Highly Immunogenic Vaccine for Prevention and Therapy of Malignant Mesothelioma

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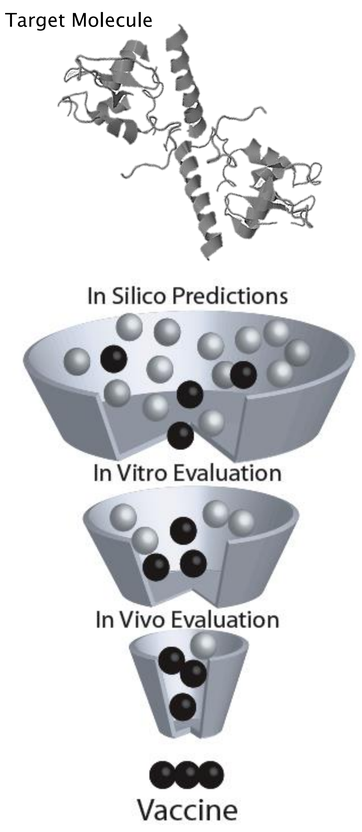
Abstract

Malignant mesothelioma (MM) is a deadly cancer with increasing incidence and no effective treatment options. We have previously constructed recombinant Fowlpox virus (FP) vectors encoding full-length survivin protein (*FP-surv*), which induced complete tumor regression in 40% of mice bearing subcutaneous MM. Macro- and microscopic evaluation of the tumor tissue showed that the tumors in vaccinated mice had large necrotic cores resulting from enhanced survivin-specific cytotoxic T lymphocyte (CTL). We aim to refine our novel FP vaccine by focusing on the most immunodominant survivin epitopes, thus enhancing its efficacy. We have identified additional high quality antigenic targets specifically upregulated in MM that will increase the breadth of T cell responses. We have used the EpiMatrix system to identify CD4+ and CD8+ T cell epitopes for synthesis and testing. Following T cell assays, highly immunogenic peptides will be selected that specifically stimulate CTLs, without activating T regulatory cells (Treg) that interfere with anti-cancer immune responses. We will include the selected peptides in the next-generation vaccine, *FP-surv.2*, which we expect to be more effective for inducing an immune response against MM cells than FP carrying the entire survivin gene (*FP-surv*). In addition, we anticipate that this response may include a higher number of long-lived memory T cells that can be exploited to prevent MM development in high-risk predisposed subjects.

Approach



In silico

EpiMatrix: Maps T-cell epitopes across HLA Class I & II

ClustiMer: Identifies promiscuous epitopes (regions of high epitope density across HLA)

JanusMatrix: Identifies epitopes with homology to autologous human proteins or to other organisms of interest

EpiAssembler: Assembles overlapping epitopes to Immunogenic Consensus Sequences (ICS)

VaccineCAD: Minimizes "nonsense" immunogenicity at the junctions between epitopes in a string-of-beads construct.

In vitro

HLA Binding Assays: Validates binding predictions as predicted by EpiMatrix

ELISpot/ELISA: Measures levels of cytokine production during immune response

Flow Cytometry: Characterizes the phenotypes of cells activated during immune response

In vivo

Animal Model Challenge: Evaluate vaccine efficacy in the context of an organism's entire immune system

Results

Protein T cell epitope content predicts immunogenic potential

- **EpiMatrix** assesses binding potential to 8 "Supertype" Class II HLA alleles.
- Protein Immunogenicity Score reflects aggregate T cell epitope content.
- Random expectation: 0
- Proteins scoring >20 are potentially immunogenic.

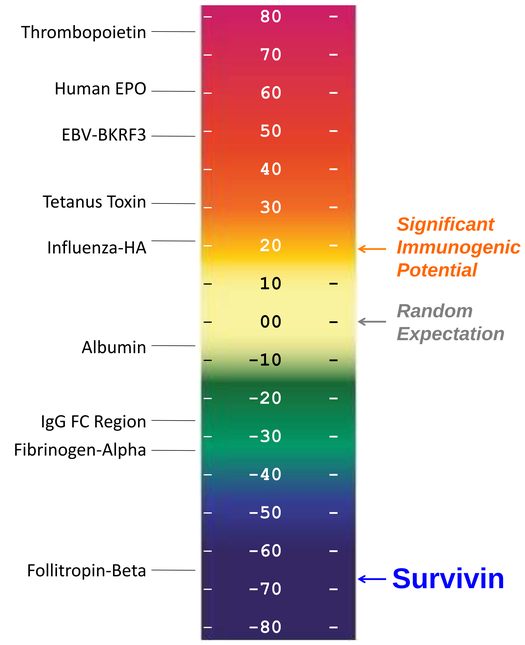
Survivin contains limited putative T cell epitope content

- EpiMatrix Class II Protein Immunogenicity Score of -69 indicates far less epitope content than we would expect for a protein of this size.
- EpiMatrix analysis was used to select regions of concentrated T cell epitopes for preliminary testing.
- Class II peptides are expected to be more promiscuous, whereas Class I peptides are expected to be more restricted.

Survivin putative epitope peptides induce naïve T cell response

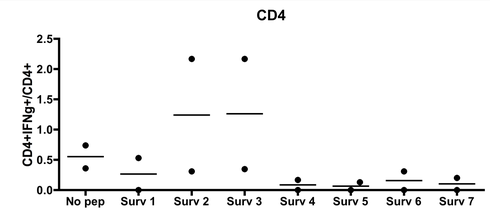
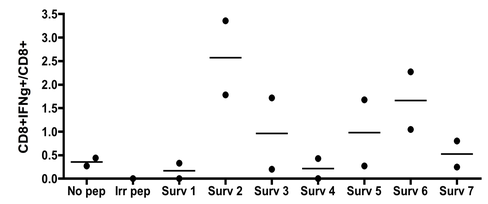
- Epitopes were tested using PBMCs collected from two healthy human donors (protocol adapted from Moser et. al. 2010[3]).
  - Samples were stimulated ex vivo with Class I or Class II T cell epitope peptides for 14 days with periodic media, IL-2 replenishment.
  - No peptide and irrelevant peptide controls were included.
- Measured response of CD4+ and CD8+ T cells
  - Population expansion
  - Intracellular IFNg production

EpiMatrix Protein Immunogenicity Scale



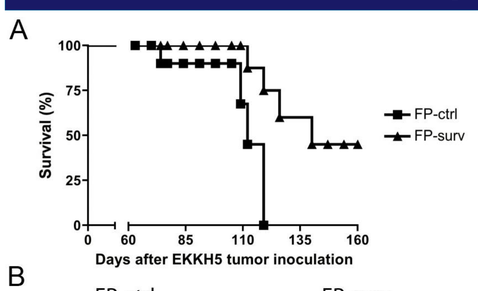
All scores normalized for protein length

CD4+ and CD8+ T cell response to selected peptides

HLA	Peptide	Alleles Targeted	IFNg+ T cell Response (n=2)
Class II	Surv 1	DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701, DRB1*0801	
	Surv 2	DRB1*0301, DRB1*0801, DRB1*1101	
	Surv 3	DRB1*0101, DRB1*0301, DRB1*0801, DRB1*1101	
Class I	Surv 4	A*0201, A*2402, B*0702, B*4403	
	Surv 5	A*2402, B*0702	
	Surv 6	A*0101	
	Surv 7	A*0301	

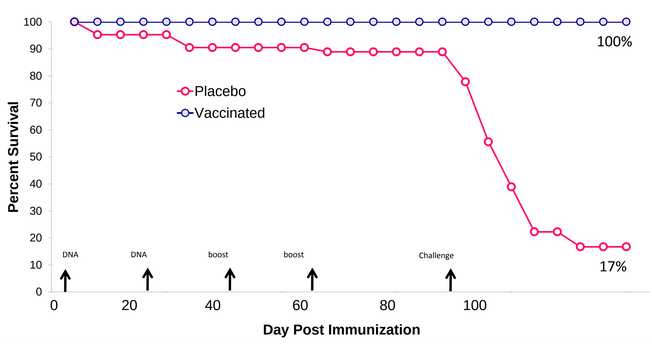
- For Class I and Class II HLA, multiple peptides induced significant T cell responses compared to controls.
- Differential response to each peptide is likely due to the HLA haplotypes of each donor (not tested).

Preliminary Data



Our malignant mesothelioma (MM) team has developed a novel vaccination approach against MM involving a recombinant Fowlpox virus (FP) expressing the survivin protein, which is highly upregulated in MM tumors. In preliminary studies, the vaccine was shown to stimulate CD8 T cells and immunostimulatory cytokines in the tumor environment Survival time was extended (A), tumor size reduced and intratumor necrosis enhanced (B) in survivin-vaccinated mice compared to control vaccinated mice.[1]

Our vaccine design team has shown proof of principle for epitope based vaccines against pathogens such as Vaccinia virus, where 100 percent of vaccinated mice survived lethal challenge (below). [2]



Conclusions / Future Directions

- **Survivin is a valuable malignant mesothelioma vaccine antigen target.**
- **We have identified several high priority HLA Class I and Class II peptide epitope candidates for inclusion in a T cell epitope-based vaccine.**
- **Human (healthy control) PBMC assays show elevated CD4+ and CD8+ T cell response to selected peptides.**
- **We aim to collect data ex vivo naïve T cell response data from at least 10 healthy human donors.**
- **Peptide-stimulated PBMCs will next be evaluated for their ability to kill mesothelioma cells in vitro.**
- **Finally, we will perform vaccination studies in mice to compare efficacy of the epitope-based vaccine containing the most immunogenic survivin peptides to a full-antigen vaccine.**

References / Acknowledgments

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