Identification of Shared Phosphopeptide Tumor Targets in Colorectal Cancer for Novel Off-the-shelf Vaccine Development

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INTRODUCTION

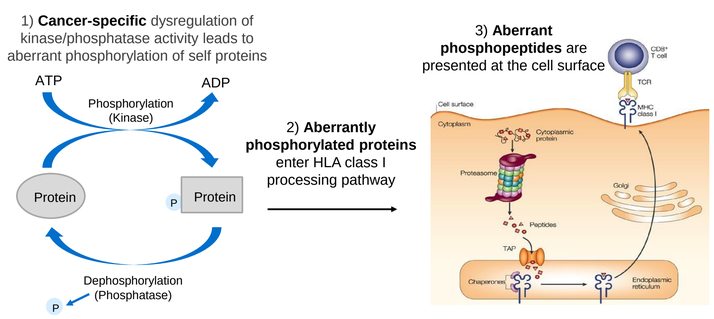
The high mortality rate of colorectal cancer (CRC) reflects limitations of current treatment modalities aimed at targeting the disease. Immunotherapy is a promising alternative to traditional chemotherapy, yet its benefit has been limited to patients with high mutational burden.

Phosphopeptide Tumor Targets (PTTs) represent a novel class of neoantigens which arise from dysregulated cellular signaling, are presented by HLA class I molecules, and are recognized as antigenic by circulating cytotoxic T cells. Furthermore, the shared nature of PTTs among CRC patients illustrates their role as excellent targets for the development of novel immunotherapeutics.

In this study, we analyze the phospholigandome profiles of 27 CRC patient tissues and identify over 500 unique PTTs. Through stringent selection criteria, we select 30 highly-prevalent, HLA-restricted targets for inclusion in a CRC PTT-based vaccine.

PHOSPHOPEPTIDE TUMOR TARGETS

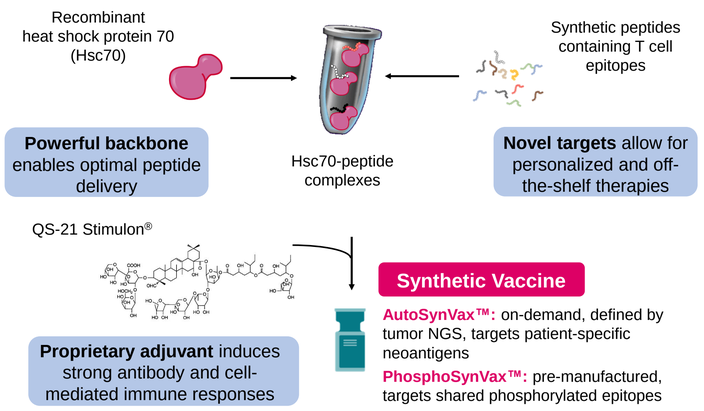
Phosphopeptide Tumor Targets are a novel class of neoantigens for immunotherapeutic development

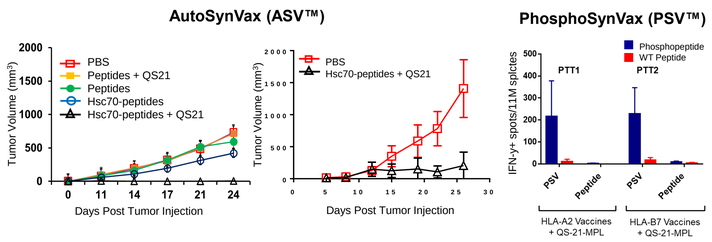


- Present due to dysregulated cell signaling pathways associated with malignant transformation
- Shared among individuals within and across indications
- Recognized by the immune system as antigenic and elicit memory immune responses in healthy individuals

AGENUS' VACCINE PLATFORM

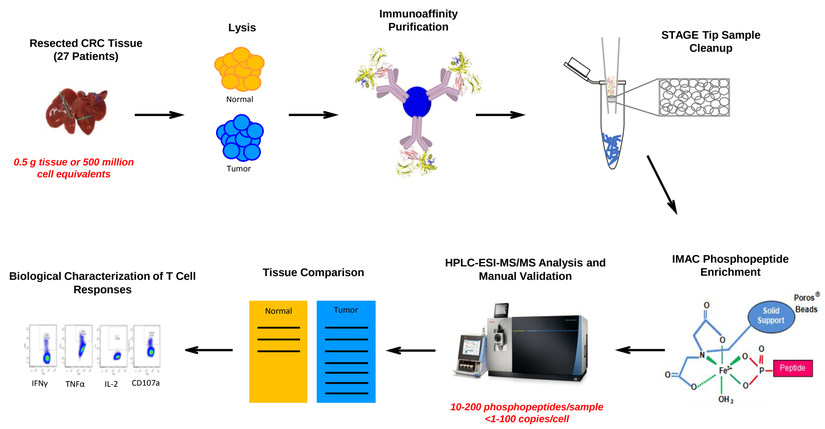
Agenus' versatile synthetic vaccine platform delivers robust immune responses



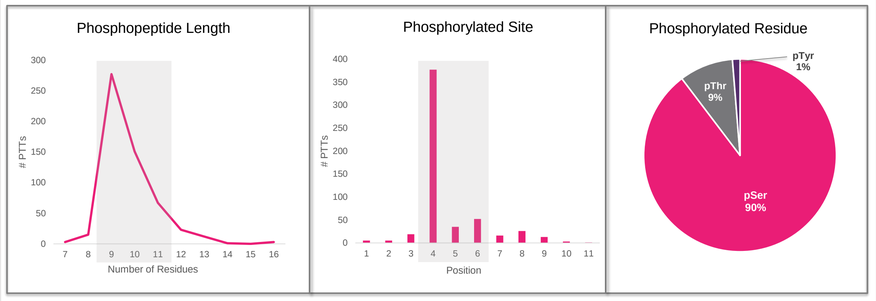


- Experiments conducted in animal models illustrate that all three components are critical for anti-tumor effect
- Agenus proprietary vaccine format demonstrates clinical safety and immunogenicity in a viral indication
- PSV demonstrates that immune response is specific to phosphorylated peptides, not unmodified counterparts

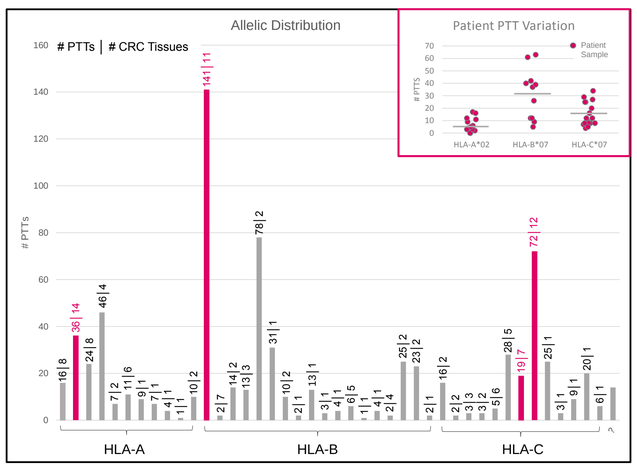
PHOSPHOLIGANDOME ANALYSIS OF CRC PATIENT TISSUES



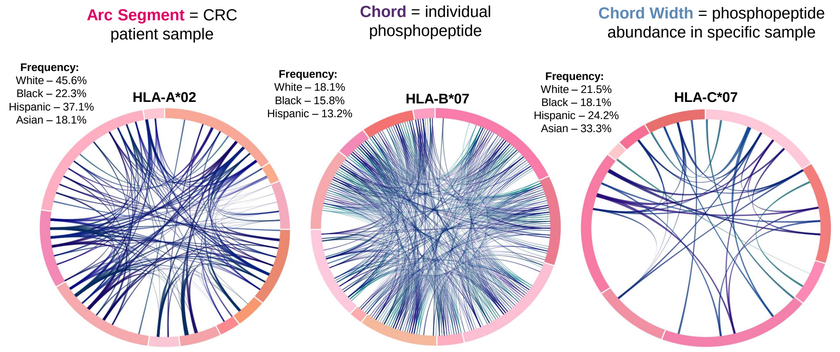
- Robust enrichment methodology coupled to highly-sensitive mass spectrometric instrumentation
- Workflow allows for routine identification of low-level phosphopeptides from CRC patient tumor tissues



- HLA-presented phosphopeptides are typically 9- to 11-mers with a phosphorylated serine residue in positions 4-6 of the peptide sequence
- Phosphorylation can enhance binding to HLA molecules: Peptides phosphorylated on a serine residue in position 4 bind 10x stronger to HLA-A*0201 than their unmodified counterpart
- A phosphate group on positions 4-6 is oriented upwards and solvent exposed, available for interaction with a TCR



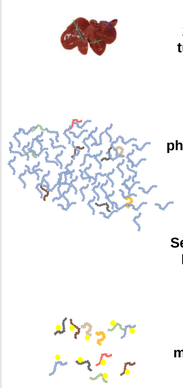
- PTTs are assigned to HLA haplotypes through predictive algorithms and binding assays
- 42/49 alleles represented present PTTs
- PTT presentation varies among alleles and patients
- HLA-B*07 is consistently a strong presenter of PTTs



- Chord diagrams illustrate the connectivity of the phospholigandome among patients
- Many CRC-associated PTTs are common to allele-matched patients within the indication
- The shared nature of PTTs allows for development of an off-the-shelf immunotherapeutic for CRC with pan-indication potential

PHOSPHOSYNVAX-CRC: PRODUCT DEVELOPMENT

Phosphopeptide Identification → **Target Selection** → **Therapeutic Benefit**



27 CRC patient tumors analyzed

Indication	CRC			**Age (Years)**	Median	66	
Patient Tissues	27				Range	45-87	
		# Patients	% Patients			# Patients	% Patients
Gender	Male	12	44%	**Tissue Type**	Primary	25	93%
	Female	9	33%		Metastatic	2	7%
	Unknown	6	22%				
MSI Status	MSI-H	6	22%	**Grade**	High	4	15%
	MSI-L	1	4%		Low	17	63%
	MSS	10	37%		Unknown	6	22%
	Unknown	10	37%				

> 500 phosphopeptides identified

Selection criteria-based filtering

- ✓ Reflect frequently occurring HLA types (A*02, B*07, C*07)
- ✓ Prevalence in allele-matched patients
- ✓ Relevance of source protein to malignant transformation
- ✓ Presence in other indications of interest
- ✓ Expression in tumor vs. normal tissues
- ✓ Memory T cell responses in healthy individuals

30 specific molecular targets for PSV-CRC

6 HLA-A, 14 HLA-B, 10 HLA-C PTTs selected for PSV-CRC

HLA Type	PTT	% CRC Patients (Allele-Matched)
A*02	PTT 1	50-80%
A*02	PTT 2	50-80%
A*02	PTT 3	14-30%
A*02	PTT 4	14-30%
A*02	PTT 5	14-30%
A*02	PTT 6	14-30%
B*07	PTT 7	>80%
B*07	PTT 8	>80%
B*07	PTT 9	>80%
B*07	PTT 10	>80%
B*07	PTT 11	50-80%
B*07	PTT 12	50-80%
B*07	PTT 13	50-80%
B*07	PTT 14	50-80%
B*07	PTT 15	50-80%

HLA Type	PTT	% CRC Patients (Allele-Matched)
B*07	PTT 16	50-80%
B*07	PTT 17	50-80%
B*07	PTT 18	30-50%
B*07	PTT 19	50-80%
B*07	PTT 20	30-50%
C*07	PTT 21	>80%
C*07	PTT 22	>80%
C*07	PTT 23	50-80%
C*07	PTT 24	>80%
C*07	PTT 25	50-80%
C*07	PTT 26	>80%
C*07	PTT 27	>80%
C*07	PTT 28	50-80%
C*07	PTT 29	50-80%
C*07	PTT 30	30-50%

- >70% of tested PTTs are memory targets
- 80% of PTTs are presented by ≥50% of allele-matched CRC patients
- 100% of PTTs are present in other cancers

% CRC Patients: 0% – 100%

Haplotype	A*02	B*07	C*07
% US Population	43%	13%	36%
# PTTs in PSV-CRC	6	14	10
% Patients with ≥1 PTT	~85%	~100%	~100%
Avg # Peptides / Patient with ≥1 PTT	2.7	9.2	6.3

- ~70% of US/EU patients are eligible for vaccine based on HLA haplotype
- 100% of eligible CRC patients' tumors express ≥ 1 PTT
- CRC tumors express on average 7-10 PTTs

CLINICAL OPPORTUNITY

Product

- Neoantigen vaccine comprised of 30 cancer-specific phosphopeptide tumor targets prioritized based on:
  - ➢ Prevalence among patients within CRC
  - ➢ Relevance of source protein to malignant transformation
  - ➢ Recognition by memory T cells from healthy individuals – validation of safety
- Agenus proprietary vaccine format – clinical safety and immunogenicity demonstrated in a viral indication

Opportunity

- PSV is designed to train the patient's immune system to recognize and kill tumor cells, promoting lasting anti-tumor immunity and improved survival
- Multi-epitope targeting minimizes the chance for minimal residual disease and relapse

CONCLUSIONS

The development of a novel PTT-based vaccine for CRC may improve efficacy outcomes compared to standard-of-care treatments.

Phospholigandome analysis of a diverse subset of CRC patient tumors allowed for selection of novel targets for immunotherapeutic development. The inclusion of highly-prevalent, multiple allele-associated epitopes from diverse source proteins expands the eligible patient population and increases the likelihood of stimulating an effective anti-tumor immune response.

Furthermore, the shared frequency observed in the PTT repertoire allows for advancement towards the development of an off-the-shelf cancer vaccine with broad therapeutic potential. Additional PSV™ formulations are in development for AML and a variety of solid tumors – including lung, cervical, head & neck, and ovarian cancers.