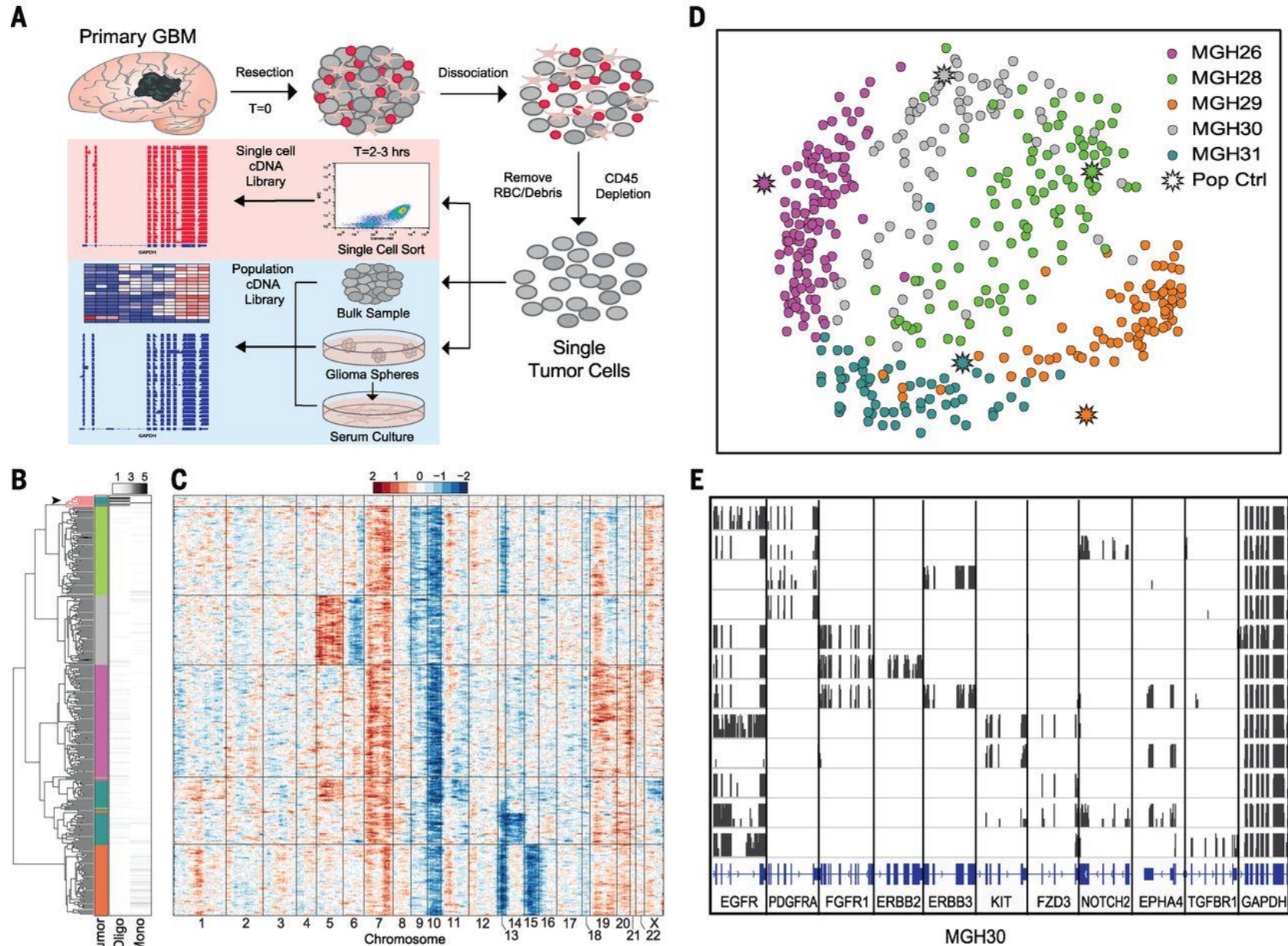
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# Disclosures

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- **Advisory board (paid to me):** Abbvie, Advantagene, Agenus, Amgen, BMS, Cavion, Celldex, EMD Serono, Genentech/Roche, Inovio, Juno Pharmaceuticals, Merck, Midatech, Momenta Pharmaceuticals, Novartis, Novocure, Oncorus, Oxigene, Regeneron, Stemline Therapeutics
- **Lab Research Support (paid to Dana-Farber):** Acerta Pharma, Agenus, Celldex Therapeutics, EMD Serono, Incyte, Inovio, Midatech, Tragara
- **Speaker (paid to me):** Genentech/Roche, Merck

# Glioblastoma: Intra-Tumoral Heterogeneity Fosters Tumor Evolution and Clonal Resistance



# Tumor Evolution: A Central Obstacle to Curative Cancer Therapy

**INTRA-TUMORAL HETEROGENEITY**

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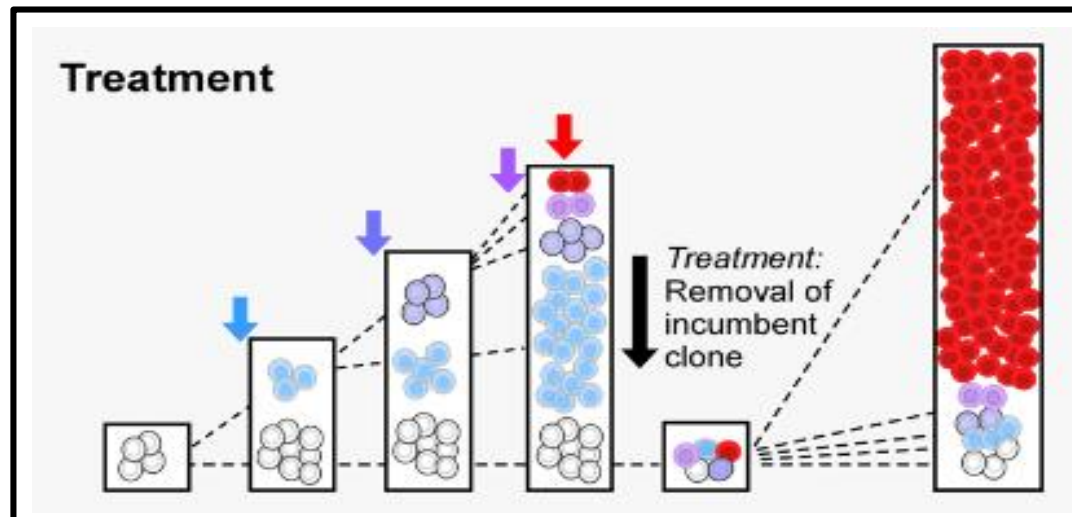
**EFFECTIVE TREATMENT**



*Cytotoxic agents*

*Novel targeted agents*

**TUMOR EVOLUTION = RESISTANCE**





*“Fighting fire with fire”:  
Overcoming tumor heterogeneity*

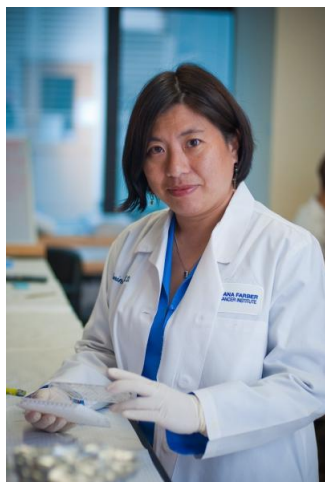
**Phase I Study of Personalized NeoAntigen Cancer  
Vaccine (“NeoVax”) in Glioblastoma**

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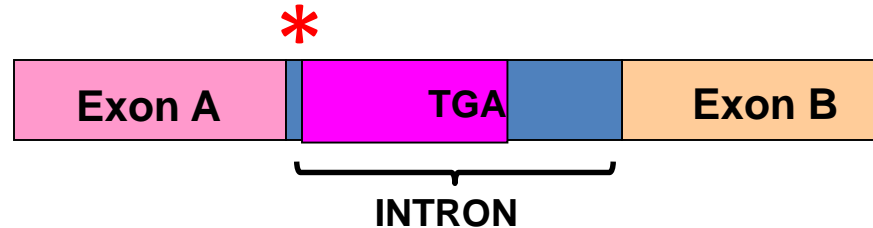
# Classes of mutations that can generate potential tumor neoepitopes

Potential neoORFs

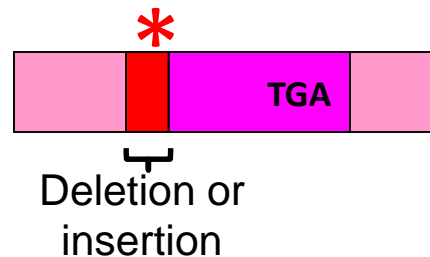
**Missense**

\*  
LMPK**H**FIR (parental)  
LMPK**L**FIR (Mutated)

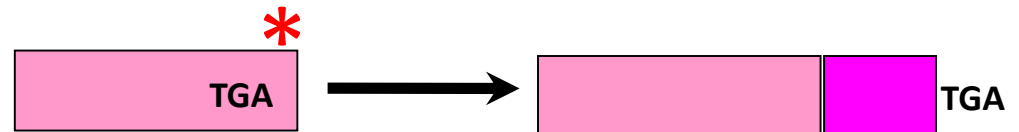
**Splice-site**



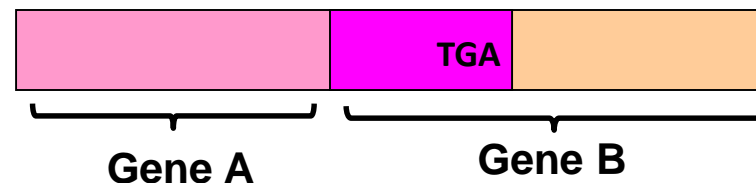
**Frame-shift**



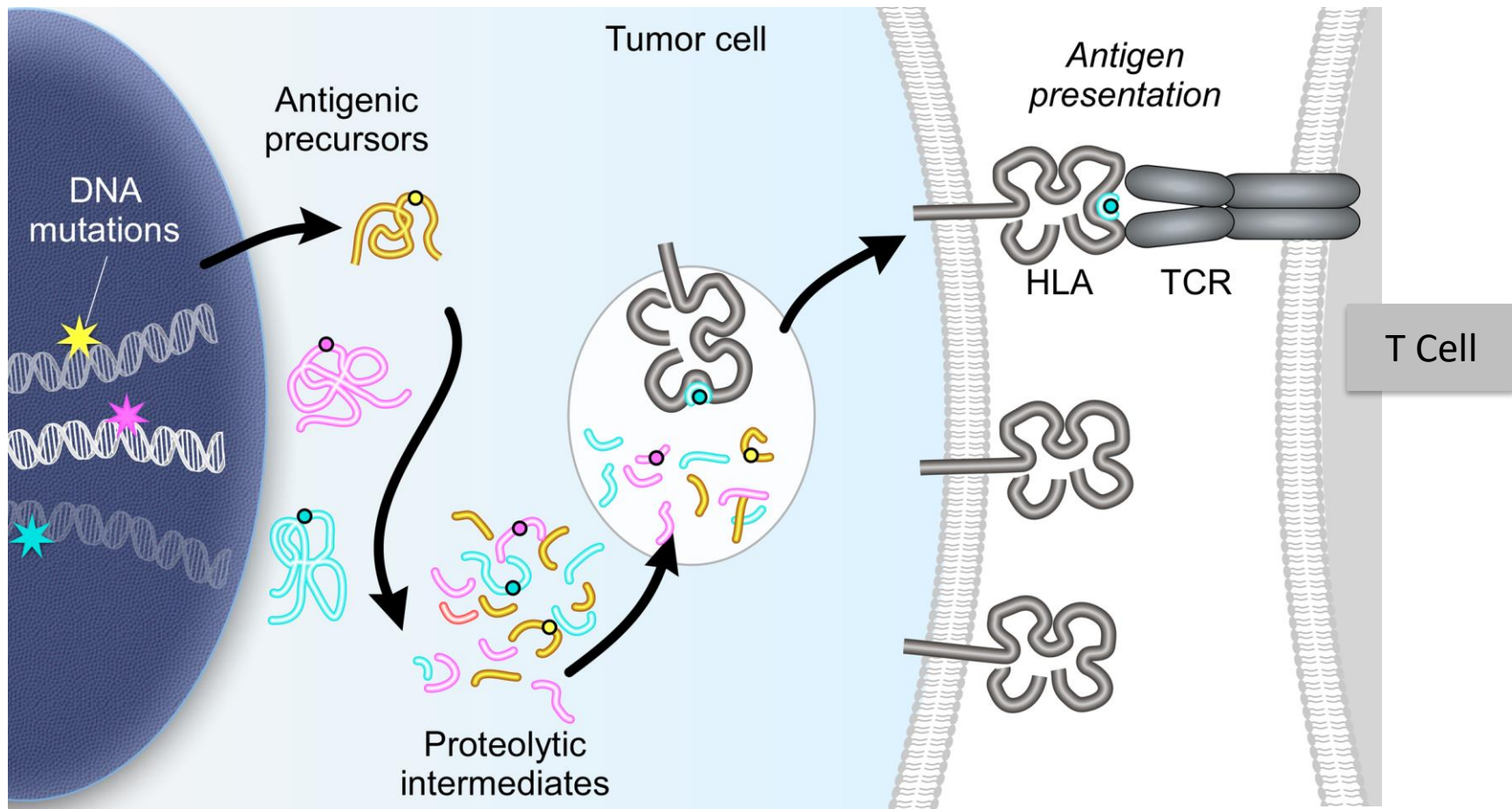
**Read-through**



**Gene fusion**



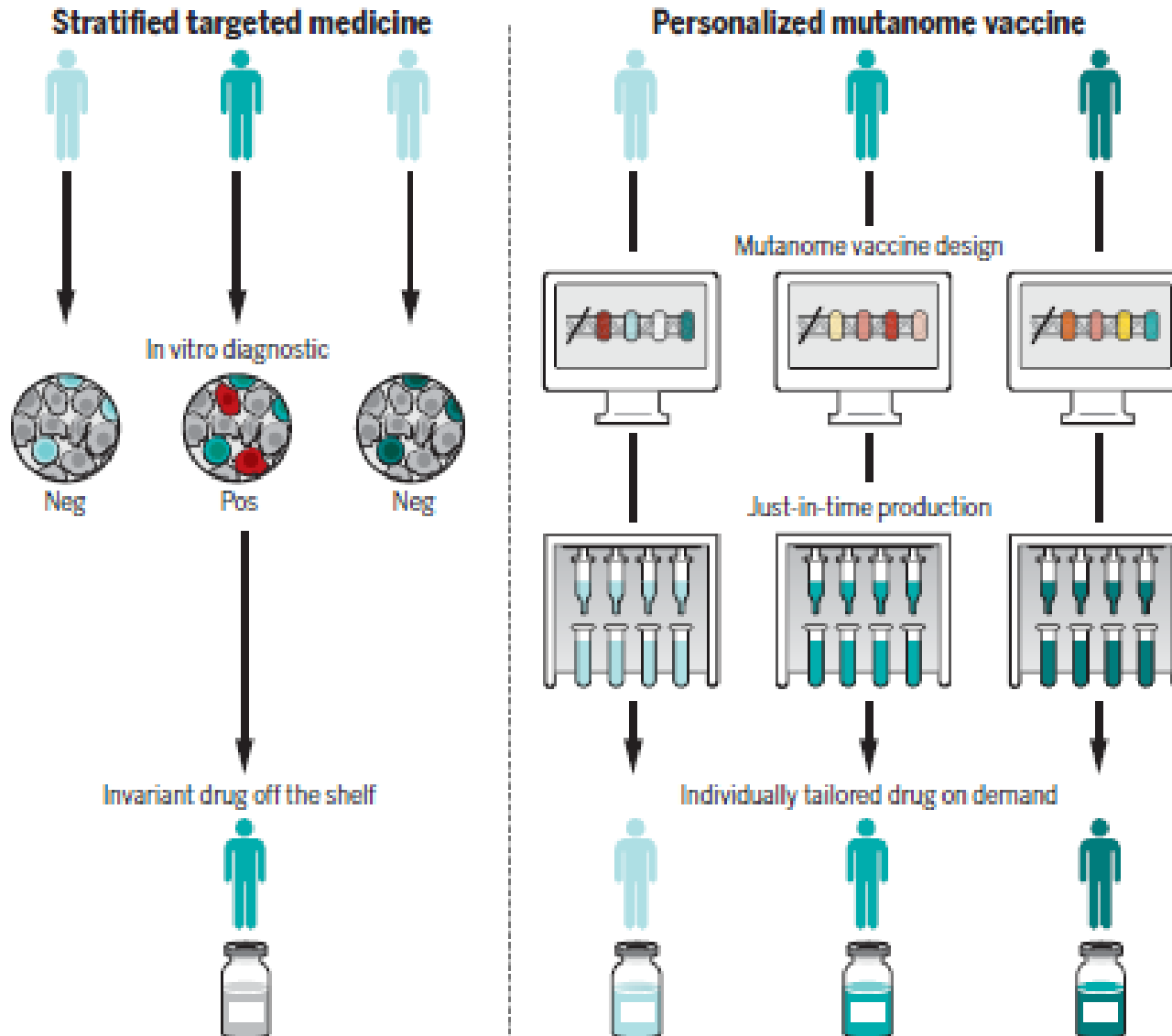
# Somatic mutations have the potential to generate neoantigens



*Lennerz et al, PNAS 2005; Mandelboim et al, Nat Med 1995; Basker et al, JCI 2004; Fritsch et al CIR 2014; Sensi & Anichini, Clin Cancer Res, 2006; Van Buuren Oncolmunology, 2014*

*Purroy & Wu (in press)*

# Personalized Cancer Medicine: Targeted vs Immunotherapy Approaches



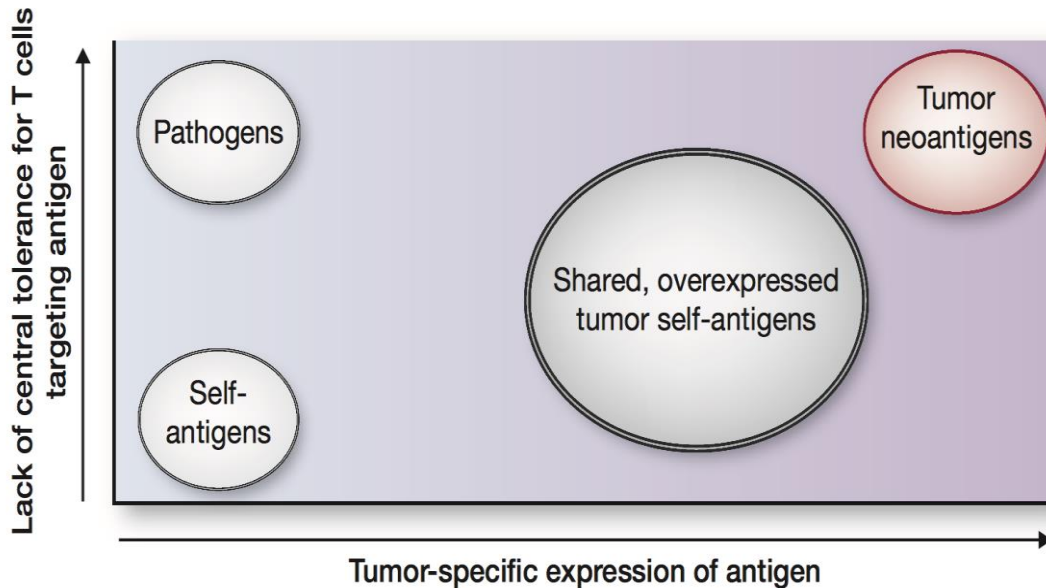
# Neoantigens: targets of effective tumor directed T cell responses

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- High neoantigen load is associated with better outcome  
*(Brown Gen Res 2014; Snyder NEJM 2014; Rivzi Science 2015; Van Allen Science 2015, Le NEJM 2015)*
- Neoantigen-reactive T cells expand in the setting of effective clinical response
  - HSCT *(Cai Clin Can Res 2012; Rajasagi 2014)*
  - TILs *(Robbins Nat Med 2013 ; Prickett CIR 2016)*
  - CPB *(Gubin Nat 2014; van Rooij JCO 2013; Rivzi Science 2015)*
- Neoantigen-specific T cells kill tumor in vivo *(Castle Cancer Res 2012, Kreiter Nature 2015; Gubin Nat 2014; Yadav 2014 Tran Science 2014)*

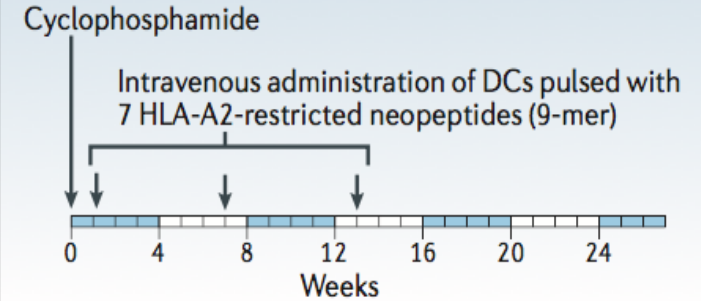
# Neoantigen Targeting Therapies: Safe, feasible and immunogenic personalized immunotherapeutics

Neoantigens are ideal cancer vaccine targets

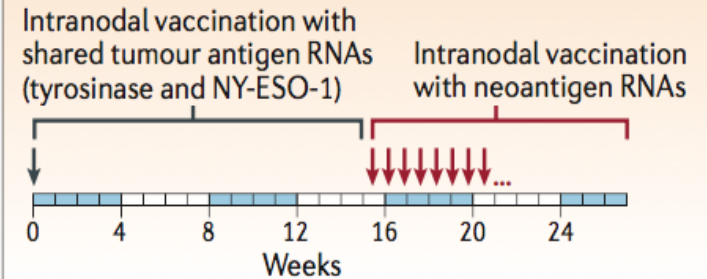


Carreno, B. *Science* 2015; Sahin, U. *Nature* 2017; Ott, P.A. *Nature* 2017

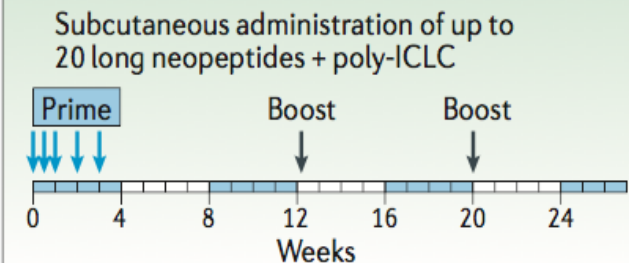
## Neoantigen-based DC vaccine Three patients previously treated with ipilimumab



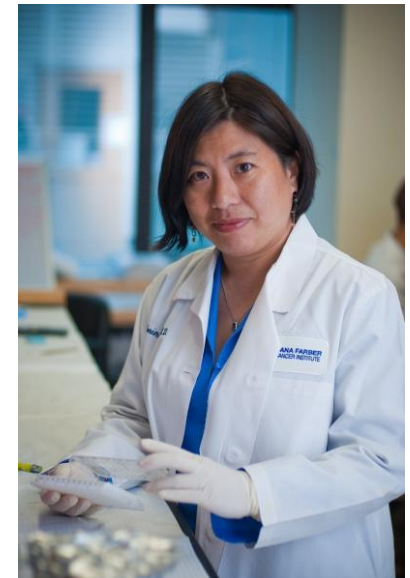
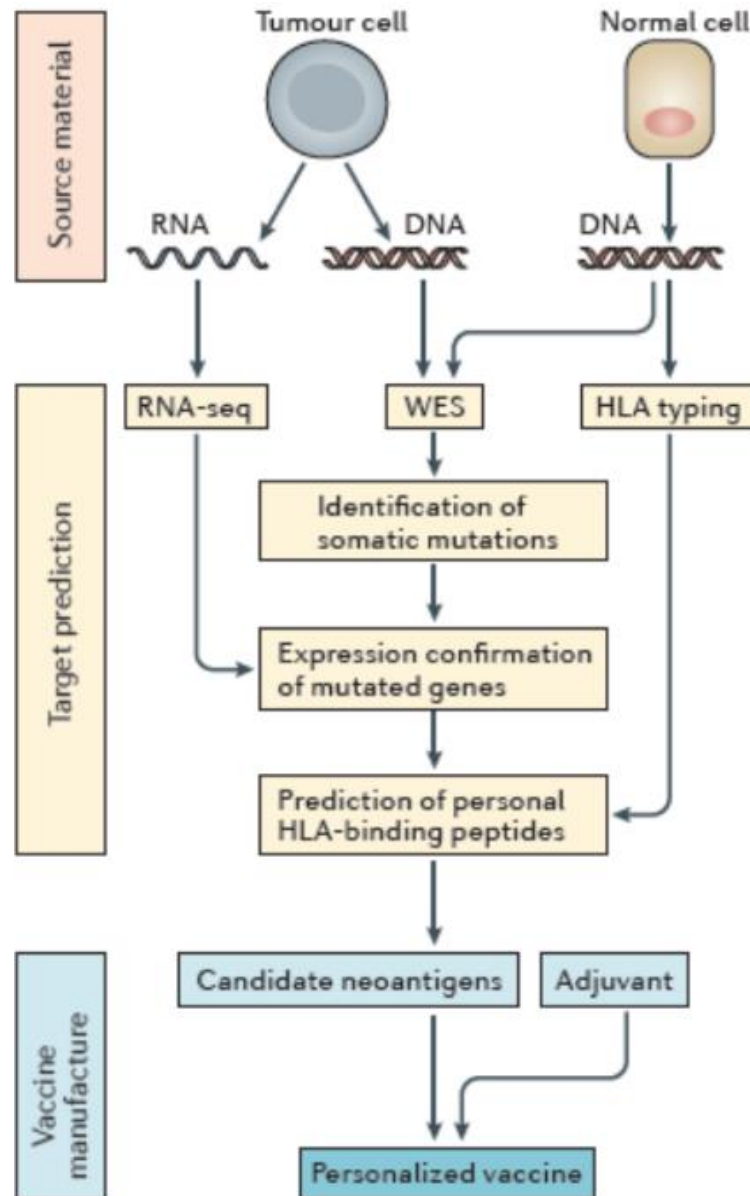
## IVAC MUTANOME Thirteen patients



## NeoVax Six patients

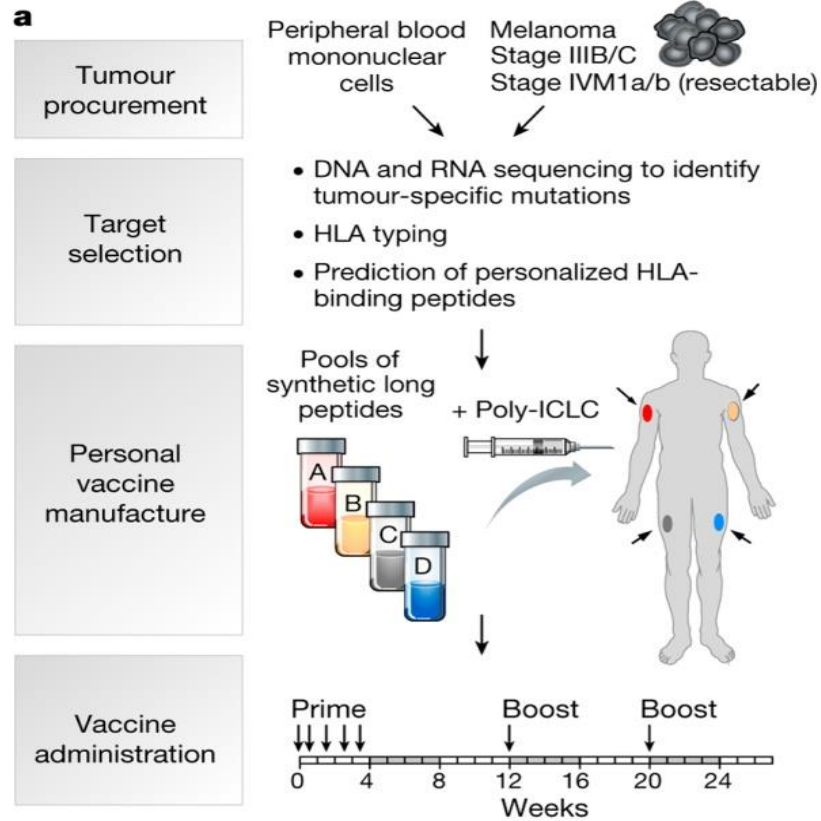
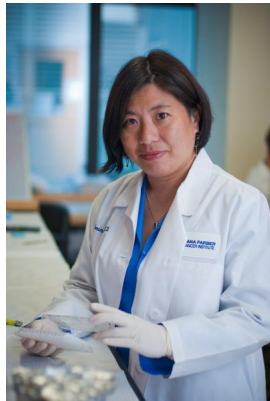


# DFCI Personalized Vaccine Pipeline

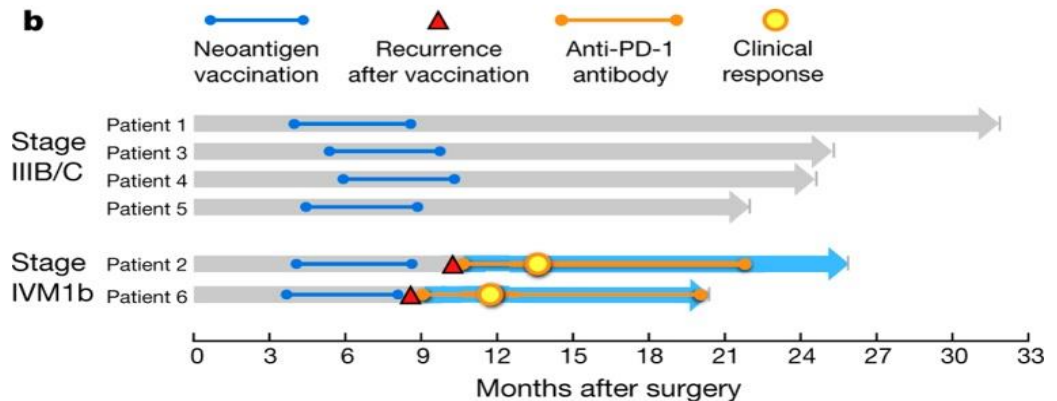


Cathy Wu, MD

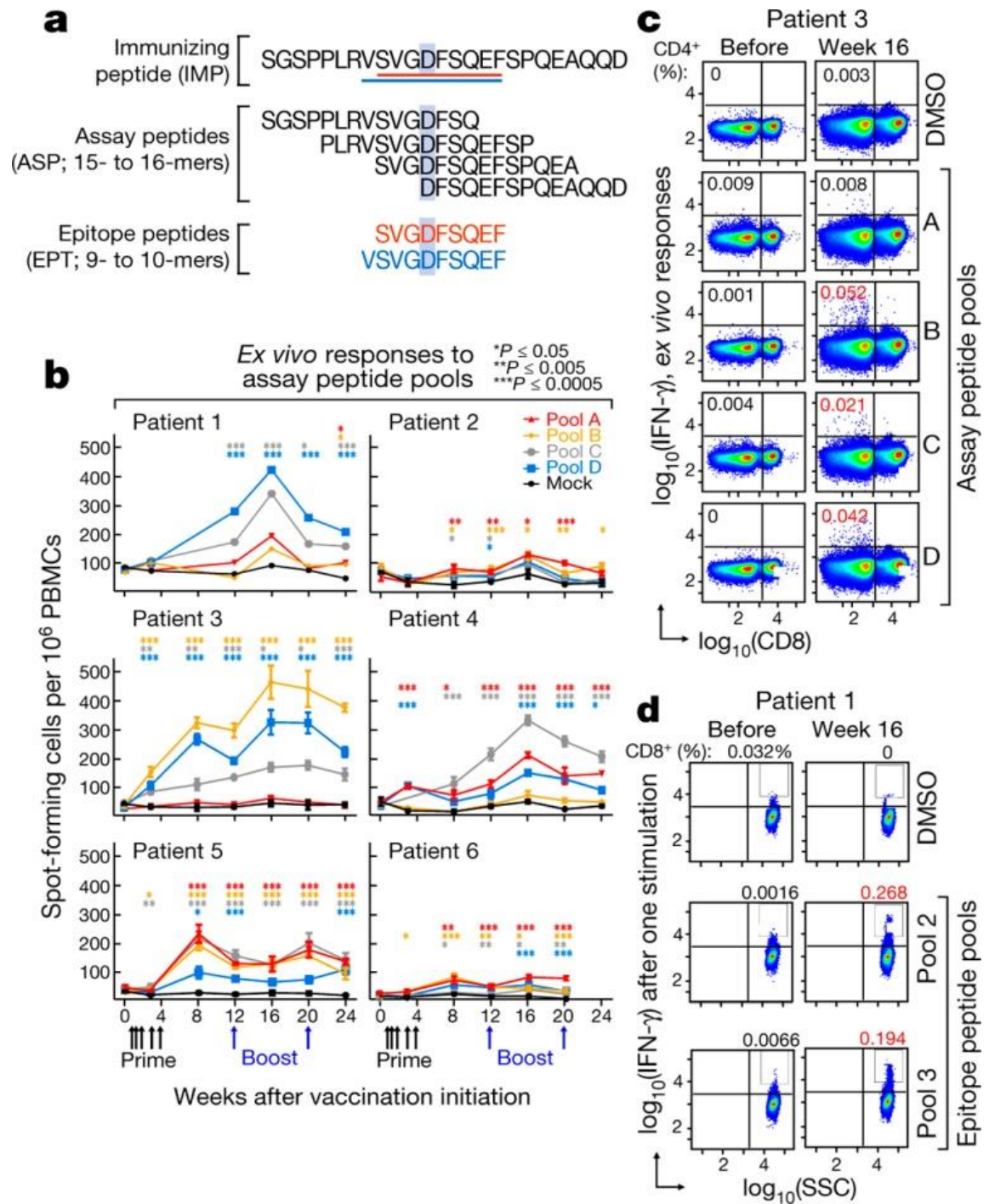
# Neoantigen Vaccination for Melanoma



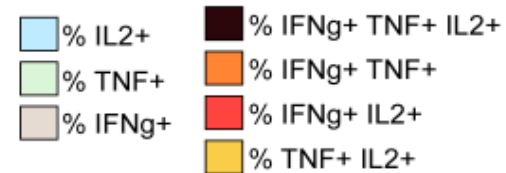
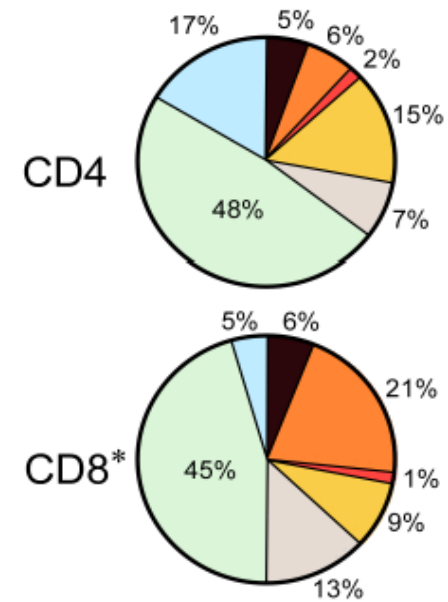
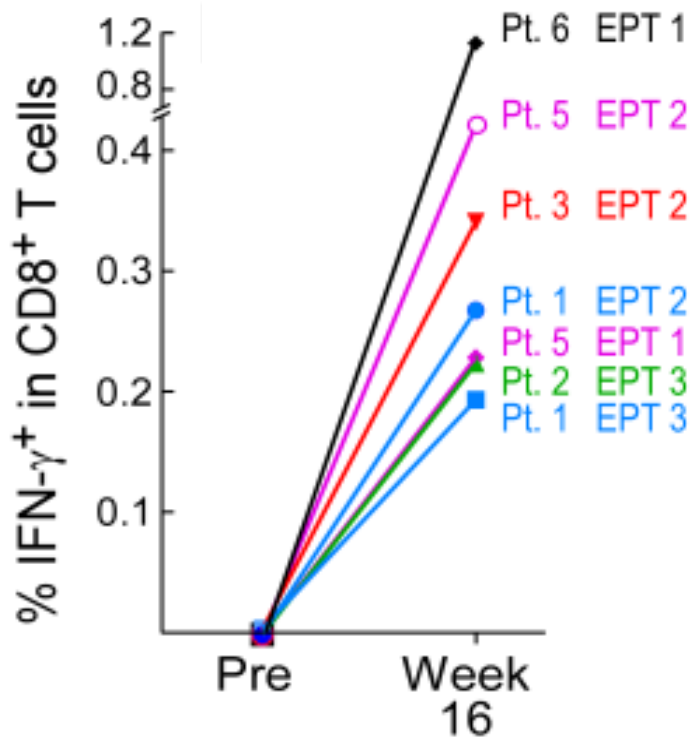
Ott/Wu Nature 547:217-21, 2017



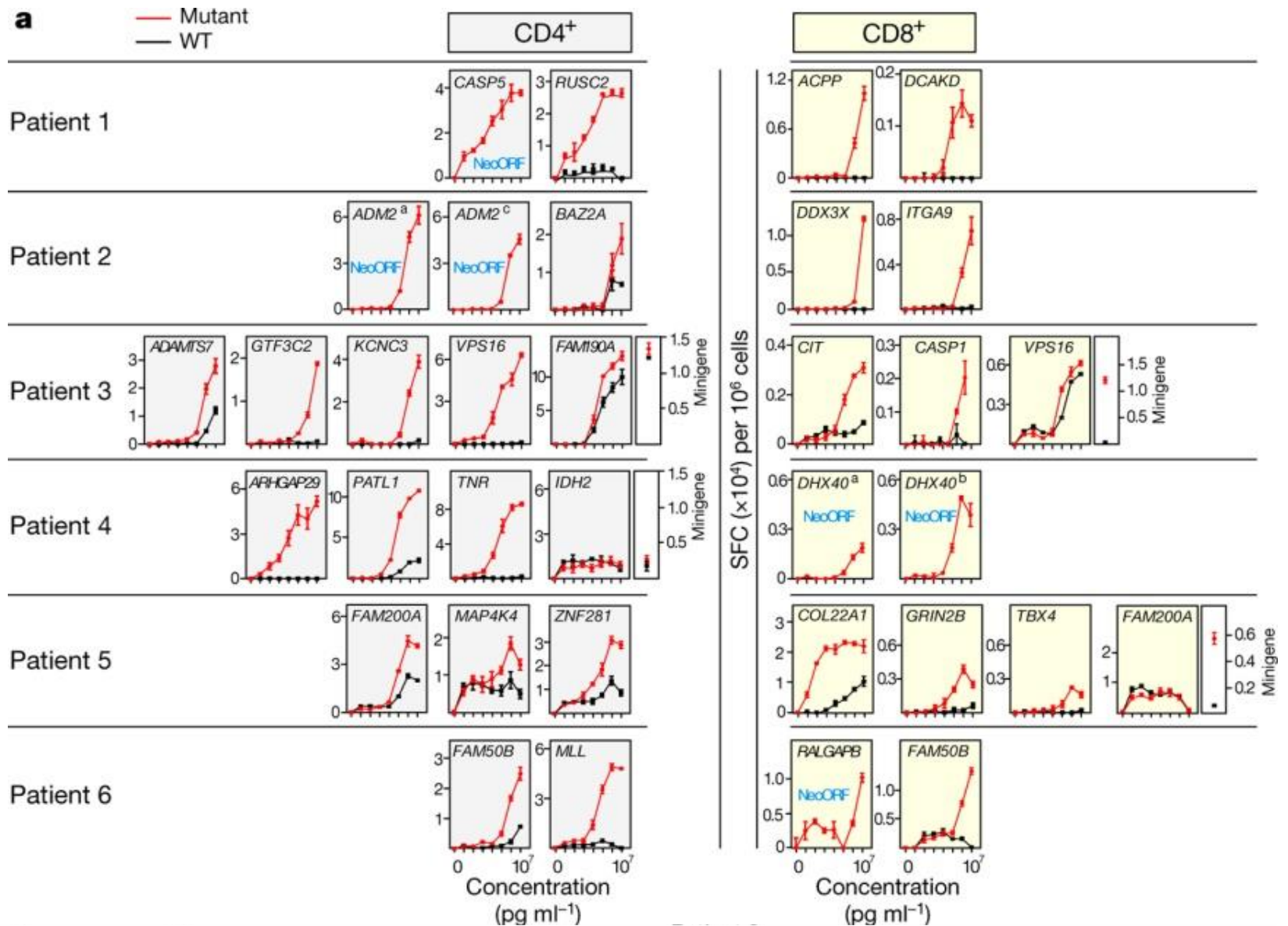
# Dana-Farber NeoVax Melanoma Trial: Significant *ex vivo* IFN- $\gamma$ responses against neopeptide pools by ELISPOT



# Dana-Farber NeoVax Melanoma Trial: Polyfunctional de novo CD4 and CD8 T cell responses against neoepitope pools



# Vaccine induced T cells discriminate mutated from WT antigens



# Glioblastoma: Additional Considerations/Challenges for NeoVax

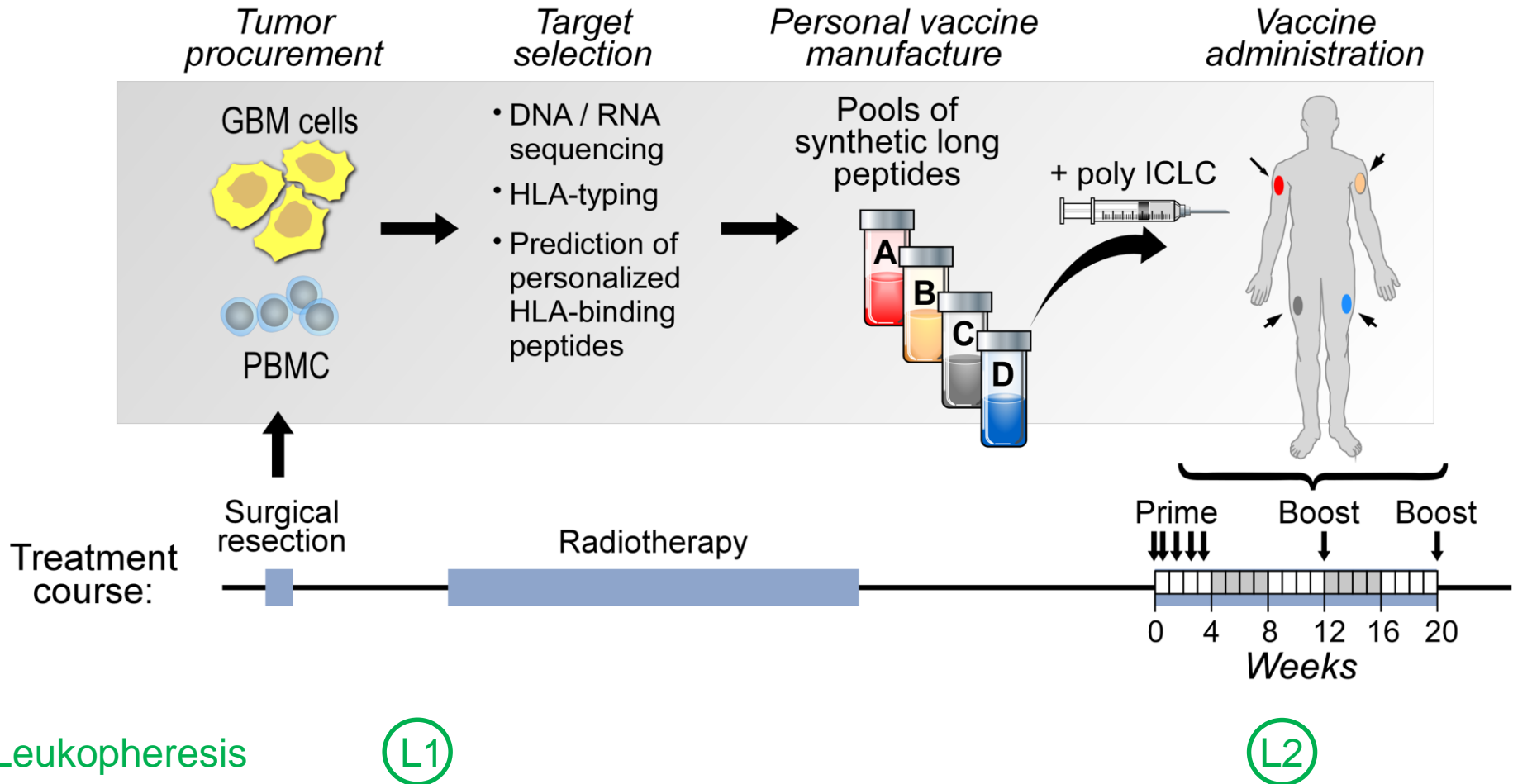
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- Is it feasible to incorporate a tumor specific neoantigen vaccine into standard therapy (radiation +/- TMZ)?
- What is the impact of CNS immune privilege?
- Do concurrently administered corticosteroids (frequently administered to alleviate symptoms from cerebral edema) impact immune responses?
- Can meaningful immune responses be generated in a tumor with relatively low mutational rate and an immunologically “cold” tumor microenvironment?

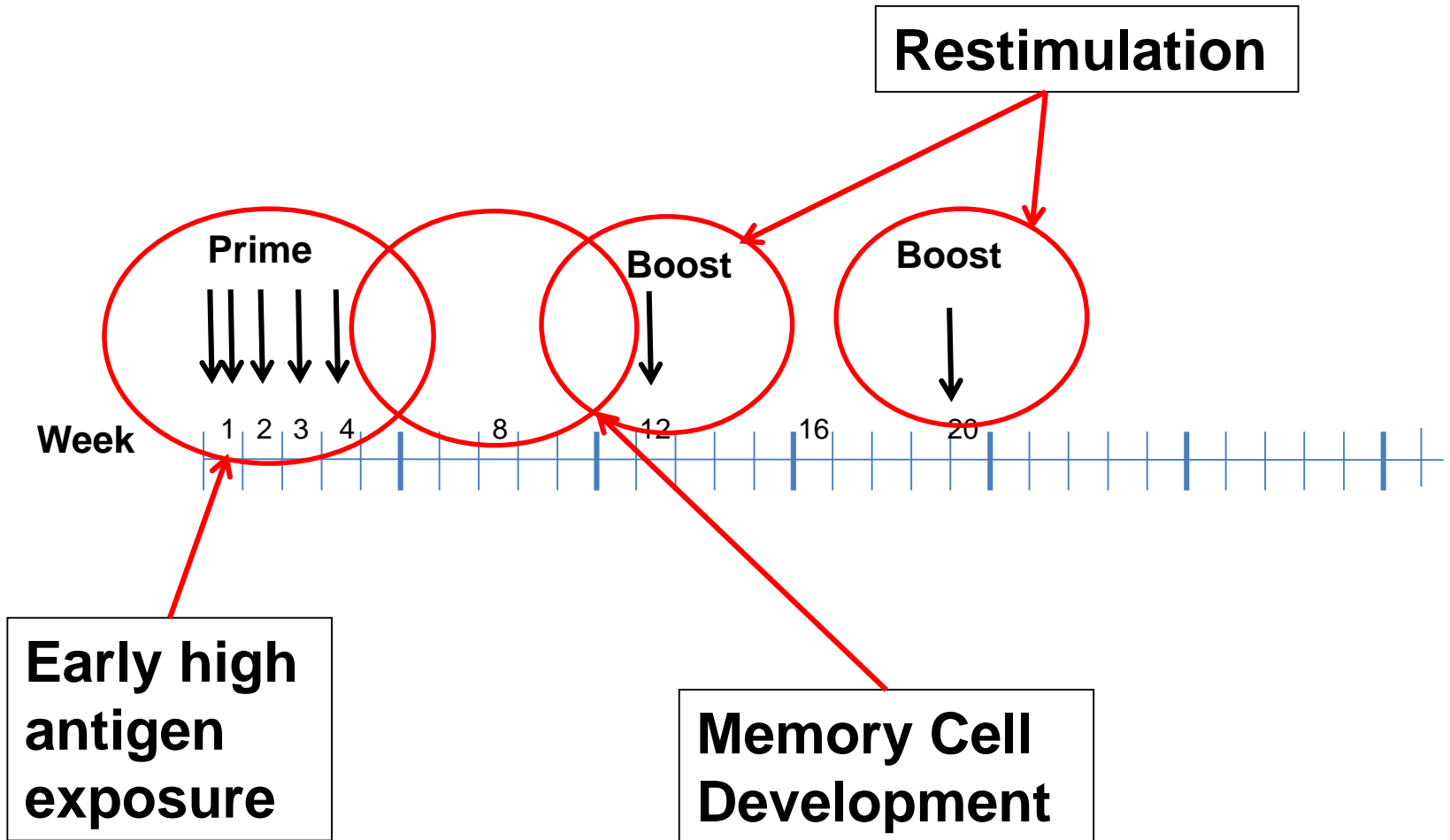
# DFCI/BWH NeoVax: Glioblastoma Trial Design

**Study Design:** Open-label, phase I study to enroll 15 newly diagnosed glioblastoma patients with **MGMT unmethylated tumors**

**Primary Objectives:** 1) Safety; 2) Feasibility (# patients with at least 10 actionable peptides; time from surgery to 1<sup>st</sup> vaccination)



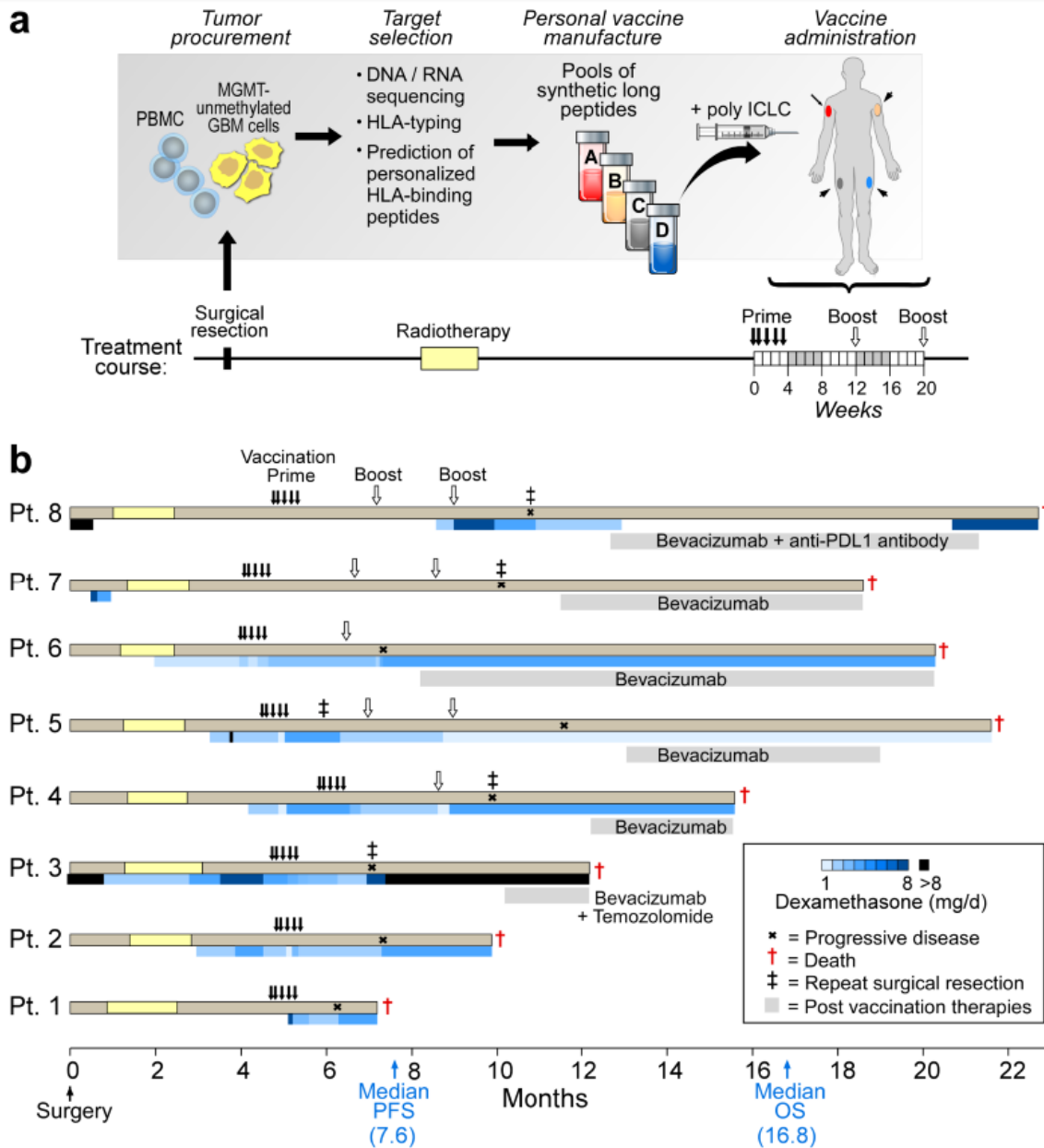
# DFCI/BWH NeoVax: Immunization Schedule



# DFCI/BWH NeoVax: Patient Characteristics (n=9)

Characteristic	Number
Age (median; years)	63 (range: 45-73)
Female	7 (78%)
KPS	
90	7 (78%)
80	1 (11%)
70	1 (11%)
Surgery	
STR	5 (56%)
GTR	4 (44%)
On dexamethasone	7 (78%; dose: 2-4 mg/day)
Absolute lymphocyte count (range)	924 (271-2450)
Surgery to 1 <sup>st</sup> NeoVax (months)	5.0 (range: 4.3-6.2)
# vaccinated peptides/patient (median)	13 (7-20)

# DFCI/BWH NeoVax: Patient Disposition



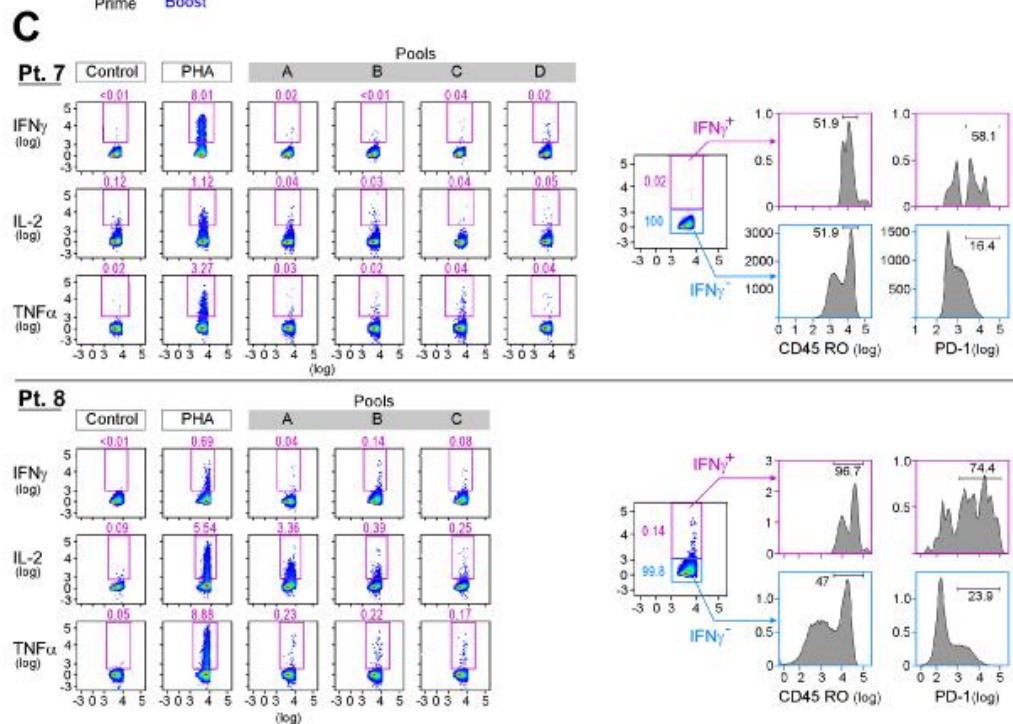
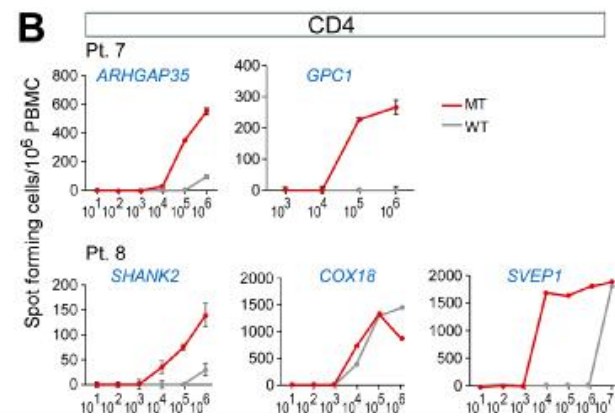
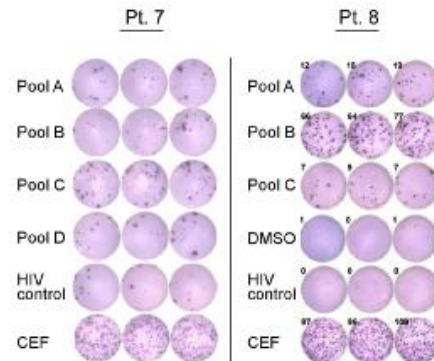
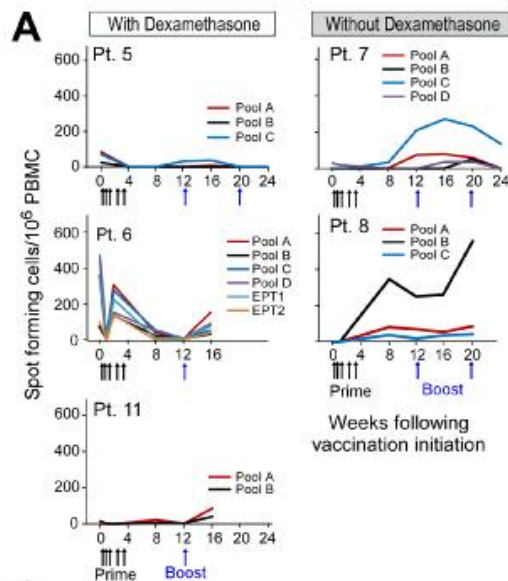
# DFCI/BWH NeoVax: Adverse Events

Adverse Event	Grade	# Patients	Relationship
Chills	1	1 (11%)	Possibly
Dizziness	1	1 (11%)	Possibly
Fatigue	2	1 (11%)	Possibly
Flushing	1	1 (11%)	Possibly
Headache	2	1 (11%)	Possibly
Myalgia	1	2 (22%)	Probably
Nausea	1	1 (11%)	Possibly
Site reaction	1	1 (11%)	Probably

No DLTs

No dose delays

Question 1. Does vaccination induce circulating neoantigen-specific T cell responses?

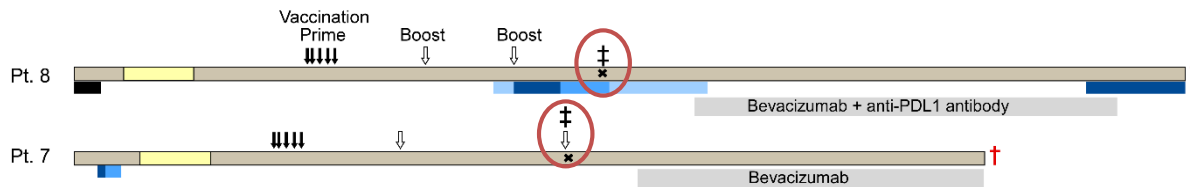


Multiple de novo, polyfunctional T cell responses noted primarily against mutant and not WT peptide among patients not on dexamethasone

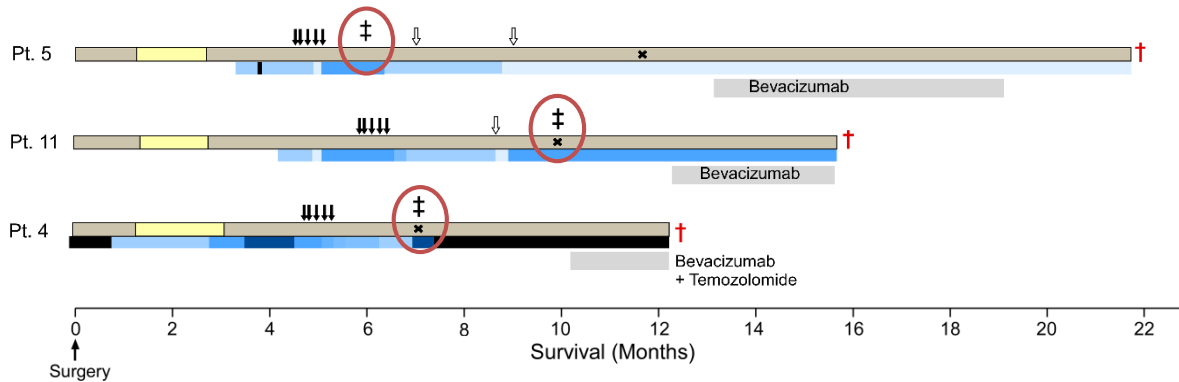
Question 2. Does vaccination alter the immune microenvironment of GBM tumors (can it turn an immunologically cold tumor into an immunologically active one)?

# Relapse resection samples were used to assess post-vaccination changes

## Without Dexamethasone

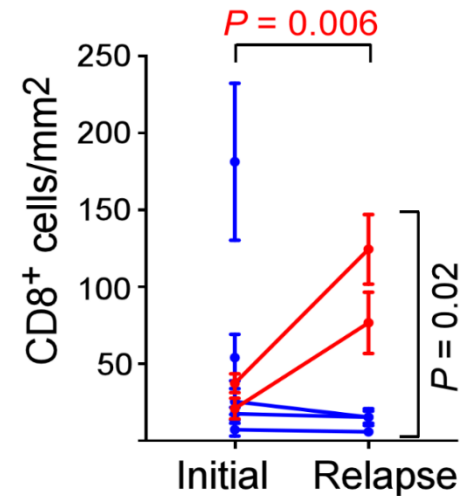
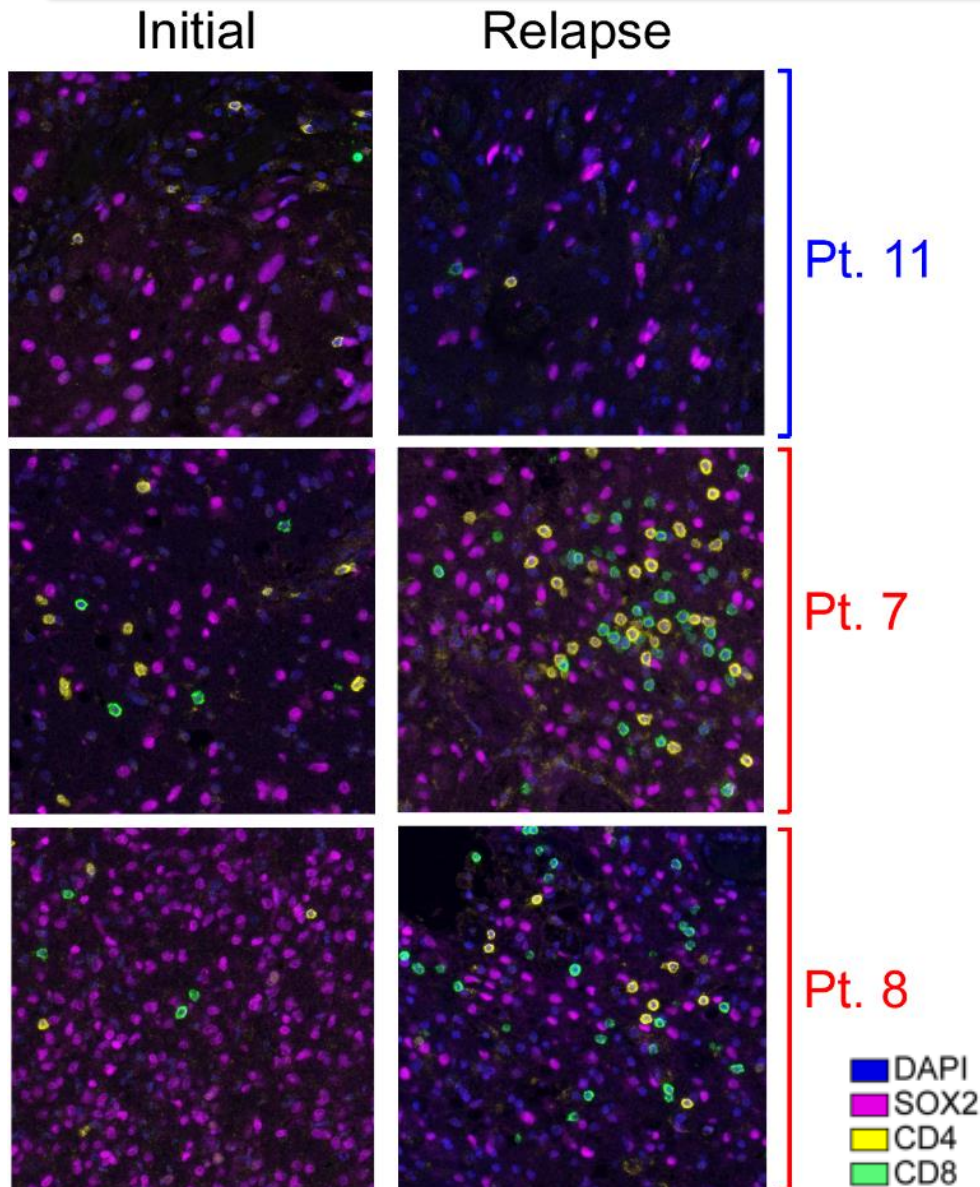


## With Dexamethasone

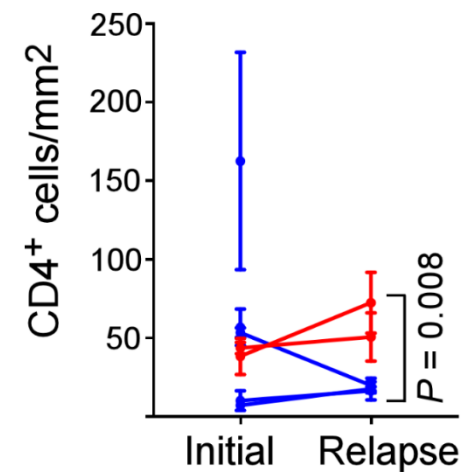


† = Repeat surgical resection

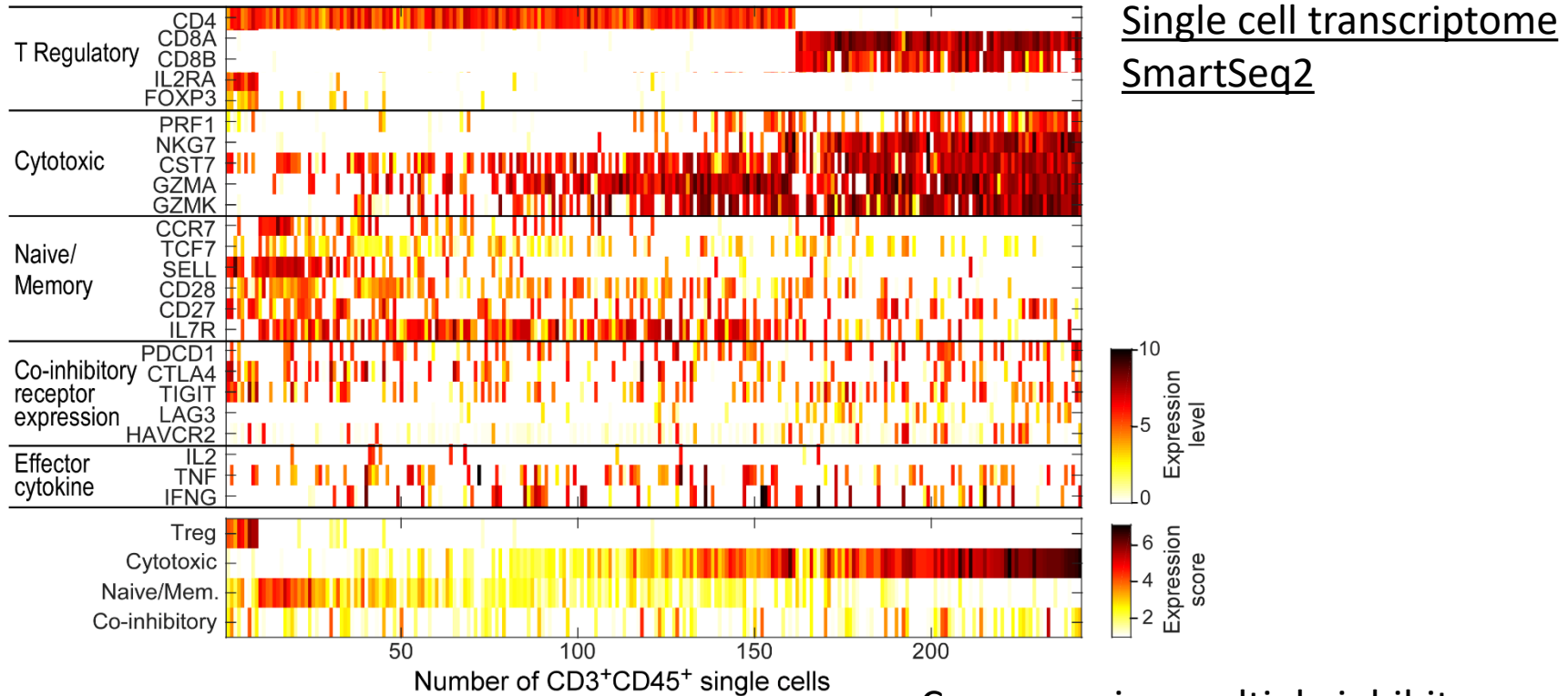
# Increased T cell infiltrate observed post-vaccination



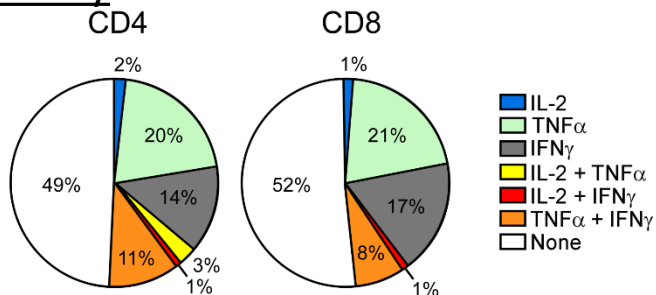
+ Dexamethasone  
- Dexamethasone



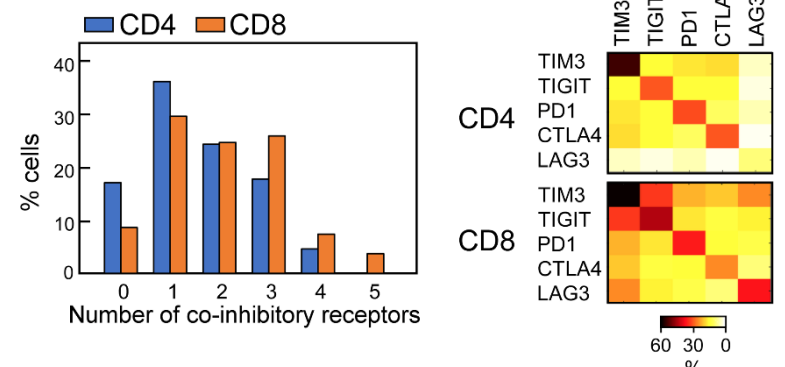
# Functional state of infiltrating CD3+ T cells: Patient 7



## Polyfunctionality



## Co-expression multiple inhibitory molecules

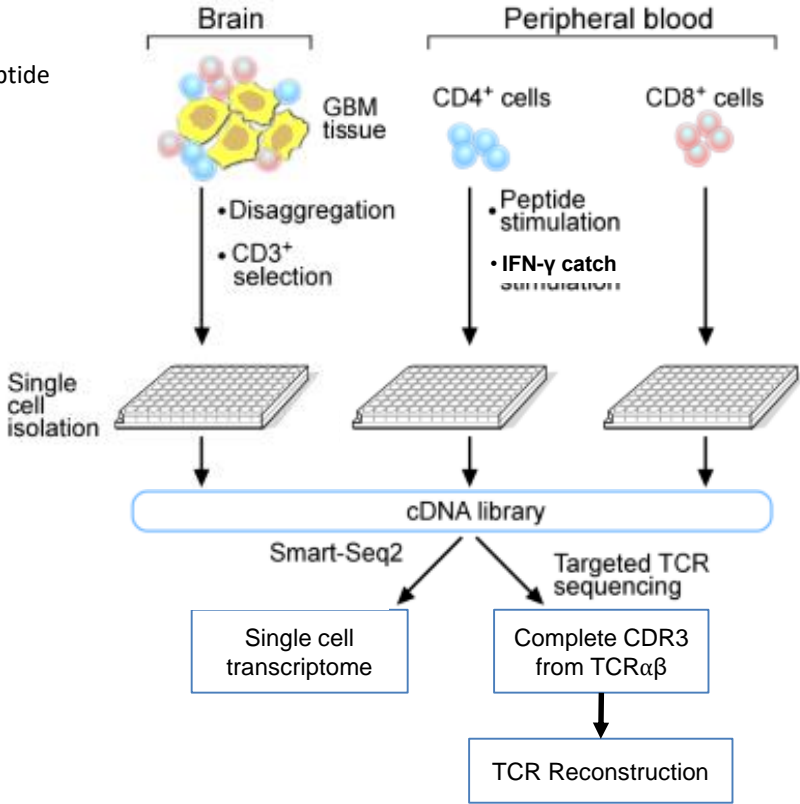
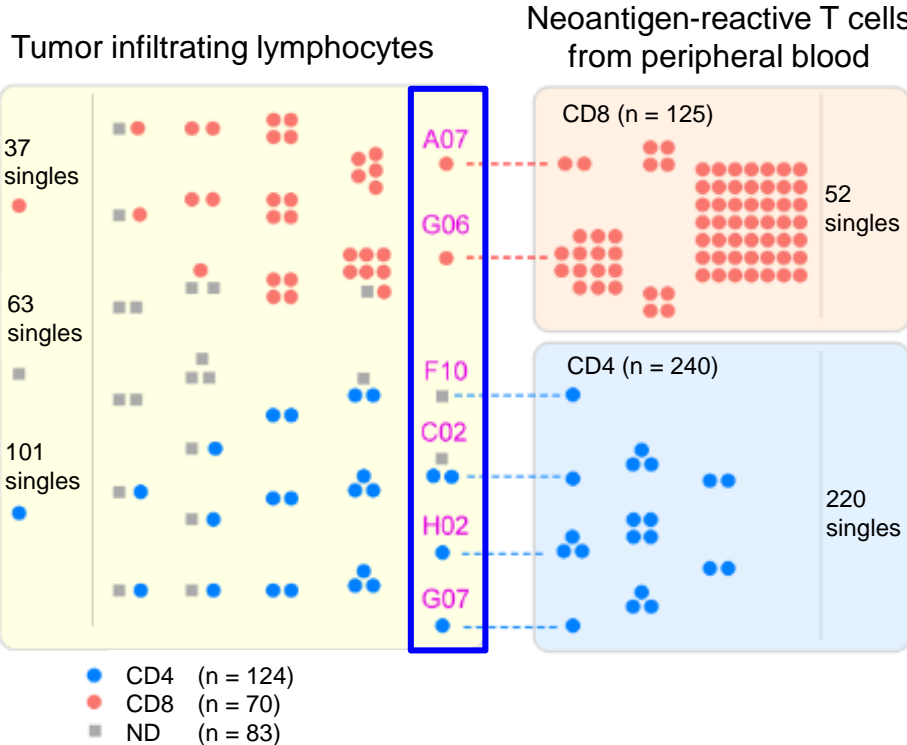


**Question 3. Are infiltrating T cells detected at the intracranial tumor site neoantigen-specific?**

# Patient 7: Identification of intratumoral neoantigen-specific T cells

**Methods**

1. Single cell TCR sequencing of CD3+ TILs isolated from fresh tumor post NeoVax and peptide stimulated T cells from peripheral blood to identify unique TCR clonotypes

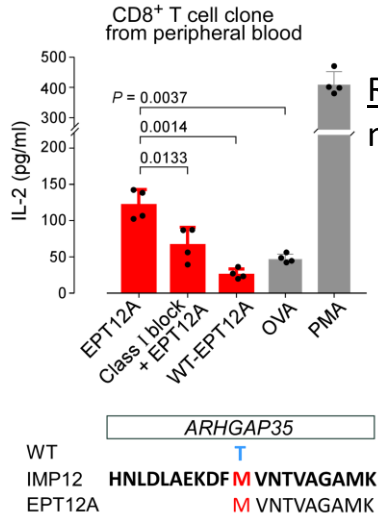


**Results**

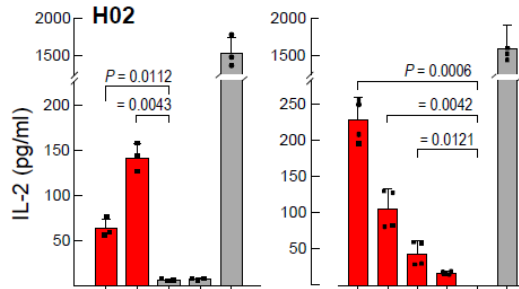
1. TCR sequences from four CD4+ and two CD8+ circulating neoantigen-reactive T cell clonotypes were identical to those from relapsed tumor

# ARHGAP35-specific T cells trafficked from peripheral blood to intracranial GBM

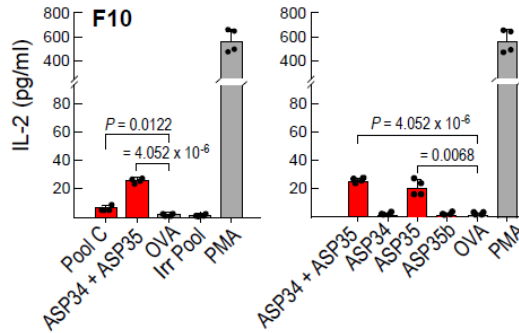
**Methods:** Cloned and expressed TCR clones in TCR deficient Jurkat cells; tested reactivity against neopeptide peptides



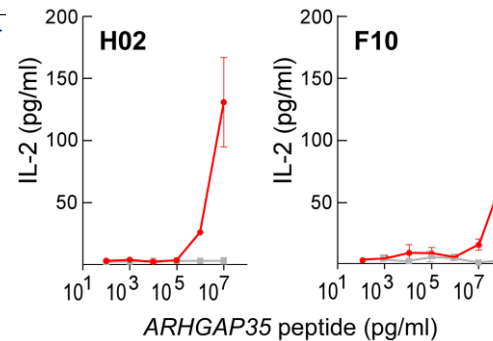
**Results:** Predominant CD8+ TCR clone reacted to EPT12A, a predicted epitope for immunizing neoantigen mut-ARHGAP35



**Results:** Two of four CD4+ TCR clones (H02 and F10) reacted to ASP34 and ASP35, predicted epitopes for immunizing neoantigen mut-ARHGAP35



-●- Mutant  
 -■- Wildtype  
 -▲- OVA



**Results:** TCR clones H02 and F10 discriminated between mutant and wildtype ARHGAP35

*Likelihood blood contamination of tumor?*

Frequency ARHGAP35 specific T cells

Blood: 39 and 29/360,000

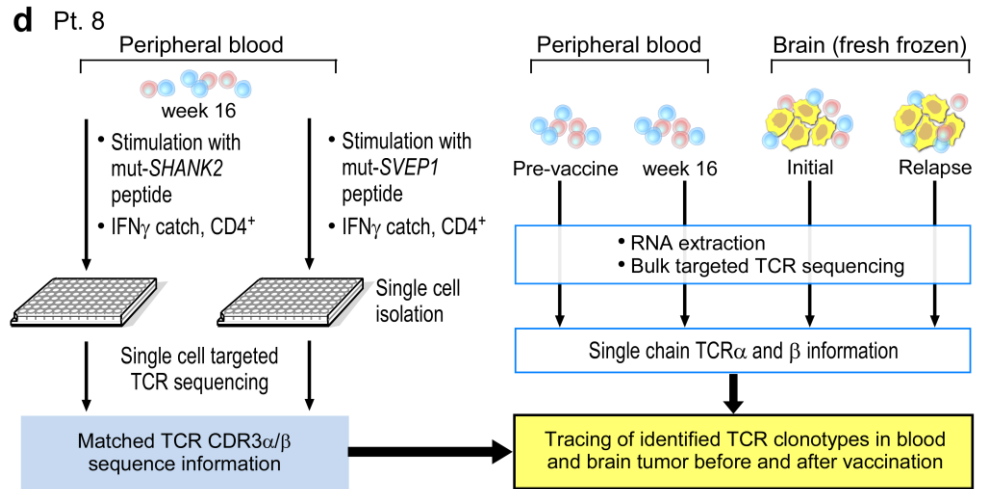
GBM: 1/277

p=0.03, 0.023 (Poisson)

# Patient 8: Identification of intra-tumoral neoantigen-specific T cells

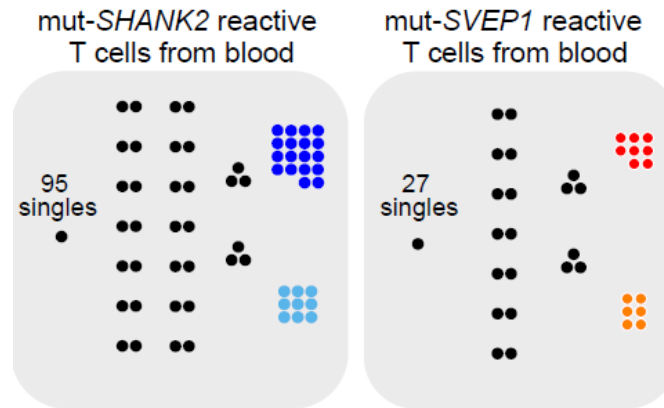
## Methods

1. Single cell TCR sequencing of in vitro neopeptide mut-SHANK2 and mut-SVEP1 stimulated T cells from peripheral blood post-NeoVax (wk 16)
2. Bulk TCR sequencing PBMCs and brain (pre&post NeoVax)

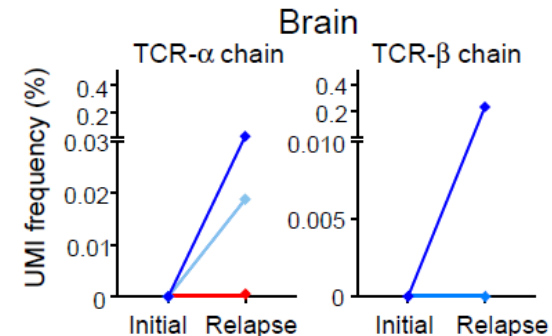
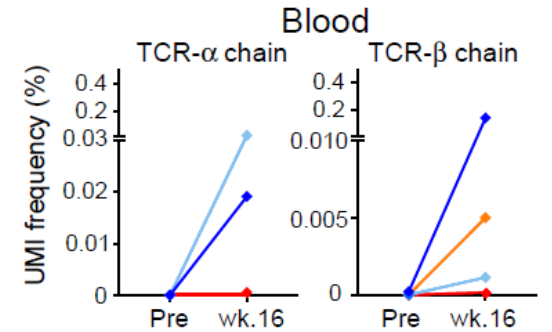


## Results

1. Dominant TCR $\alpha\beta$  mut SHANK 2 and mut-SVEP1 clones detected in week 16 peripheral blood but not pre-vaccine blood
2. Only mut-SHANK2 TCR $\alpha\beta$  directly detected in GBM sample post NeoVax

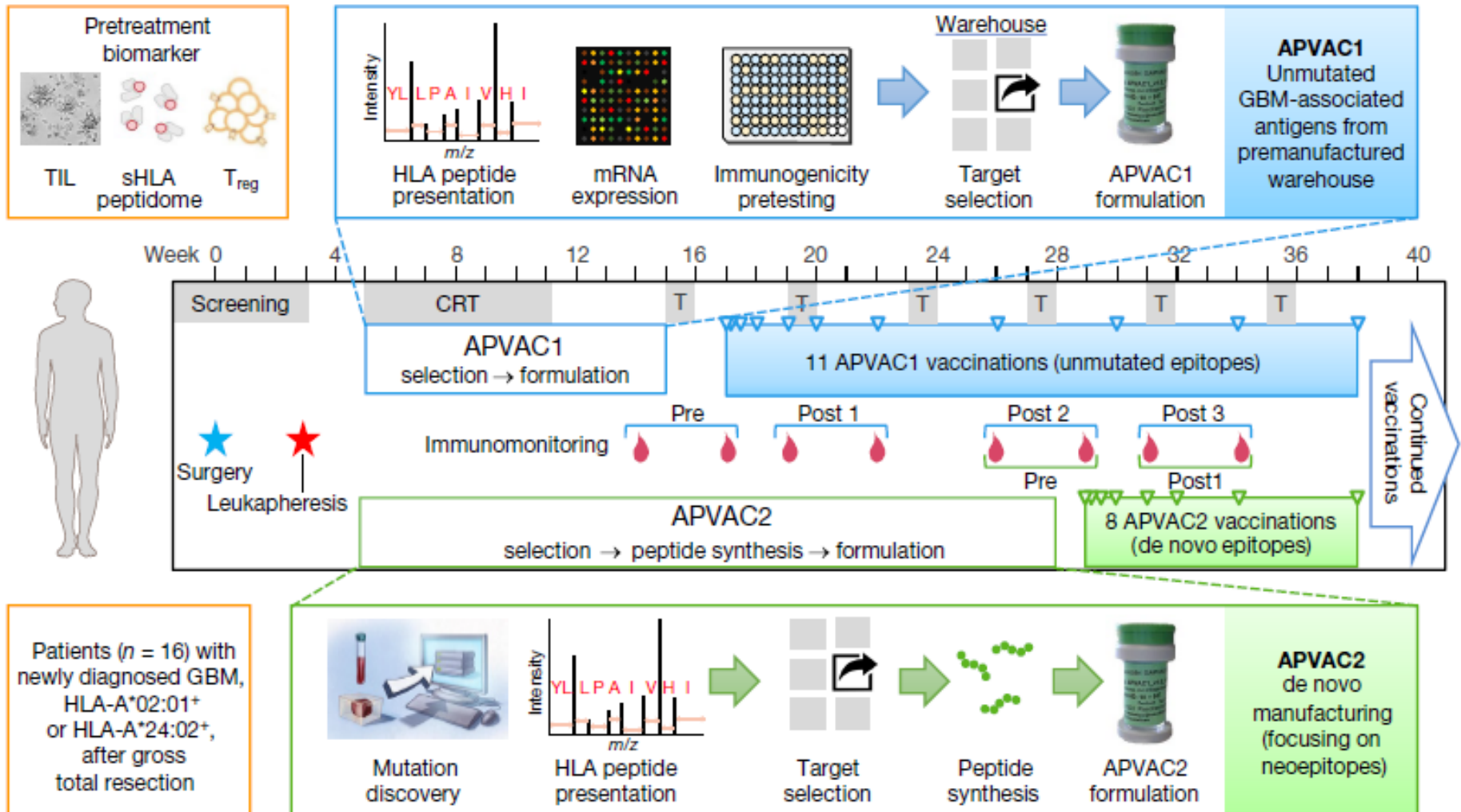


Clonotype	TCR- $\alpha$ chain	TCR- $\beta$ chain
#1	CAGPRDSNYQLIW	CASSLGNQPQHF
#2	CAASAESSNTGKLIF	CASSQDLGKELFF
#3	CIVLGRGYKLSF	CASSAQTGGELFF
#4	CGASPQGGSEKLVF	CASSVGLGRGYEQYF



# Actively personalized vaccination trial for newly diagnosed glioblastoma

Norbert Hilf<sup>1,26</sup>, Sabrina Kuttruff-Coqui<sup>1,26</sup>, Katrin Frenzel<sup>2</sup>, Valesca Bukur<sup>2</sup>, Stefan Stevanovic<sup>3,4</sup>, Cécile Gouttefangeas<sup>3,4,5</sup>, Michael Platten<sup>6,7,8</sup>, Ghazaleh Tabatabai<sup>3,4,9</sup>, Valerie Dutoit<sup>10</sup>, Sjoerd H. van der Burg<sup>5,11</sup>, Per thor Straten<sup>5,12,13</sup>, Francisco Martínez-Ricarte<sup>14</sup>, Berta Ponsati<sup>15</sup>, Hideho Okada<sup>16,17</sup>, Ulrik Lassen<sup>18</sup>, Arie Admon<sup>19</sup>, Christian H. Ottensmeier<sup>20</sup>, Alexander Ulges<sup>1</sup>, Sebastian Kreiter<sup>2,5</sup>, Andreas von Deimling<sup>6,7</sup>, Marco Skardelly<sup>9</sup>, Denis Migliorini<sup>10</sup>, Judith R. Kroep<sup>11</sup>, Manja Idorn<sup>12,13</sup>, Jordi Rodon<sup>14,22</sup>, Jordi Piró<sup>15</sup>, Hans S. Poulsen<sup>18</sup>, Bracha Shraibman<sup>19</sup>, Katy McCann<sup>20</sup>, Regina Mendrzyk<sup>1</sup>, Martin Löwer<sup>2</sup>, Monika Stieglbauer<sup>3,5</sup>, Cedrik M. Britten<sup>2,5,23</sup>, David Capper<sup>6,7,24</sup>, Marij J. P. Welters<sup>5,11</sup>, Juan Sahuquillo<sup>14</sup>, Katharina Kiesel<sup>1</sup>, Evelyn Derhovanessian<sup>2</sup>, Elisa Rusch<sup>3,5</sup>, Lukas Bunse<sup>6,7</sup>, Colette Song<sup>1</sup>, Sandra Heesch<sup>2</sup>, Claudia Wagner<sup>1</sup>, Alexandra Kemmer-Brück<sup>2</sup>, Jörg Ludwig<sup>1</sup>, John C. Castle<sup>2,25</sup>, Oliver Schoor<sup>1</sup>, Arbel D. Tadmor<sup>21</sup>, Edward Green<sup>7,8</sup>, Jens Fritsche<sup>1</sup>, Miriam Meyer<sup>1</sup>, Nina Pawlowski<sup>1</sup>, Sonja Dorner<sup>1</sup>, Franziska Hoffgaard<sup>1</sup>, Bernhard Rössler<sup>1</sup>, Dominik Maurer<sup>1</sup>, Toni Weinschenk<sup>1</sup>, Carsten Reinhardt<sup>1</sup>, Christoph Huber<sup>2</sup>, Hans-Georg Rammensee<sup>3,4</sup>, Harpreet Singh-Jasuja<sup>1</sup>, Ugur Sahin<sup>2</sup>, Pierre-Yves Dietrich<sup>10</sup> & Wolfgang Wick<sup>6,7\*</sup> Nature 2019



APVAC1 unmutated

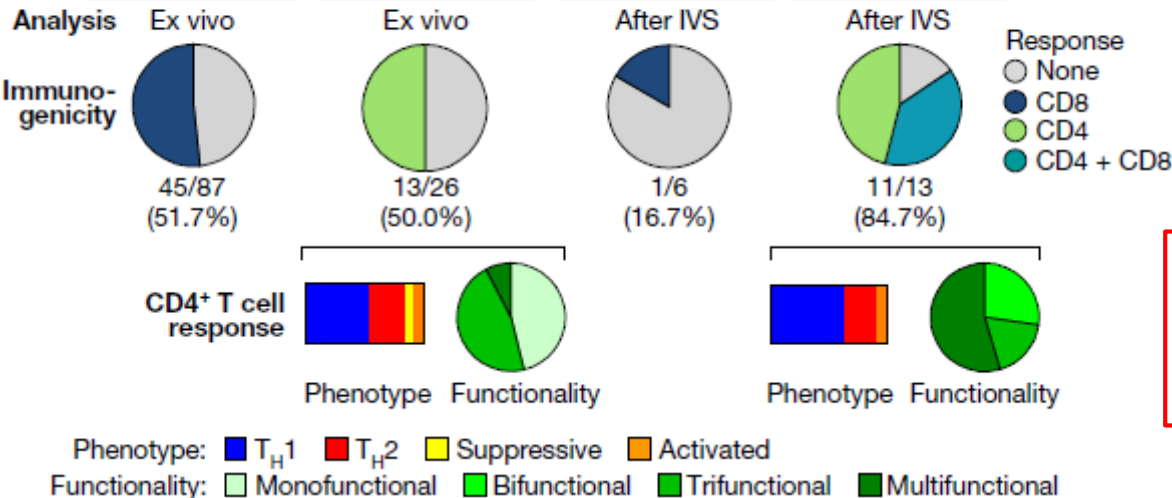
APVAC2 de novo

Class I (9-10 aa)

Class II (14-18 aa)

Unmutated (9-10 aa)

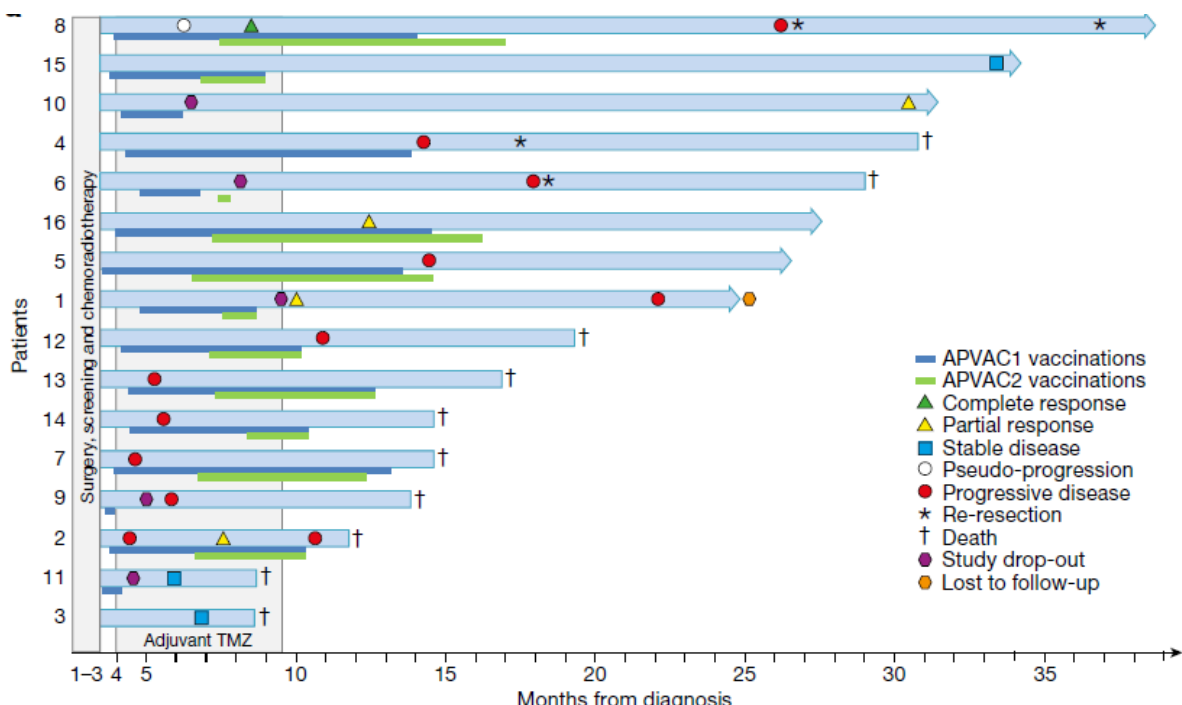
Mutated (19 aa)



High rate of systemic immune responses to APVAC1 and APVAC2 vaccinated antigens

Newly dx'd GBM (n=16)  
 HLA-A\*02:01 or HLA-A\*24:02  
 GTR  
 <= 2 mg decadron/day

Median follow-up: 31.5 months  
 Median PFS: 14.2 months  
 Median OS: 29.0 months



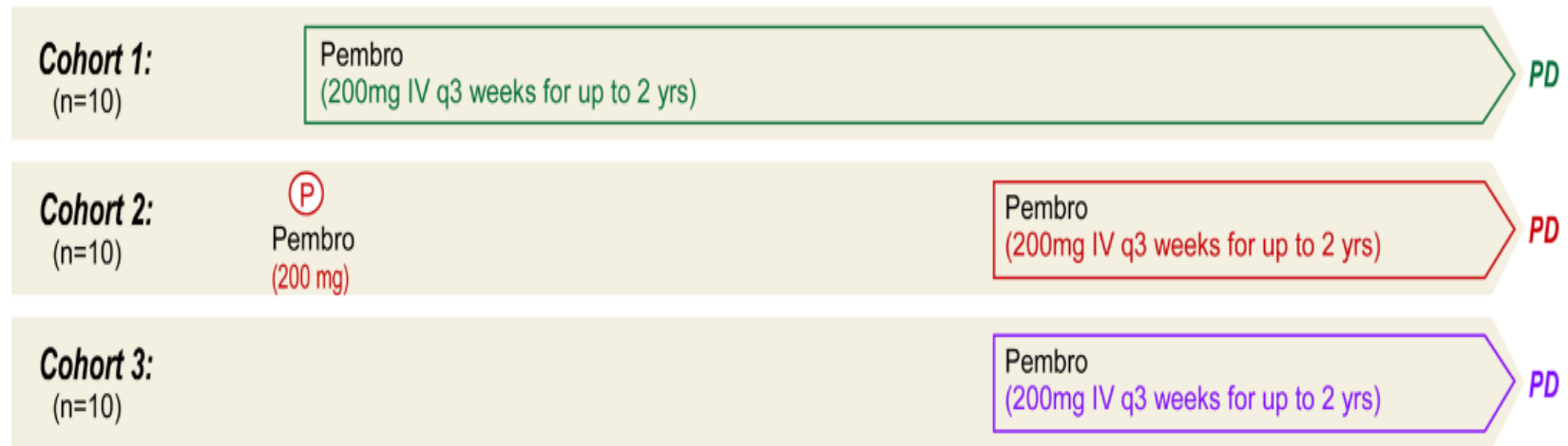
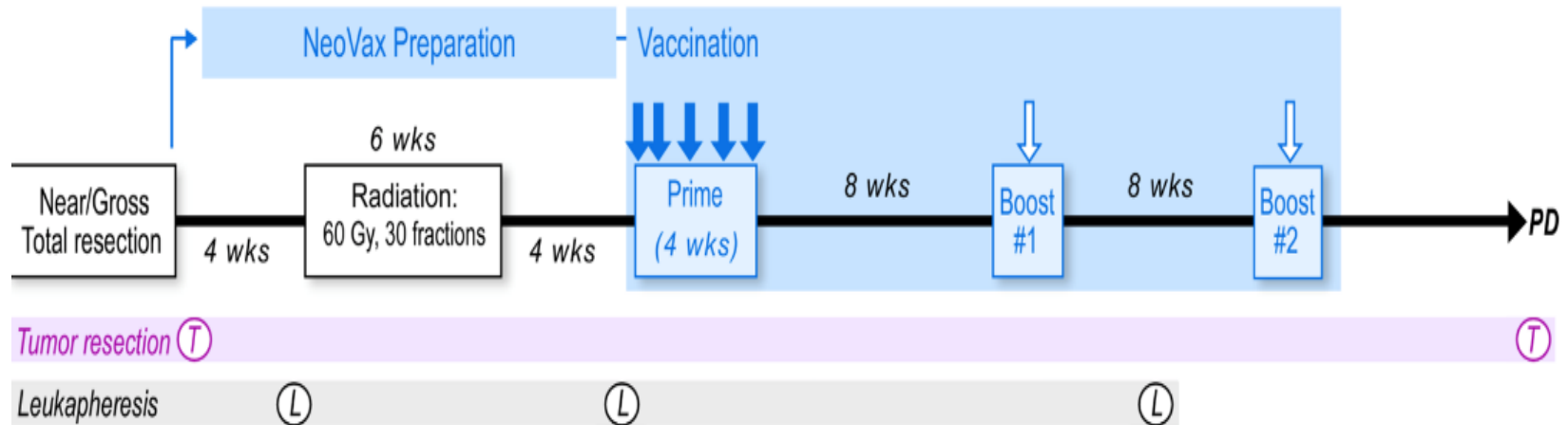
# Next Step

- NeoVax can stimulate tumor-specific immune cells to activate in the bloodstream and successfully traffic into the tumor in the brain.
- Can we block immunosuppression generated by the tumor to allow the NeoVax generated tumor specific immune cells to a chance to successfully attack the tumor?



# NeoVax + Anti-PD-1 for Newly Diagnosed GBM

Planned Initiation: Spring 2019



# Conclusions

- I. Immunotherapy for medical oncology: tip of the iceberg?
- I. Multiple hurdles for immunotherapy in neuro-oncology
  - GBM: low inherent immunogenicity
  - GBM microenvironment: A “perfect storm” of multiple complementary mechanisms exploited to impair anti-tumor immune responses
- III. Personalized vaccination against tumor-specific neoantigens
  - strategy to therapeutically exploit tumor heterogeneity
  - feasible and safe
  - encouraging systemic and intratumoral immunogenicity
- V. Path forward
  - Increased fundamental understanding of tumor immunoevasion mechanisms in GBM
  - Rationale combinatorial regimens
  - Avoid steroids!!

# Acknowledgements

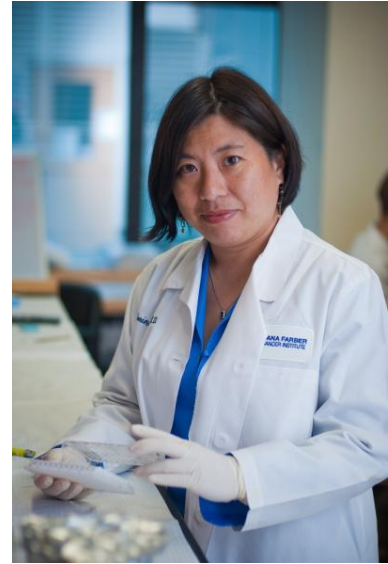
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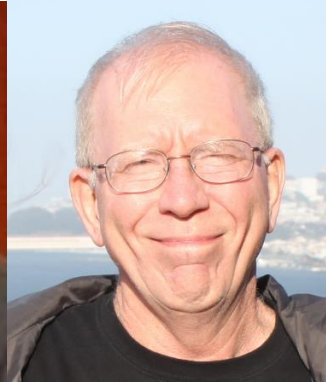
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