An immunogenic neoantigen vaccine for glioblastoma designed with machine learning-based algorithms

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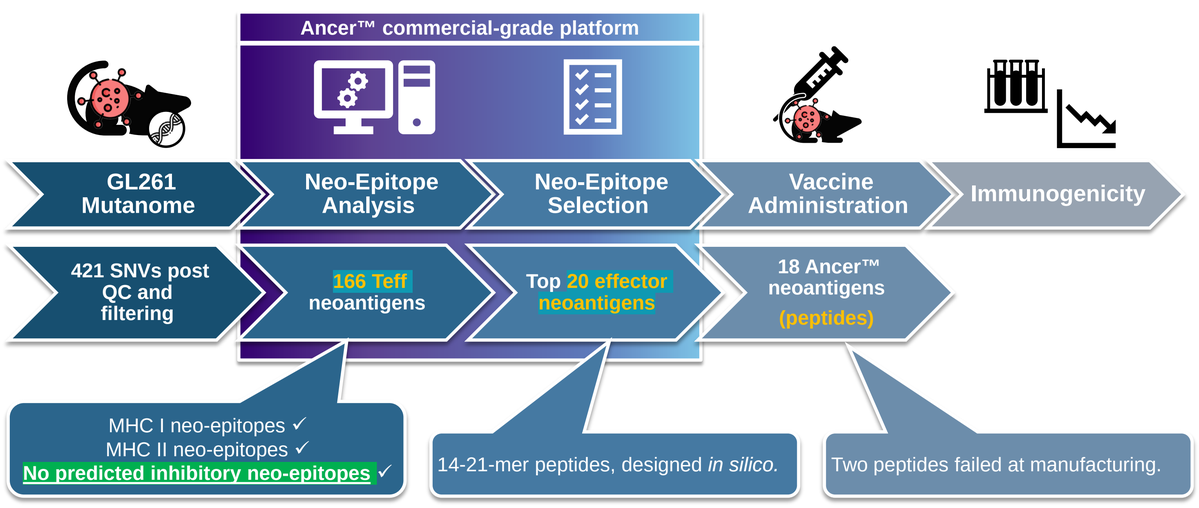
Overview

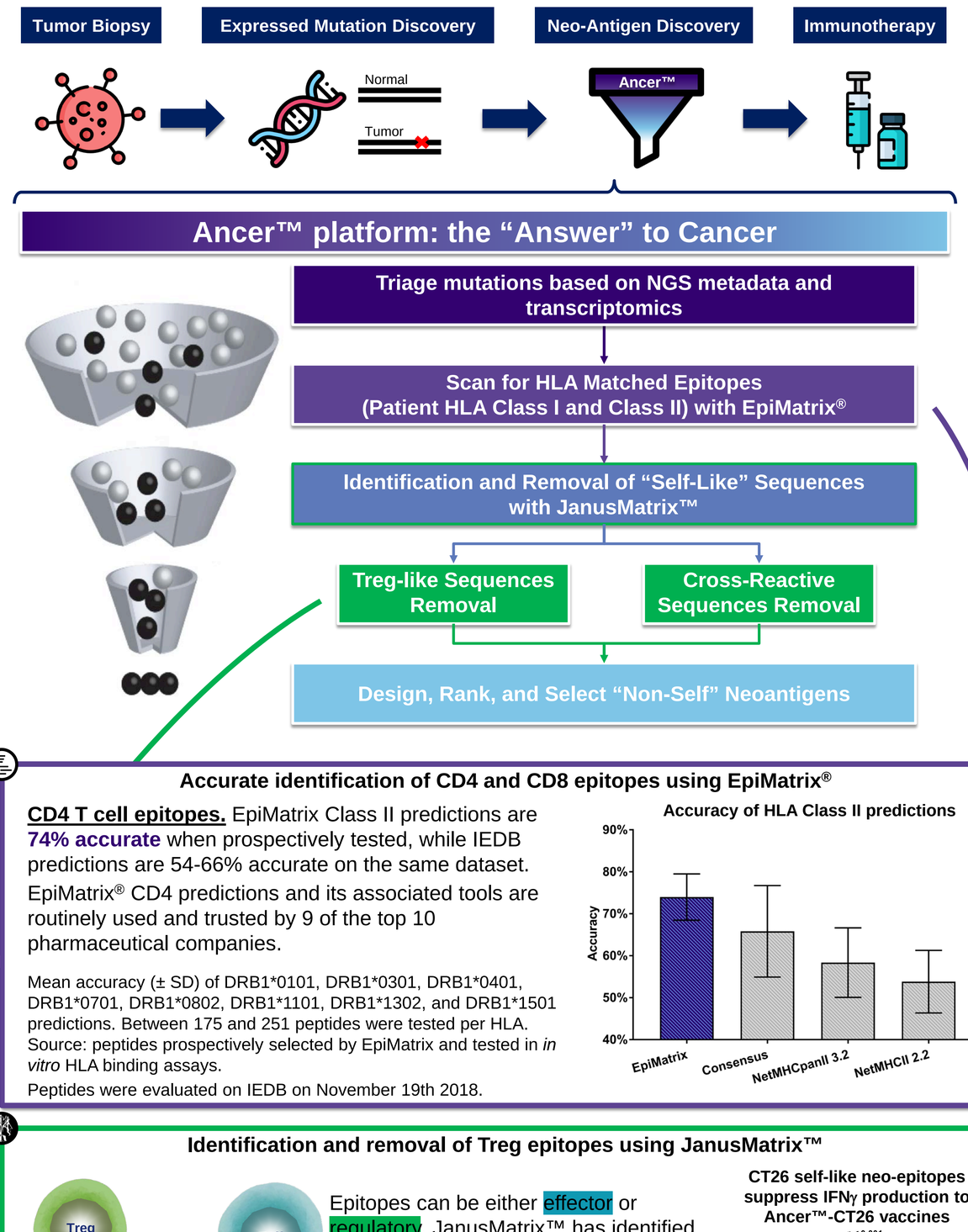
- We sequenced the **GL261 glioblastoma (GBM) orthotopic mouse model** using Next Generation Sequencing to design a **neoantigen-based GBM vaccine**.
- We used the **Ancer™ platform** to identify and rank mutations encoding **CD8 and CD4 neo-epitopes**.
- The Ancer™ platform uses **machine learning-based** algorithms and **specialized homology** tools to remove putative **regulatory T cell epitopes** from vaccine designs.
- Our Ancer™-GBM vaccines (peptides + poly-ICLC) was administered in GL261 tumor bearing animals and its **immunogenicity was tested in LAMP1 (CD107A) degranulation and IFNγ secretion assays**.
- **Ancer™-GBM** vaccine induced **high levels of cytotoxic T cells** and **elevated IFNγ production** in tumor bearing animals.
- ***In silico* screening** of neoantigen sequences using **specialized** tools offers the possibility of enriching and designing **new vaccines with higher quality candidates**.
- Follow-up studies include assessment of **T cell infiltration in brain tissues** collected from immunized mice and the **impact of the Ancer™-GBM vaccine on animal survival.**

In silico design of a glioblastoma neoantigen vaccine



- The GL261 glioblastoma mouse cell line exome and transcriptome was sequenced and its mutanome was analyzed with the Ancer™ platform.
- Ancer™ selects sequences with the highest potential for inducing effector T cell responses while minimizing the risk of inducing regulatory T cell responses.
- Effector neoantigen sequences were identified and ranked based on their MHC Class I and MHC Class II immunogenicity.
- Top sequences were synthesized as peptides (Ancer™-GBM vaccine) and their immunogenicity tested in GL261 tumor bearing animals.

Mutanome-Directed Cancer Immunotherapy Design Platform



Accurate identification of CD4 and CD8 epitopes using EpiMatrix®

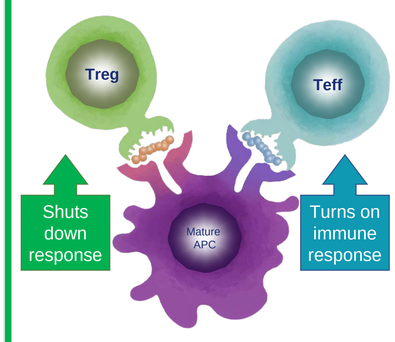
CD4 T cell epitopes. EpiMatrix Class II predictions are **74% accurate** when prospectively tested, while IEDB predictions are 54-66% accurate on the same dataset.

EpiMatrix® CD4 predictions and its associated tools are routinely used and trusted by 9 of the top 10 pharmaceutical companies.

Mean accuracy (± SD) of DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701, DRB1*0802, DRB1*1101, DRB1*1302, and DRB1*1501 predictions. Between 175 and 251 peptides were tested per HLA. Source: peptides prospectively selected by EpiMatrix and tested in *in vitro* HLA binding assays.

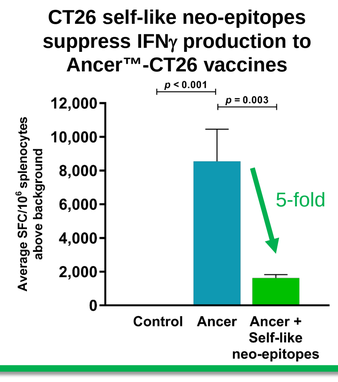
Peptides were evaluated on IEDB on November 19th 2018.

Identification and removal of Treg epitopes using JanusMatrix™



Epitopes can be either effector or regulatory. JanusMatrix™ has identified immunosuppressive (e.g. Treg) T cell epitopes in pathogens [1, 2, 3].

JanusMatrix™ has also identified **immunosuppressive neo-epitopes in cancer mutanome** (e.g. in the CT26 colon carcinoma mouse model).

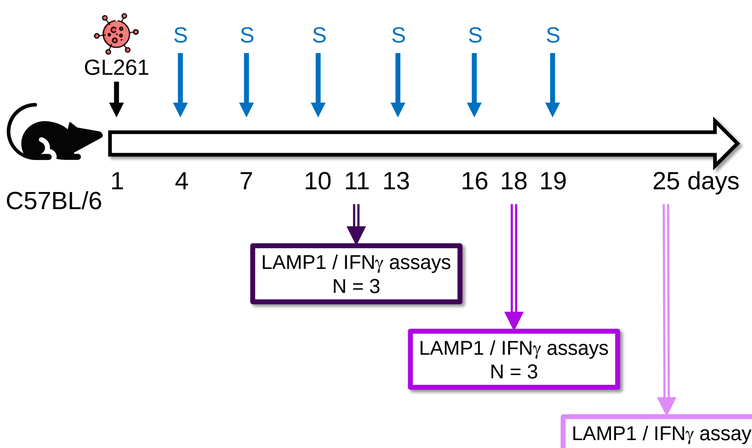


Ancer™-GBM vaccines induce cytotoxic T cells and IFNγ production in tumor bearing animals

Experimental design

Groups (N=9):

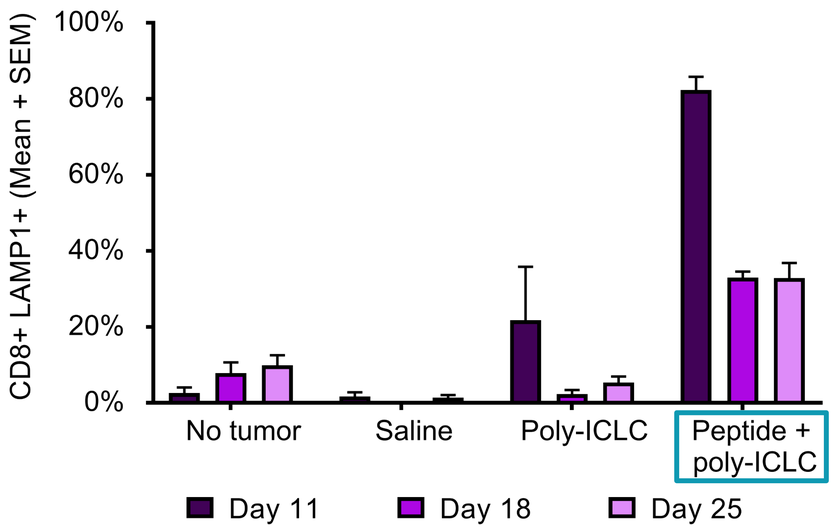
1. Control (no tumor, saline only)
2. Saline only
3. Saline + poly-ICLC
4. Saline + poly-ICLC + Ancer™-GBM



S = Saline formulations

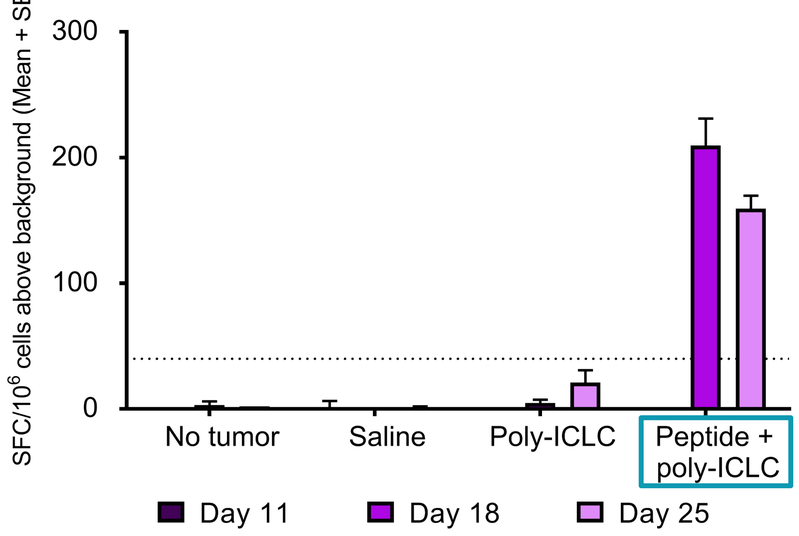
Cells from spleens and tumor draining lymph nodes collected at days 11, 18, and 25 were evaluated in LAMP1 degranulation and IFNγ ELISpot assays.

LAMP1 Degranulation Assay



- Ancer™-GBM vaccines induce cytotoxic T cells and the release of tumor cell killing molecules.
- Diminished cytotoxic T cell levels over time suggest tumor killing cells may migrate to the tumor from immune induction sites.

IFNγ ELISpot Assay



- Ancer™-GBM vaccines induce vaccine-specific IFNγ production.
- IFNγ response to Ancer™-GBM vaccines is expected to support an effective immune response against tumors.
- Ancer™-GBM vaccines stimulate sustained IFNγ production.

Conclusions

- **EpiVax's immunogenicity screening tools (EpiMatrix® and JanusMatrix™) are integrated into the Ancer™ platform for streamlined designs of personalized cancer vaccines.**
- **Analysis of the MHC- and TCR-facing residues of T cell epitopes by JanusMatrix™ enables prediction of epitope phenotype.**
- **The GL261 glioblastoma cell line was sequenced and its mutanome was analyzed with Ancer™ to design a GBM neoantigen vaccine (Ancer™-GBM).**
- **Ancer™-GBM Saline and Montanide vaccine formulations induced cytotoxic T cells and IFNγ production in GL261 tumor bearing animals. Immune responses to Ancer™-GBM vaccines are expected to control tumor growth.**
- **Follow-up studies include assessment of T cell infiltration in brain tissues collected from immunized mice and the impact of the Ancer™-GBM vaccine on animal survival.**

References and Acknowledgments

1) Moise L. et al., iVAX: An integrated toolkit for the selection and optimization of antigens and the design of epitope-driven vaccines. Hum Vaccin Immunother. 2015;11(9):2312-21.

2) Liu R. et al., H7N9 T-cell epitopes that mimic human sequences are less immunogenic and may induce Treg-mediated tolerance, Hum Vaccin Immunother. 2015 11:9, 2241-2252

3) Wada Y. et al., A humanized mouse model identifies key amino acids for low immunogenicity of H7N9 vaccines. Sci Rep. 2017 Apr 28;7(1):1283

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