Lymph Node Targeted AMP-peptide Vaccines Generate Functional T cell Immunity Against Mutant p53 and BRAF

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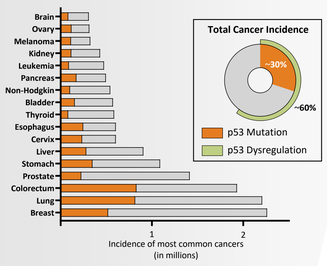
Clinical Relevance: p53 and BRAF Mutations in Cancer

Mutant p53

- Nearly **60% of human tumors** exhibit dysregulated p53[1] including mutations, copy number alterations, upstream deregulation
- **~50% of those tumors have missense mutations in p53**[2,3]
  Annual incidence: 5.2M patients worldwide and 700K patients in the US
- **Prevalent** in nearly all tumor types; sign of **advanced disease**
- **Poor clinical prognosis**[4]

p53 Mutations by Cancer Type
Estimated Worldwide Annual Incidence[2,3]

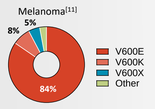


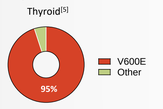
Mutant BRAF

Mutations are concentrated in **one hotspot**[5-11]
Up to 97% of BRAF mutations occur at the V600 location

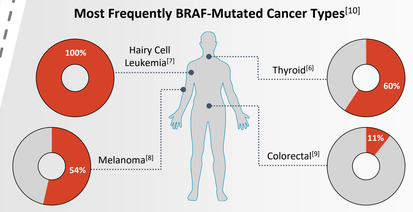
High prevalence in melanoma and thyroid tumors[10,11]
Overall, BRAF mutations are present in 8% of solid tumors
Representing approx. 1 million cases globally each year

BRAF Mutation Hotspots





Most Frequently BRAF-Mutated Cancer Types[10]



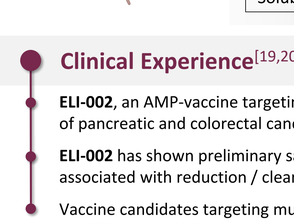
Therapeutic Vaccination: Benefits of Targeting Mutated p53 & BRAF

	Mutant p53	Mutant BRAF
Truncal Mutation	These mutations are present early on in tumor progression and **shared among all progeny** as they apply positive selection for survival in the cancer cell population and allow for clonal expansion[12].	
Driver Mutation	Signaling is required for tumor survival and growth, making **loss of these mutations unlikely**, thus preventing immune escape.	
High Prevalence	Occurs in nearly **all types of tumors** and is involved in **~30% of overall cancer** cases[3].	Major driver in [10] 100% of hairy cell leukemia 60% of thyroid cancer 54% of melanoma 11% of colorectal cancer
Public Neoantigen	Mutant peptides are **not centrally tolerized**; cognate TCRs are present in naïve repertoire[13-16]	
Proven Clinical MOA	Mutant p53- and BRAF-specific T cells are **known to mediate anti-tumor efficacy**[14,15]	

The AMP-Platform Delivers Cargo Directly to the Lymph Nodes[17,18]

- **Smart trafficking to the lymph nodes** after subcutaneous dosing generates immune responses with increased magnitude, function, and durability.
- **Takes advantage of potent lymph node immune mechanisms**, including activation of innate and adaptive immune cells, antigen-spreading, and improved tumor T cell trafficking / infiltration.
- Mutant p53/BRAF peptides provide **validated antigens** for application of the Amphiphile platform.
- Lymph node delivery of potent adjuvants **minimizes systemic exposure to improve safety.**

Lymph Node Biodistribution



AMP Platform Design

AMP MOA

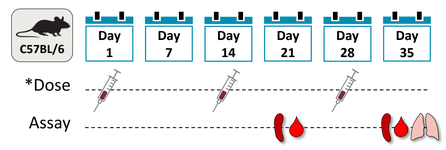
Biodistribution of AMP Vaccines[17]

Clinical Experience[19,20]

- **ELI-002**, an AMP-vaccine targeting mKRAS, is currently being investigated in clinical trials for treatment of pancreatic and colorectal cancer (**AMPLIFY-201:** NCT04853017 and **AMPLIFY-7P:** NCT05726864).
- **ELI-002** has shown preliminary safety / tolerability, and significant increases in mKRAS T cell responses, associated with reduction / clearance of tumor biomarkers, and reduced risk of progression and death.
- Vaccine candidates targeting mutant p53 / BRAF have been built from the AMP-platform, and utilize the ELI-002 adjuvant, **AMP-CpG**, providing the potential for favorable clinical tolerability and activity.

Repeat-dose Immunization Strategy

Schema



*Control groups were immunized with equivalent amounts of unmodified "soluble (SOL)" CpG and antigen peptide.

Preclinical Murine Doses:

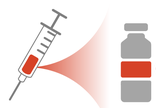
	AMP-CpG		AMP-Peptide
ELI-007	10 nmol	+	20 nmol
ELI-008	5 nmol	+	1.25 nmol

Preclinical Assays:

Spleen	ELISpot, FluoroSpot ICS, Luminex
Blood	ICS
Lung	ELISpot, ICS

ELI-007 is Designed to Address ~95% of BRAF Mutations

ELI-007



Adjuvant [TLR9 Agonist]
AMP-CpG 7909

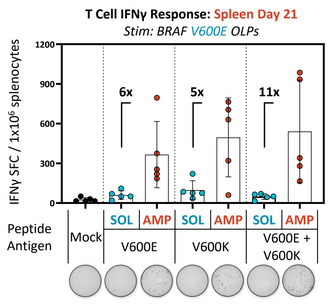
Antigen [29mer Peptides]
AMP-BRAF V600E
AMP-BRAF V600K

	Mutant	% Patients
1	V600E	~90%
2	V600K	~5%

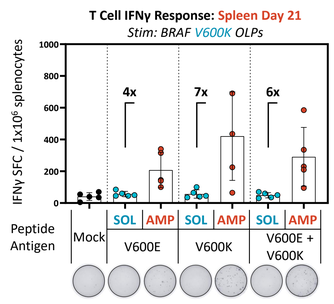
~95% of all patients with BRAF mutations

ELI-007 Generates Strong Immune Responses to V600E and V600K

T Cell IFNγ Response: Spleen Day 21
Stim: BRAF V600E OLPs

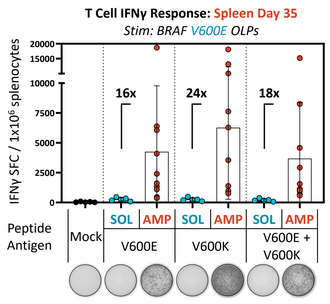


T Cell IFNγ Response: Spleen Day 21
Stim: BRAF V600K OLPs

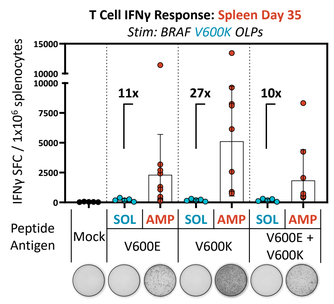


T cell responses are detectable after only two doses of the AMP-BRAF V600E, V600K, and combination (ELI-007) vaccines.

T Cell IFNγ Response: Spleen Day 35
Stim: BRAF V600E OLPs



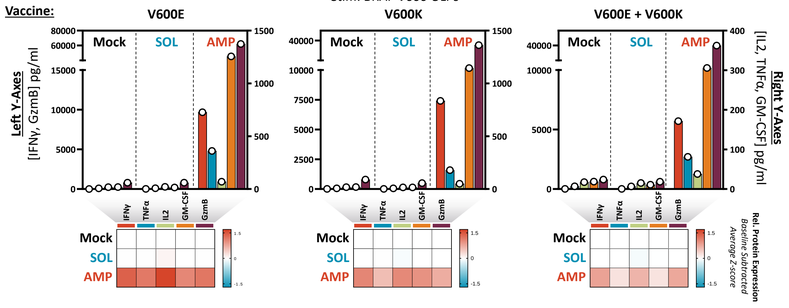
T Cell IFNγ Response: Spleen Day 35
Stim: BRAF V600K OLPs



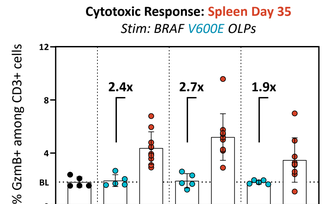
Further immunization with the AMP-BRAF vaccines increases the T cell response substantially.

Polyfunctional T Cell Responses: Cytokines and Granzyme

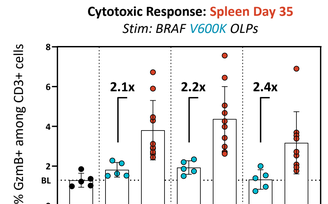
Multiplexed Immune Proteomics: Spleen Day 35
Stim: BRAF V600 OLPs



Cytotoxic Response: Spleen Day 35
Stim: BRAF V600E OLPs



Cytotoxic Response: Spleen Day 35
Stim: BRAF V600K OLPs



Polyfunctional T cells induced by AMP-BRAF vaccines produce high levels of cytokines as well as cytolytic GzmB.

TAKE HOME MESSAGES

- AMP enhances vaccine potency via targeted lymph node delivery.
- ELI-007 and ELI-008 substantially improved T cell responses over soluble comparator vaccines:
  - Polyfunctional T cells that produce T_H1-associated cytokines: (IFNγ / TNFα / IL2 / GM-CSF).
  - Secretion of Granzyme B, potent cytolytic function
- AMP-vaccines have the potential to address a high, unmet medical need for millions of patients with BRAF / p53 mutations annually.
- The AMP-platform technology is simple, rapid and scalable for broad clinical application.

ELI-008 Is Designed to Target >30% of p53 Mutations

ELI-008

Adjuvant [TLR9 Agonist]
AMP-CpG 7909

Antigen [30mer Peptides]
AMP-p53 Mutant Peptides
Potential candidates: 12 of the most frequently occurring p53 mutations[21].

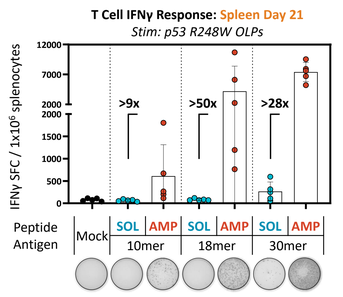
	Mutant	% Patients
1	R248W	3.53%
2	R248Q	4.37%
3	R175H	5.60%
4	R273H	3.95%
5	R273C	3.31%
6	R282W	2.83%
7	G245S	2.11%
8	R249S	2.04%
9	Y220C	1.83%
10	C135Y	0.43%
11	R158H	0.52%
12	H214R	0.39%

~30% of all patients with p53 mutations

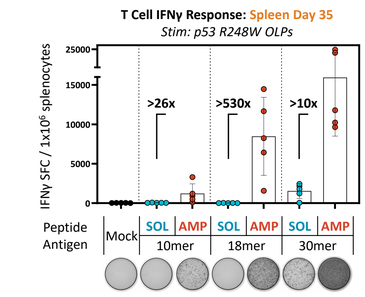
AMP-p53 R248W Produces Strong T Cell Responses in Mice

The 10mer, 18mer, and 30mer AMP-peptides are centered around the same R248W mutation.

T Cell IFNγ Response: Spleen Day 21
Stim: p53 R248W OLPs



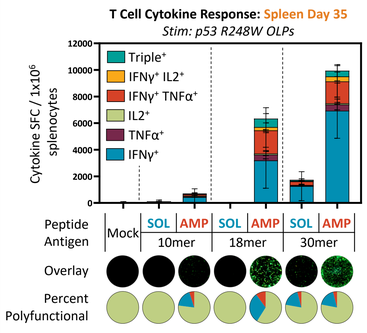
T Cell IFNγ Response: Spleen Day 35
Stim: p53 R248W OLPs



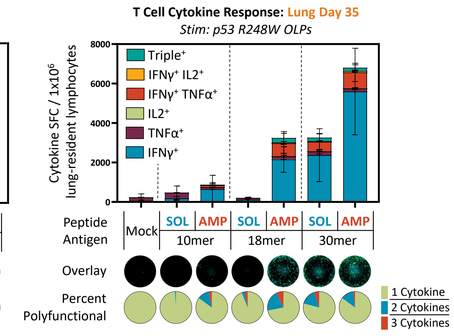
Longer antigen peptides contain greater numbers of potential epitopes, increasing the probability of a strong immune response in various HLA haplotypes.

Potent T cell responses are observed after only two doses with AMP-p53 R248W, which are further improved upon after a third bi-weekly dose.

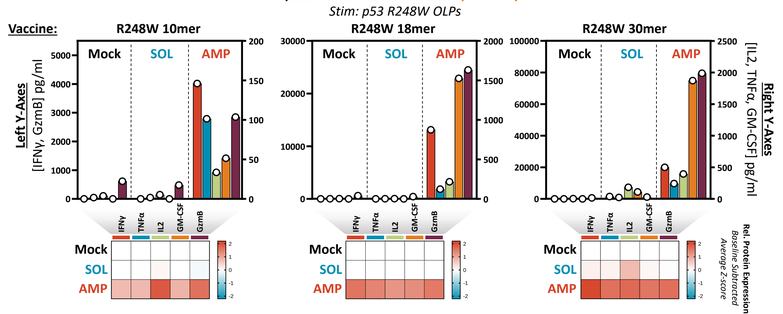
T Cell Cytokine Response: Spleen Day 35
Stim: p53 R248W OLPs



T Cell Cytokine Response: Lung Day 35
Stim: p53 R248W OLPs



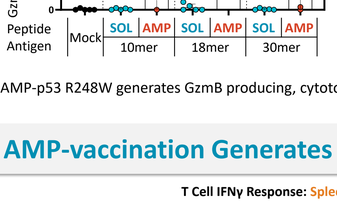
Multiplexed Immune Proteomics: Spleen Day 35
Stim: p53 R248W OLPs



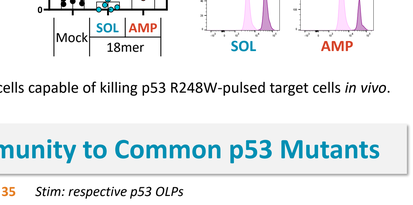
Polyfunctional T cells reside not only in secondary lymphoid tissues like spleen, but in peripheral organs such as the lung, which is one of the predominant sites for metastatic spread in melanoma and colorectal carcinoma.

AMP-p53 R248W Generates Cytotoxic T Cells

T Cell Cytotoxic Response: Spleen Day 35
Stim: p53 R248W OLPs



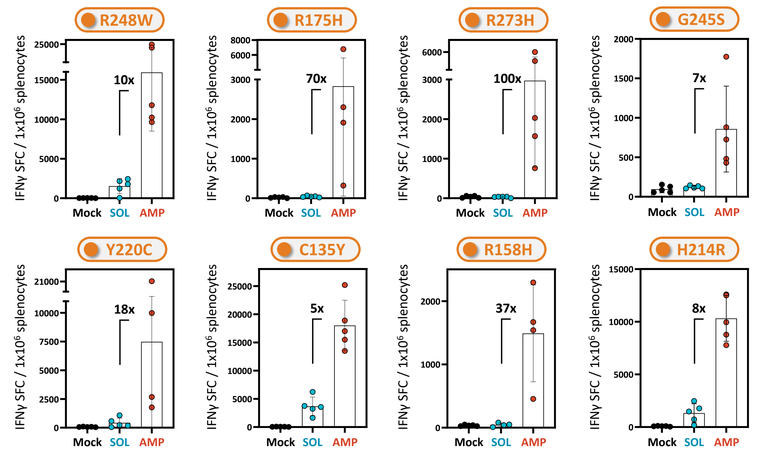
***In Vivo* Target Cell Killing: Spleen Day 35**
Stim: p53 R248W OLPs



AMP-p53 R248W generates GzmB producing, cytotoxic T cells capable of killing p53 R248W-pulsed target cells *in vivo*.

AMP-vaccination Generates Immunity to Common p53 Mutants

T Cell IFNγ Response: Spleen Day 35 *Stim: respective p53 OLPs*



8 candidate p53 mutant 30mer AMP-peptides elicit strong T cell responses upon immunization, demonstrating the versatility, ease of application and potency of the AMP-platform.

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