Targeting the Lymph Nodes to Enhance Mutant KRAS-Specific Vaccine Responses

Peter DeMuth, PhD, VP of Research
RAS Targeted Drug Discovery Summit
September 15, 2020
Why Immunotherapy?

mKRAS is a Promising Target for Immunotherapy

- **Truncal**: mutations occur early in the development of tumors, expressed with good uniformity
- **Driver**: mKRAS signaling is required for tumor growth and survival
- **Highly prevalent**: involved in ~25% of solid tumors
- **Public neoantigen**: not centrally tolerized, reactive TCRs present in naïve repertoire
- **Promiscuous HLA presentation**: potential for off-the shelf use in diverse patient population
- **Proven Clinical MOA**: mKRAS-specific T cells are known to mediate anti-tumor efficacy
- **Multi-targeting potential**: recognition of clonal and subclonal mKRAS variants to prevent escape

But Substantial Challenges Remain:

- Conventional vaccines have induced **low frequency T cell responses**
- ACT is effective but **difficult and expensive to manufacture**
- Historical studies have focused on advanced tumor patients with **bulky disease burden**
- Advanced tumors develop **suppressive microenvironment** (physical, immunological)
Clinical Experience with mKRAS Immunotherapy
## Low Frequency mKRAS Immune Responses Deliver Measurable but Inadequate Clinical Benefit

### Important Lessons:

- Peptide, autologous DC vaccines produce measurable T cell responses to mKRAS
- **T cell responses are low frequency**: 7 day ex vivo expansion is required to observe T cell responses
- **T cell responses are mKRAS-specific** with little cross-reactivity to WT KRAS
- Responses are both **CD4 and CD8**, restricted by various common HLA
- Numerous studies have show statistically significant **association of mKRAS immune response with DFS**

### Clinical Evidence of T Cell Efficacy Against mKRAS Tumors

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<th>mKRAS</th>
<th>Indication</th>
<th>Treatment</th>
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Adoptively Transferred mKRAS-specific T Cell Proof of Principle – Elimination of Large Metastatic Tumors

Key Results:
• Patient had 7 metastatic mKRAS lung lesions at the time of treatment
• All lesions showed objective regressions following therapy

Important Lessons:
• Spontaneously arising T cells can detect and eliminate even large mKRAS tumors
• Naïve TCRs specific for mKRAS exist in native repertoire
• mKRAS is effectively presented by tumors
• Efficacy requires sufficient T cell expansion (~15% of peripheral T cells), functionality, and persistence
The Amphiphile (AMP) Platform
Lymph Nodes are Where the Immune Response Against Cancer is Orchestrated

- Natural Site for Immune Surveillance
- Site of Residence for Immune Cells
- The “School House” for T-Cells
- Potent Deterministic Immune Signaling

Conventional Soluble Subunit Vaccines do not get to Lymph Nodes

Poor exposure to target immune cells gives Limited Efficacy
Albumin is the Ideal Carrier to Transport Immuno-therapies and Vaccines into Lymph Nodes

- Molecular size dictates trafficking fate of immuno-therapies and vaccines
- Albumin in the tissues is efficiently trafficked into lymphatics because of its large size
Amphiphile (AMP) Platform Enables Lymph Node Delivery of Validated Therapeutics with Modular Application

A Modular Conjugation Approach for Delivery of Immune Therapeutics to the Lymph Node

1. Albumin-binding, ✓ Lymph Node targeting
2. Linker, ✓ Improve Solubility
3. Therapeutic Payload

- ✓ Small Molecules
- ✓ Peptides
- ✓ Nucleic Acids
- ✓ Proteins
Amphiphiles Potently Stimulate T Cells in the Lymph Nodes

1. **Subcutaneous Injection Site**
   - Tissue Albumin Binding

2. **Lymph Node Trafficking**
   - Tissue Albumin Binding

3. **Delivery to Immune Cells**
   - Tissue Albumin Binding
   - Amphiphiles Delivery to Immune Cells

4. **T Cell Stimulation**
   - Delivery of Tissue Albumin and Amphiphiles to Dendritic Cell
   - Dendritic Cell Activation
   - Cytokines Released
   - T Cell Stimulation
AMP-Vaccines are Highly Optimized to Precisely Target Delivery to the Lymph Nodes

1. SC Injection
   - Vaccine
     - 10 μg Peptide-FITC
     - 30 μg CpG

2. Lymph Node Analysis
   - Day 1: Dose
   - Day 2: Analyze Lymph Node Uptake

- **Soluble** Peptide-FITC + CpG
- **AMP** Peptide-FITC + AMP-CpG

**Peptide Fluorescent Signal**
- **Inguinal Lymph Node**
  - >30x
- **Axillary Lymph Node**
  - >20x

AMP-Vaccines are Highly Optimized to Precisely Target Delivery to the Lymph Nodes

**Day 1**
- **Dose**
- Analyze Lymph Node Uptake

**Day 2**

**Vaccine**
- 10 μg CpG-FAM

**Soluble CpG-FAM**
- AMP-CpG-FAM

**CpG Uptake in Lymph Node Cells**

- **Macrophages**
- **Dendritic Cells**

- **Soluble**
- **AMP**

Lymph Node Targeted AMP-Vaccines Potently Enhance Functional CD8 T Cell Responses

**Day 0**
- Dose
- **Day 14**
- Dose
- **Day 21**
- Blood ICS

**Vaccine**
- 10 μg antigen
- 8 ug equivalent CpG

**IFN-γ CD8+ T cell Response**

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<tr>
<th>Vaccine Type</th>
<th>IFN-γ+ CD8+ T cell Response</th>
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<tr>
<td>Soluble peptide gp100</td>
<td>2%</td>
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<tr>
<td>AMP peptide gp100</td>
<td>52%</td>
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**Graph**

- **X-axis:** % IFN-γ+ of CD8+
- **Y-axis:** Soluble AMP
- **Bar:** 22x

Moynihan, Irvine
Lymph Node Targeting Drives Unprecedented Immune Responses

- >10-fold improved lymph node delivery over conventional soluble vaccines in mice and primates
- >50-fold enhanced delivery of vaccines to immune cells in mice and primates
- >30-1000-fold increase in functional immune responses relative to conventional soluble vaccines
- Eradication and durable cures of large aggressive tumors in multiple models (lung, melanoma, breast, colorectal, head and neck, glioma)
- Broad application across many therapeutic classes and indications
  - Boosting natural immune responses
  - Enhancing Adoptive Cell Therapies (CAR-T, TIL)

Liu et al., Nature, March 2014
Moynihan et al., Nature Medicine, March 2016
Moynihan et al., Cancer Immunology Research, June 2018
Ma, et al., Science, July 2019
ELI-002 Preclinical Studies: mKRAS Vaccine
AMP Vaccination Against mKRAS Drives Powerful Functional Immunity

- >400-fold increase in functional T cell responses relative to conventional soluble vaccines in mice
  - Polyvalent responses simultaneously target all 7 mKRAS sequences
  - CD4 and CD8 T cell induction
  - >5000-fold increase in polyfunctional cytokine effector profile relative to conventional therapies
  - >100-fold increase in cytolytic effector functionality relative to conventional therapies
  - Potent in vivo killing of mKRAS-presenting cells
ELI-002: Lymph Node Targeting Polyvalent mKRAS Vaccine Immunotherapy

A Lymph Node Targeted Polyvalent mKRAS Peptide + TLR-9 Agonist CpG Vaccine Therapy

ELI-002: AMP-Peptides + AMP-CpG


AMP-CpG: CpG TLR-9 Immunostimulatory DNA

Lymph Node Targeting Linker Vaccine Payload
AMP-Vaccines Accumulate in Lymph Nodes to Enhance Uptake in Resident Immune Cells

20 µg mKRAS-G12D FITC peptide + 30 µg CpG

**Soluble** KRAS G12D-FITC + CpG

**AMP**-KRAS G12D-FITC + AMP-CpG

**Day 1**

- **Inguinal**
  - >4x

- **Axillary**
  - >4x

**Day 4**

- **Inguinal**
  - >3x

- **Axillary**
  - >2x

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AMP-Vaccines Enable Potent T Cell Responses to mKRAS

Day 0

Dose 1

Day 14

Vaccine

20 µg mKRAS peptide

30 µg CpG

Day 21

ELISPOT

Splenocyte Restimulation with:

- Soluble KRAS G12X + CpG
- AMP-KRAS G12X + AMP-CpG

T Cell Cytokine Response

IFNγ SFC / 1x10^6 splenocytes

- KRAS G12D 18-mer: 400x
- KRAS G12R 18-mer: 130x
- KRAS G12V 18-mer: 40x
AMP-Vaccines Prime and Boost Polyfunctional Cytokine Secreting T cell Responses to mKRAS

- Day 0: Dose 1
- Day 14: Dose 2
- Day 21: Cytokine CBA
- Day 28: Dose 3
- Day 34: Cytokine CBA
- Day 42: Dose 4
- Day 50: In Vivo Killing

Vaccine
- 20 μg mKRAS peptide
- 30 μg CpG
- or
- 50 μg polyI:C

T Cell Cytokine Response: Splenocyte Restimulation with G12D 18-mer

- Day 34

Graph showing cytokine response:
- TNFa
- IFNg
- IL-6
- IL-2

Legend:
- untreated
- Soluble G12D + CpG
- AMP G12D + AMP-CpG

- pg/ml (TNFa, IFNg)
- pg/mL (IL-6, IL-2)

- >5,000x

9/16/2020
AMP-Vaccination Induces Cytotoxic mKRAS-specific T Cells

Vaccine
- 20 μg mKRAS peptide
- 30 μg CpG
- or
- 50 μg polyI:C

**T Cell Cytolytic Response**

- **Long Peptide: G12D 18-mer**
  - Granzyme B SFC / 1x10^6 splenocytes
  - Untreated
  - Soluble KRAS G12D + CpG
  - AMP-KRAS G12D + AMP-CpG

- **Short Peptide: G12D 9-mer**
  - Untreated
  - Soluble KRAS G12D + CpG
  - AMP-KRAS G12D + AMP-CpG

**In Vivo Killing**

- Dose 1: day 0
- Dose 2: day 14
- Dose 3: day 27
- Dose 4: day 41
- Dose 5: day 55
- Dose 6: day 66
- ELISPOT: day 74

**Cell Count**

- CFSE high: KRAS G12D 18-mer
- CFSE low: no peptide

- Untreated: 51
- AMP: 77
- Soluble KRAS G12D + CpG: 49
- AMP-KRAS G12D + AMP-CpG: 23
ELI-002 Clinical Development
AMP 7-Peptide Vaccine with AMP-CpG Addresses 99% of Mutations Driving 25% of All Solid Tumors

- 57,000 pancreatic, 56,000 colorectal, and 58,000 lung mKRAS cancers annually in US
- Trial designs target minimal residual disease patients (MRD) post pancreatectomy / colectomy → 80% of PDAC patients will relapse within one year
  - Microscopic tumor burden – maximize immune effector : tumor target ratio – minimal immune suppression from tumor environment
- Trial design includes cross-over for progressing patients not originally randomized to treatment group to test ELI-002 for RECIST radiographic response
- Cell free DNA (ctDNA) marker can identify those patients who will relapse for study and measure vaccine impact on MRD
mKRAS+ Expansion Cohorts Will Rapidly Assess PDAC, CRC, NSCLC, and Other Solid Tumors (Endometrial/Ovarian/Bile Duct)

ELI-002 Study Cohort Schematic

- **P1 Dose Escalation**
  - Cohort 1
- **P1 Dose Escalation**
  - Cohort 2
- **P1 Dose Escalation**
  - Cohort 3
  - **P2 PDAC**
    - Cohort 4A, 4B, 4C
    - ELI-002 vs SOC
  - **P1 Expansion**
    - CRC
    - Cohort 5
  - **P1 Expansion**
    - NSCLC
    - Cohort 6
  - **P1 Expansion**
    - Other Solid Tumors
    - Cohort 6

4A – ELI-002; 4B – observation; 4C – observation crossover to ELI-002 at time of radiographic relapse
## ELI-002 (KRAS)
Operable Pancreatic Cancer Phase 1/2 Clinical Trial

### Multi-center Phase 1 3+3 Dose Escalation

<table>
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<th>Modality Therapy</th>
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<td>mKRAS – ctDNA positive</td>
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### Immunization Period (8 weeks)

3 months

### Booster Period (4 weeks)

2 months

### Endpoints: ctDNA conversion, RFS, Immunologic Responses

### Objectives
- Safety and tolerability
- Immunologic POC
- Recommended P2 dose

### Enrollment
- 9-18 MRD+ patients
- MRD+ by mKRAS ctDNA

### Treatment
- 3 progressive dose level cohorts
- Prime, boost ELI-002

### Endpoints
- Safety
- ctDNA conversion
- LN enlargement, cytokines, immune response

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ELI-002: Pancreatic/Other mKRAS+ Tumor Subjects who are MRD+ Despite Chemo and Surgery

**Objectives**
- Safety and tolerability
- Immunologic POC

**Enrollment**
- 90 PDAC patients
- MRD+ by mKRAS ctDNA

**Treatment**
- Established RP2D
- Prime, boost ELI-002

**Endpoints**
- Relapse free survival
- ctDNA conversion
- iRECIST response rate after crossover
- LN enlargement, cytokines, immune response

**Multicenter Phase 2 2:1 vs standard treatment (observation) with crossover at relapse**

- Neo adjuvant chemotherapy (FOLFIRINOX, GEM/ABRAXANE)
- Pancreatic Surgery (R0 or R1 resection) mKRAS – ctDNA positive
- Immunization Period (8 weeks)
- Booster Period (4 weeks)
- Endpoints: ctDNA conversion, RFS, RECIST Immunologic Responses

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mKRAS-targeted immunotherapy offers

- Attractive immunogen profile
- Historical CPOC signal in CRC, PDAC, NSCLC
- Broad multi-targeted G12/13 potential activity

ELI-002 shows promising immune response profile

- Precise lymph node targeting
- **High frequency** T cell responses
- **Polyvalent** mKRAS-specificity
- **Polyfunctional** effector profile
- Potent **cytotoxic** functionality

ELI-002 Clinical Development

- FIH P1 in MRD+, P2 single arm CRC, NSCLC, OST, and P2 randomize PDAC
- **Cross-over** in randomized PDAC cohort from observation to ELI-002 at time of relapse: iRECIST
  - **ctDNA** enabled patient selection and molecular disease monitoring
    - Comprehensive **immunological** profiling
  - **Designed for robust CPOC** across multiple indications
AMP-Vaccination to Discover and Develop mKRAS-specific TCR-T Cell Therapy

HLA-transgenic Mice (human immune response)

AMP-KRAS + AMP-CpG

Dose 1

Dose 2

mKRAS-specific TCRs compatible with human tumor recognition

T cells engineered to express mKRAS-specific TCR and expanded

T cells transfused to patient

T cells infiltrate and kill tumor

James Yang MD

Tran, et al. NEJM 2016
Martin Steinbuck PhD, Lochana Seenappa MS, Aniela Jakubowski MS, Chris Haqq MD PhD, Michael DiVecchia, Charles Chase PhD, Lisa McNeil PhD, Esther Welkowski, Nicole Hsu, Julian Adams PhD

Colin Weekes MD, Ryan Corcoran MD, David Ryan MD

Haipeng Lu PhD, Kelly Moynihan PhD, Darrell Irvine PhD

James Yang MD

Melody Chee