Filtering out self-like neoantigens improves immune response to cancer vaccines

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Prepared for:
AACR Annual Meeting 2019
Cancer Vaccines and Intratumoral Immunomodulation Minisymposium
Sunday Mar. 31st, 2019
Atlanta, Georgia, USA

Who are we?
EpiVax Oncology, est. 2017

EpiVax → 2017 → **EpiVax Oncology**

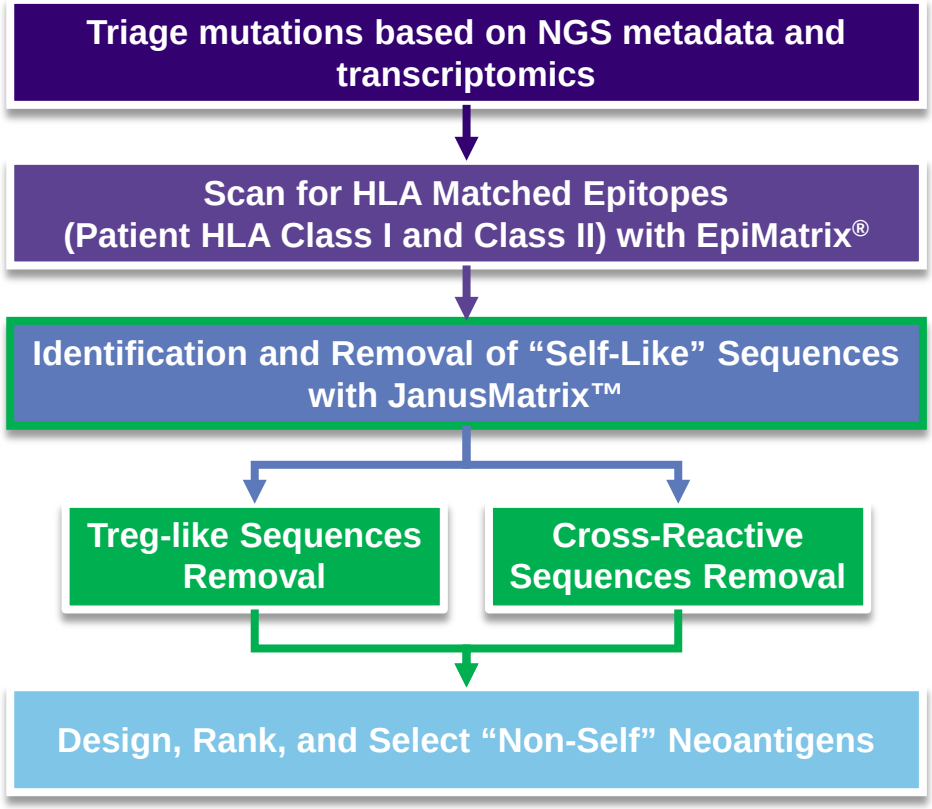
20+ years of experience in vaccinology → Ancer™ → ***Precision cancer immunotherapy***

Commercial-grade *in silico* neo-epitope discovery platform based on machine learning algorithms

- EpiVax Oncology, Inc. is company created in 2017 by EpiVax, Inc.
- EpiVax, Inc. is a 20-year-old, privately held immunoinformatics biotech.

What do we do?
Use commercial-grade tools to design precision cancer immunotherapies

Ancer™ platform



Ancer™ strengths

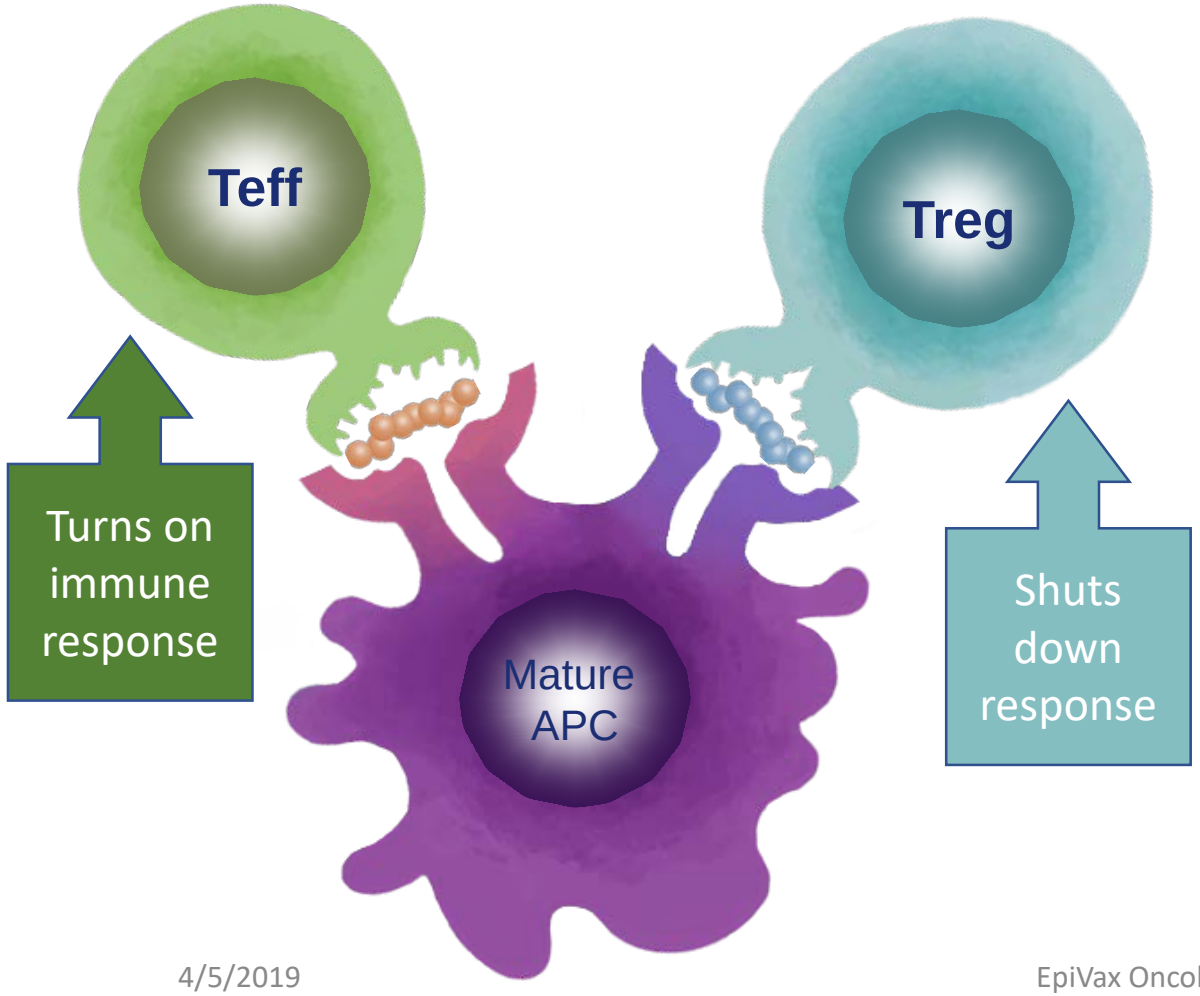
- Commercially-used, machine learning-based, CD4 and CD8 T cell prediction tools (since 1998)
- Identify and remove regulatory T cells (Treg) epitopes (since 2013)
- De-risk vaccines for Treg induction or immune adverse events
- Precise design of neoantigen-based vaccines

Talk Overview?
It's all about finding and removing Treg neo-epitopes.

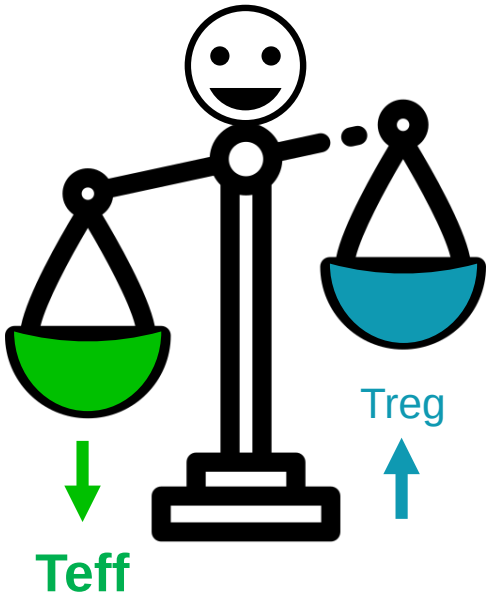
- Some MHC II T cell epitopes are recognized by Tregs and reduce vaccine efficacy.
- In this presentation I will show you that:
  1. We find self-like (i.e. putative Treg) neo-epitopes in cancer mutanomes and remove them from vaccines.
  2. We design highly immunogenic vaccines by precisely selecting MHC I and MHC II effector neo-epitopes.
  3. Some self-like MHC II neo-epitopes reduce vaccine immunogenicity by 5-fold.
- Inclusion of Treg neo-epitopes in cancer immunotherapies may be a cause for lack of efficacy.

Some T cell epitopes may engage Tregs.
Achieving the right balance between Teff and Treg epitopes is important.

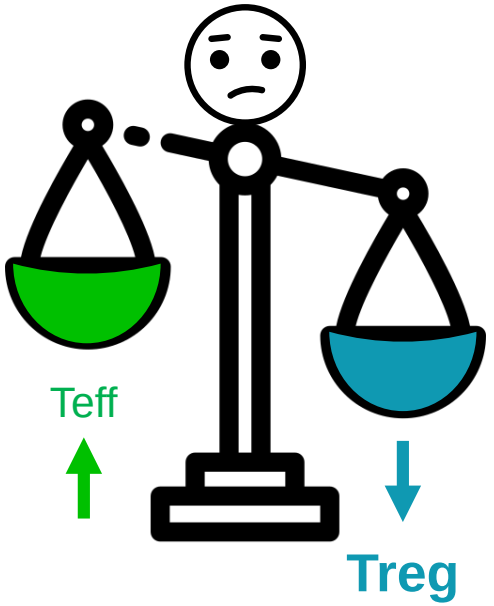
Epitopes can be either effector or regulatory



Inclusion of Treg epitopes may hinder vaccine efficacy



Good vaccines: balance shifted toward inflammation



Poor vaccines: balance shifted towards regulation

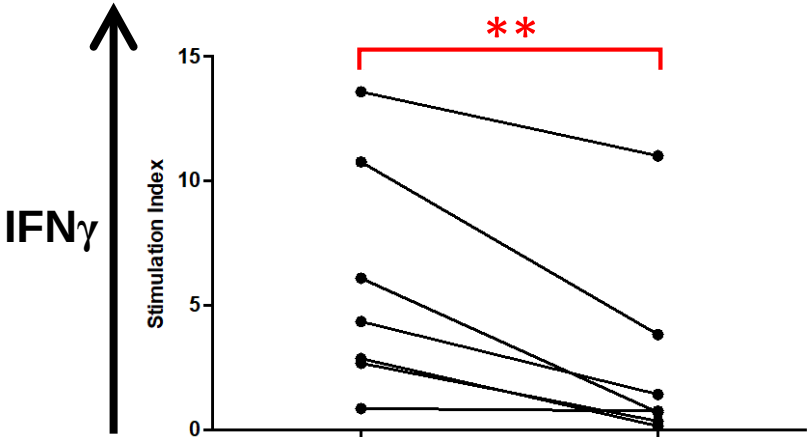
How to eliminate Treg responses?

We identified Treg epitopes in pathogens.
Can lessons learned from infectious disease be translated to oncology?

In silico tools help us identify immunosuppressive T cell epitopes.

Moise, Hum Vaccin Immunother 2013

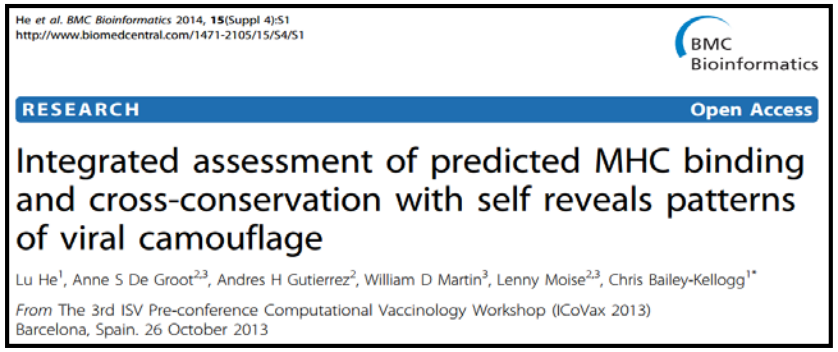
Flu case study:



Lines link experiments performed with the same donor PBMCs.

In silico-derived influenza (H7N9) Treg epitope reduces IFNg responses to effector peptides.

Liu, Hum Vaccin Immunother 2015


He *et al. BMC Bioinformatics* 2014, **15**(Suppl 4):S1
http://www.biomedcentral.com/1471-2105/15/S4/S1

BMC Bioinformatics

RESEARCH Open Access

Integrated assessment of predicted MHC binding and cross-conservation with self reveals patterns of viral camouflage

Lu He[1], Anne S De Groot[2,3], Andres H Gutierrez[2], William D Martin[3], Lenny Moise[2,3], Chris Bailey-Kellogg[1*]

From The 3rd ISV Pre-conference Computational Vaccinology Workshop (ICoVax 2013)
Barcelona, Spain. 26 October 2013

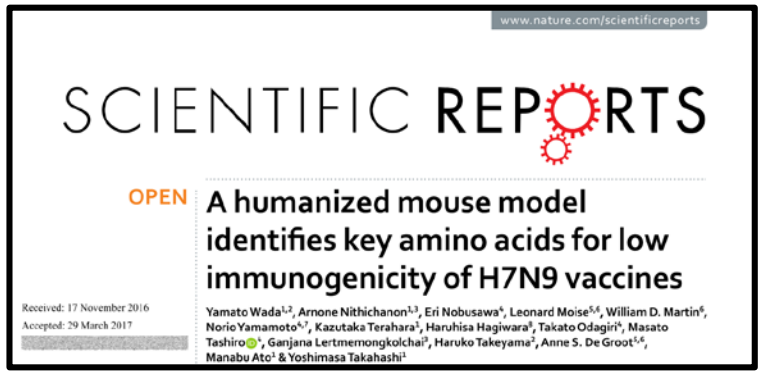

RESEARCH PAPER

Human Vaccines & Immunotherapeutics 11:9, 2241–2252; September 2015; Published with license by Taylor & Francis Group, LLC

H7N9 T-cell epitopes that mimic human sequences are less immunogenic and may induce Treg-mediated tolerance

Rui Liu[1], Leonard Moise[1,2], Ryan Tassone[1], Andres H Gutierrez[1], Frances E Terry[2], Kotou Sangare[3], Matthew T Ardito[2], William D Martin[2], and Anne S De Groot[1,2,*]

[1]Institute for Immunology and Informatics; University of Rhode Island; Providence, RI USA; [2]EpiVax Inc.; Providence, RI USA; [3]Laboratory of Applied Molecular Biology (LBMA); University of Bamako; Bamako, Mali


www.nature.com/scientificreports

SCIENTIFIC REPORTS

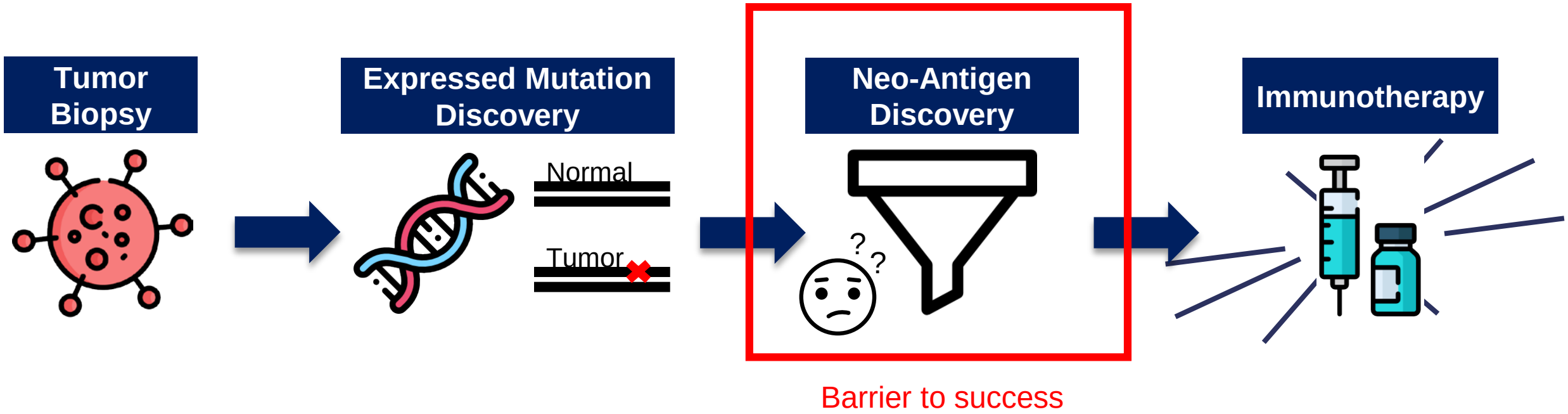
OPEN

A humanized mouse model identifies key amino acids for low immunogenicity of H7N9 vaccines

Received: 17 November 2016
Accepted: 29 March 2017

Yamato Wada[1,2], Arnone Nithichanon[1,3], Eri Nobusawa[4], Leonard Moise[5,6], William D. Martin[6], Norio Yamamoto[4,7], Kazutaka Terahara[1], Haruhisa Hagiwara[8], Takato Odagiri[4], Masato Tashiro[4], Ganjana Lertmemongkolchai[3], Haruko Takeyama[2], Anne S. De Groot[5,6], Manabu Ato[1] & Yoshimasa Takahashi[1]

Finding the right neo-epitopes to include in personalized immunotherapies remains a challenge.



Which neo-epitopes should be included in personalized immunotherapies?

Traditional considerations:

- Variant Expression
- Variant Clonality
- Class I MHC binding (CD8 T cells)

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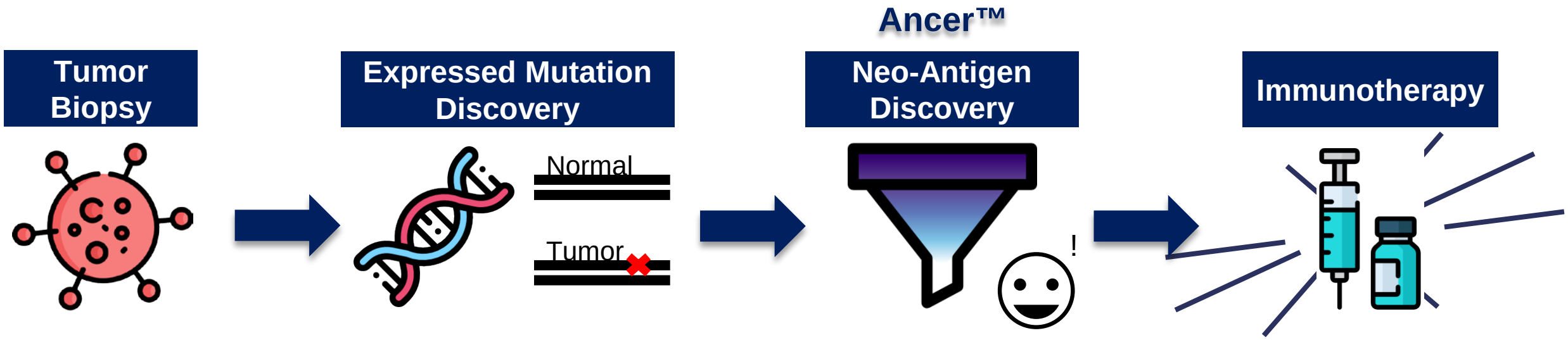


Which neo-epitopes should be included in personalized immunotherapies?

Traditional considerations:

- Variant Expression
- Variant Clonality
- Class I MHC binding (CD8 T cells)

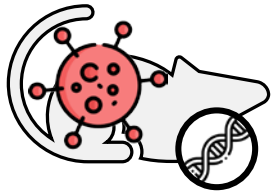
Additional novel considerations:

- **Class II MHC binding (CD4 T cells)**
- **Type of T cell response (Teff or Treg)**

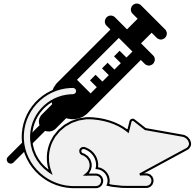
Outline

- Background
- Can our *in silico* platform generate immunogenic neo-epitope-based vaccines?
- Can certain neo-epitopes negatively affect the outcome of immunotherapies?
- Summary and next steps

We precisely designed a new CT26 vaccine enriched for Teff content and with reduced risk of engaging Tregs



Ancer™ commercial-grade platform



CT26 Mutanome	Neo-Epitope Analysis	Neo-Epitope Selection	Vaccine Administration	Immunogenicity Efficacy
378 SNVs post QC and filtering	135 Teff neoantigens	Top 20 neoantigens	20 Ancer™ peptides	ELISpot Flow Cytometry Tumor Volume

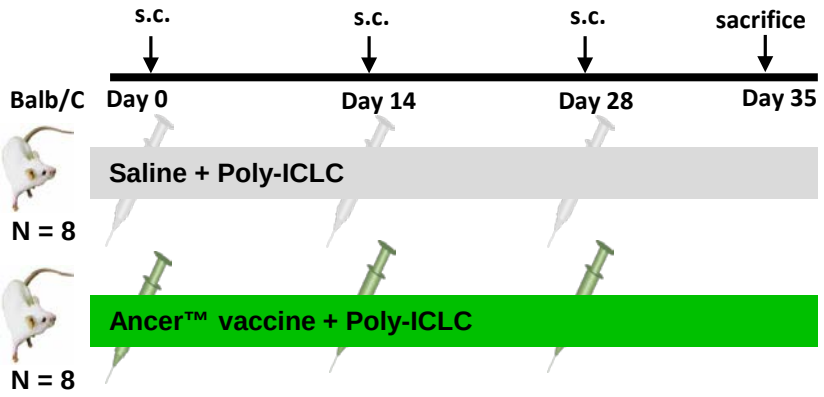
MHC I neo-epitopes ✓
MHC II neo-epitopes ✓
No predicted inhibitory neo-epitopes ✓

11-18-mer peptides, designed *in silico.*

Only two peptides overlap mutations previously reported as immunogenic (Kreiter, Nature 2015)

Immunization of naïve Balb/c mice with our CT26 vaccine induced strong IFNγ ELISpot responses

Protocol

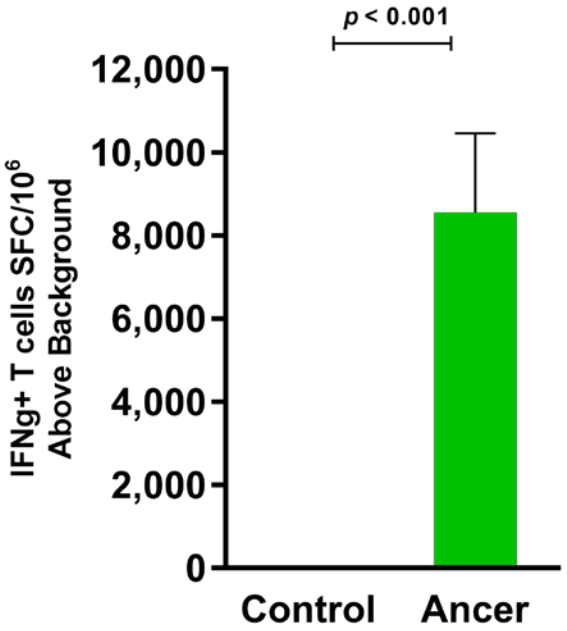


Prime + boost x2, bi-weekly

Subcutaneous injection

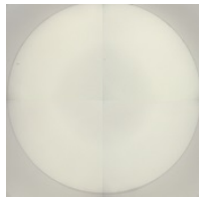
Splenocytes collected at Day 35

IFNγ ELISpot Results

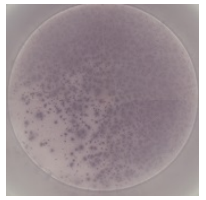


Mean + SEM

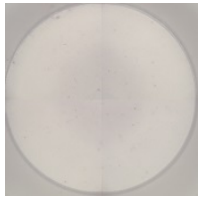
Strong neo-epitope responses



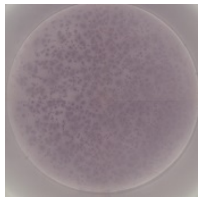
Assay negative control (media)



Assay positive control (ConA)



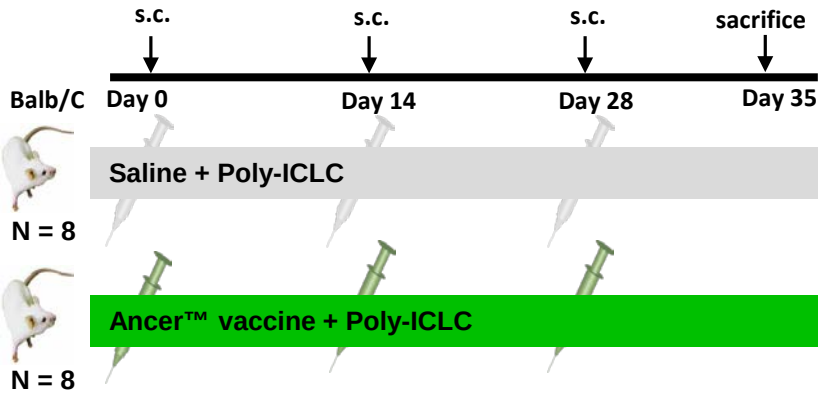
Control vaccine



Ancer™ peptides

Flow cytometry confirmed that our CT26 vaccine stimulated multifunctional CD4+ and CD8+ T cells

Protocol

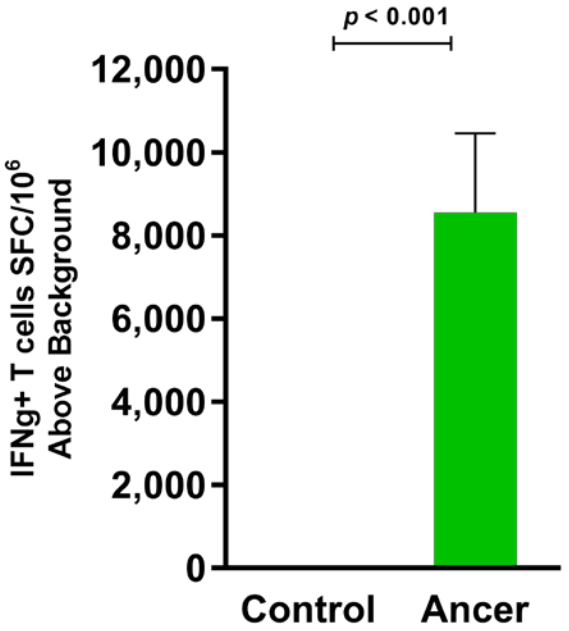


Prime + boost x2, bi-weekly

Subcutaneous injection

Splenocytes collected at Day 35

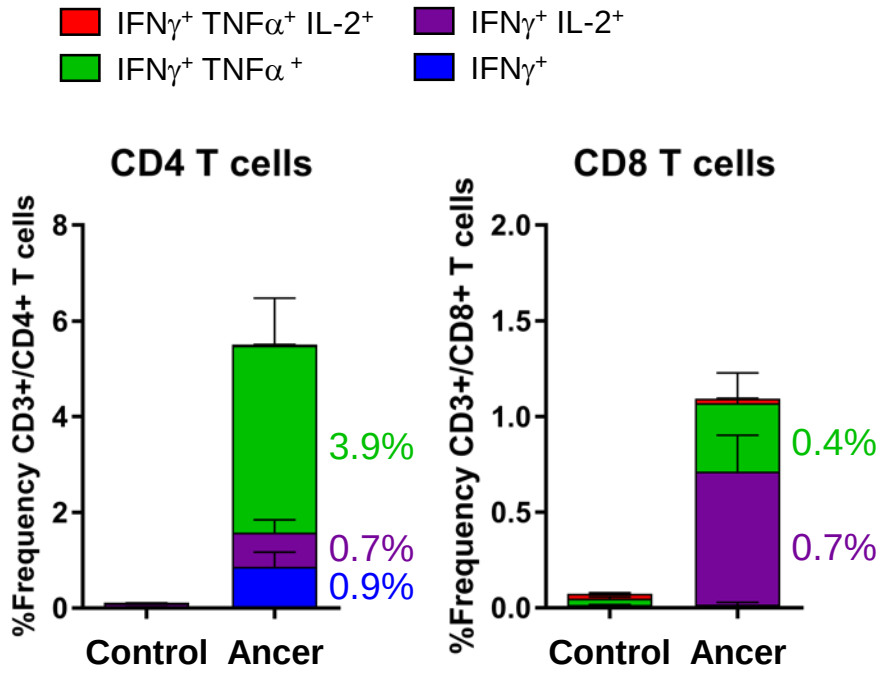
IFNγ ELISpot Results



Mean + SEM

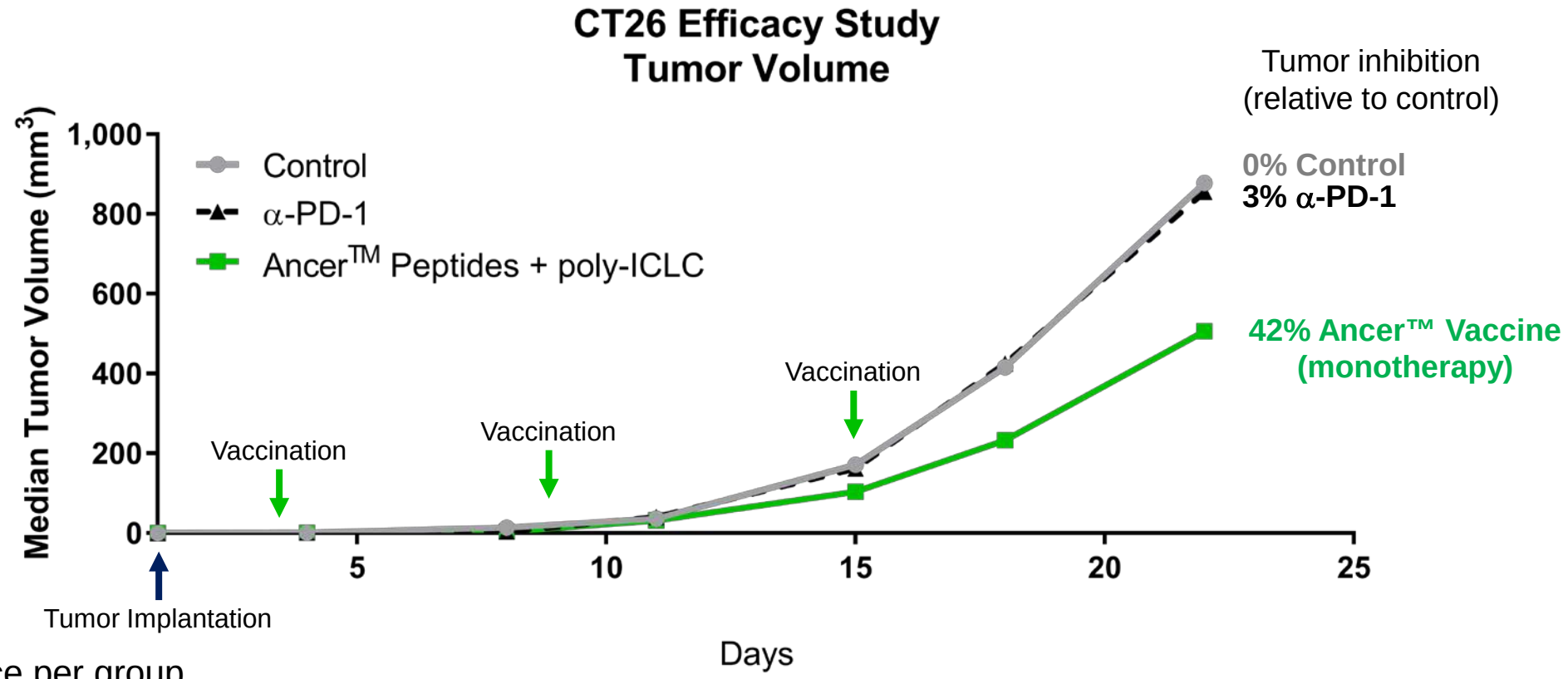
Strong neo-epitope responses

Flow Cytometry Results



Multifunctional CD4+/CD8+ response

Preliminary results show a 42% reduction in tumor burden with our CT26 vaccine (unoptimized dosing schedule)



N = 12 mice per group

Ancer™ vaccine alone reduces median CT26 tumor burden by 42% at day 22. Additional efficacy studies are ongoing.

Outline and preliminary conclusions

- Background
- Can our *in silico* platform generate immunogenic neo-epitope-based vaccines?

  YES
- Can certain neo-epitopes negatively affect the outcome of immunotherapies?
- Summary and next steps

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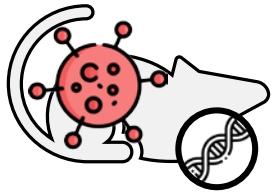
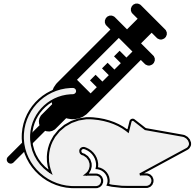
We selected 10 MHC II “self-like” CT26 neo-epitopes. We hypothesize these may be Treg epitopes.



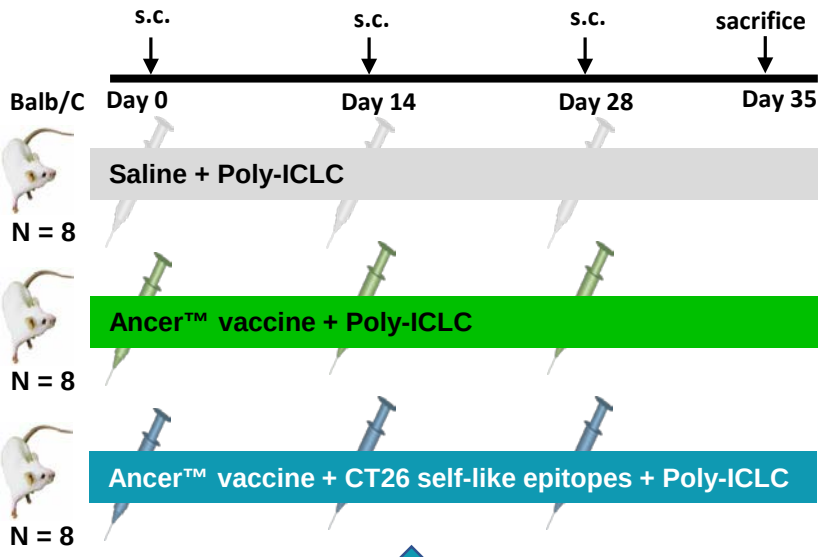


Ancer™ commercial-grade platform

CT26 Mutanome	Neo-Epitope Analysis	Neo-Epitope Selection	Vaccine Administration	Immunogenicity Efficacy
378 SNVs post QC and filtering	135 Teff neoantigens	Top 20 neoantigens	20 Ancer™ peptides	ELISpot Flow Cytometry Tumor Volume
	27 MHC II self-like neo-epitopes	10 putative Treg neo-epitopes (MHC II)	20 Ancer™ + 10 self-like peptides	ELISpot

Co-administration of CT26 self-like neo-epitopes with our immunogenic vaccine diminished IFNγ ELISpot responses by 5-fold

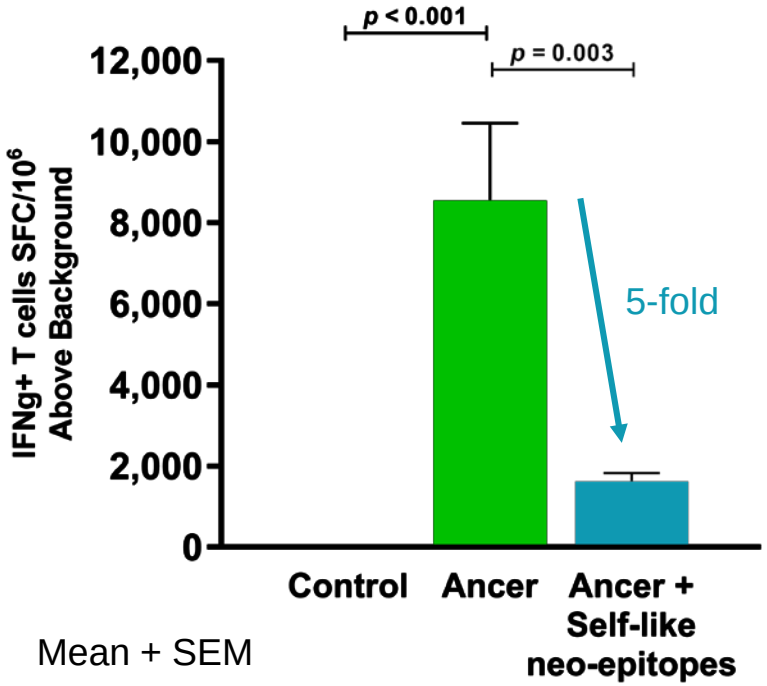
Protocol



Does adding back "Treg" neo-epitopes suppress the immune response?

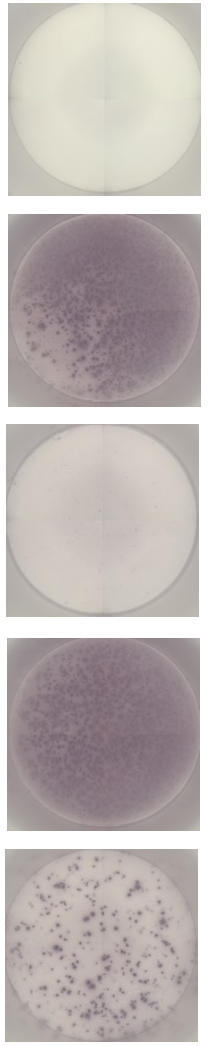
Prime + boost x2, bi-weekly
Subcutaneous injection
Splenocytes collected at Day 35

IFNγ ELISpot Results



Mean + SEM

Immunogenicity reduced 5-fold when including MHC II self-like neo-epitopes



Assay negative control (media)

Assay positive control (ConA)

Control vaccine

Ancer™ peptides

Ancer™ + self-like peptides

Outline

- Background
- Can our *in silico* platform we generate immunogenic neo-epitope-based vaccines? YES
- Can certain neo-epitopes negatively affect the outcome of immunotherapies? YES
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Summary

- ***Not all neo-epitopes are created equal!***
- **Some neo-epitopes may suppress immune responses due to their homology with self-sequences.**
- We designed a new highly immunogenic CT26 neoantigen-based vaccine **enhanced for both CD4 and CD8 T effector content and devoid of self-like epitopes**.
- Our vaccine induced **strong IFNγ ELISpot responses** and **multifunctional CD4+ and CD8+ T cell responses**.
- Co-administration of **computationally predicted inhibitory MHC II neo-epitopes** with our vaccine reduced its immunogenicity by **5-fold**.

Next Steps

- Perform bystander suppression (Treg) assays to confirm the suppressive effect of the CT26 self-like neo-epitopes.
- Determine if CT26 self-like neo-epitopes affect both CD4 and CD8 T cell responses.
- Efficacy studies are ongoing and will clarify the role of self-like neo-epitopes on tumor growth and survival.

Collaborations are welcomed!

Acknowledgments to the EpiVax and EpiVax Oncology family

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