



# Approaches to Improve the Clinical Performance of Genetically Engineered T Cells

CAR-TCR Annual Summit

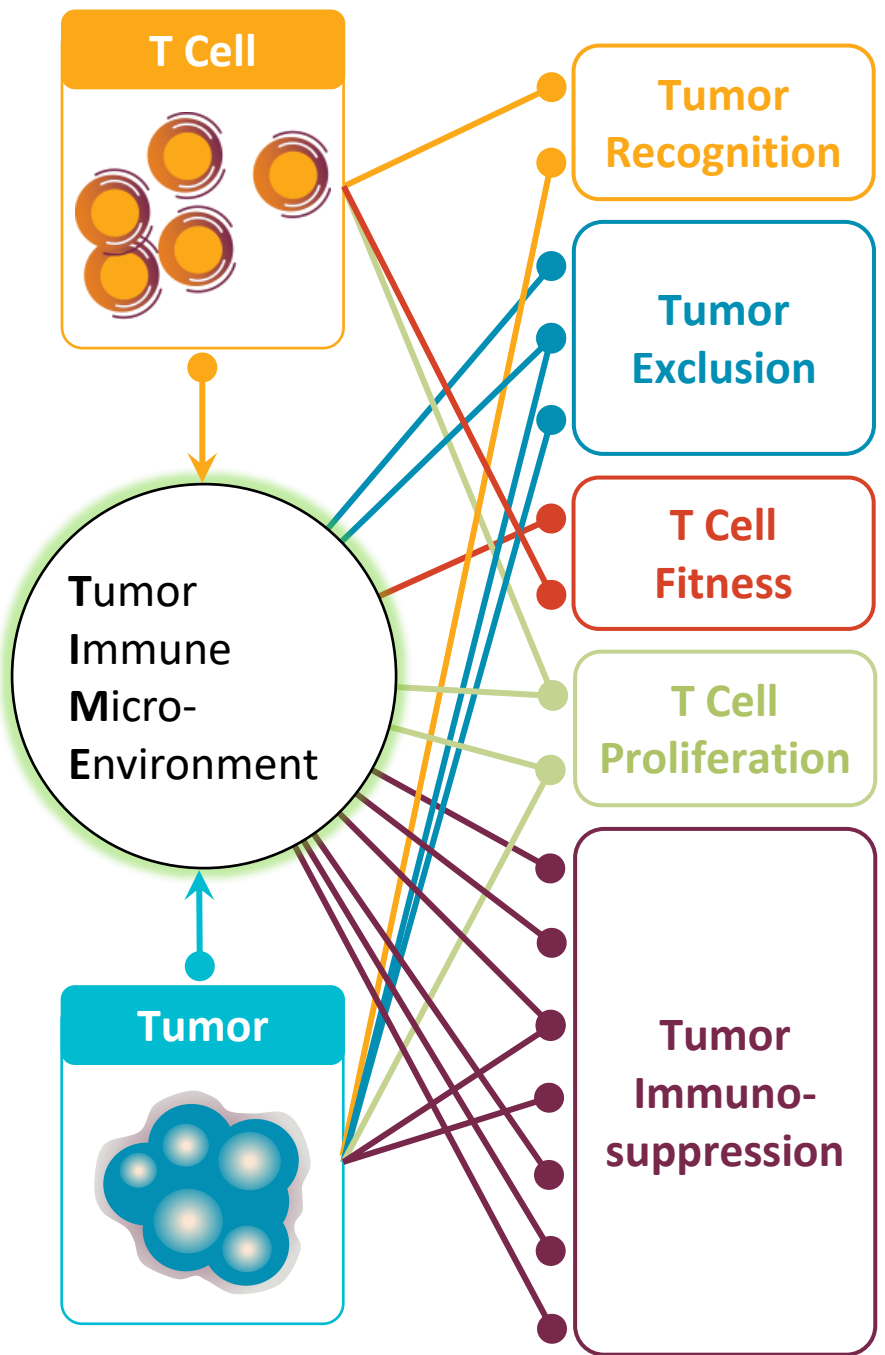
Pete DeMuth, PhD

Vice President of Research

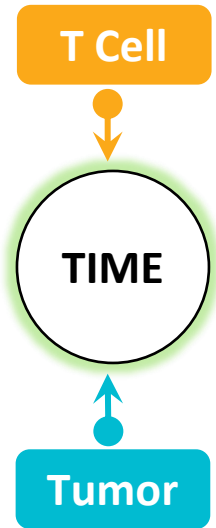
Cambridge, Massachusetts

**What are the major challenges to improving engineered T cell therapeutic efficacy?**





Challenge		Underlying Factor(s)
Tumor Specificity	Antigen Escape	Normal tissue expression, immune tolerance
		Ag heterogeneity, loss, & presentation defects
Recruitment	Penetration	Aberrant chemokine profile
	Infiltration	Physical barriers (peripheral, internal)
		Dysregulated vasculature (topology, adhesion)
Exhaustion	Exhaustion	TME-induced chronic activation
		Tonic signaling
Limited Proliferation	Pro-apoptotic Factors	Terminal phenotype, lack of APC & co-stim
		TME-derived FasL
Functional Inhibition	Functional Inhibition	Suppressor Cells (Treg, MDSC, TAMs)
	Functional Inhibition	Inhibitory Cytokines (TGFβ, IL-10)
	Functional Inhibition	Tumor-intrinsic Factors (PD-L1)
	Functional Inhibition	Hypoxia, acidosis
Metabolic Suppression	Metabolic Suppression	Glucose limitation
		Amino acid shortage (tryptophan, arginine)
Oxidative Stress		Elevated ROS production



**What solutions are most promising to address these challenges?**



## Therapeutic Engineering Strategies

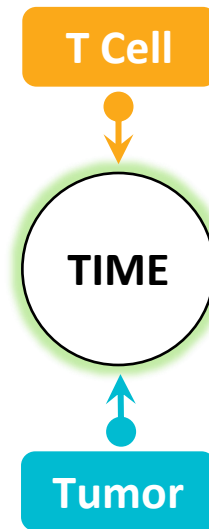
Promote Tumor Recognition

Promote T cell Fitness

Overcome Tumor Exclusion

Promote T cell Proliferation

Resist TIME Inhibition



## Clinical Practice Strategies

Early Line & Combinations

- Opportunity to maximize efficacy & durable response
- Potential to treat **lower tumor burden**
- Prevent sequential lines of therapy with little marginal effect on disease
- Minimize opportunity for **tumor-intrinsic** acquired mechanisms of **resistance**
- Minimize potential for **T cell-intrinsic** acquired mechanisms of **resistance**



## Therapeutic Engineering Strategies

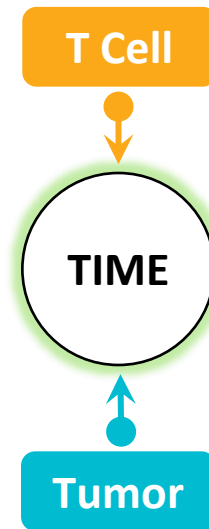
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## Clinical Practice Strategies

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- Minimize potential for **T cell-intrinsic** acquired mechanisms of **resistance**



**Promote Tumor Recognition**

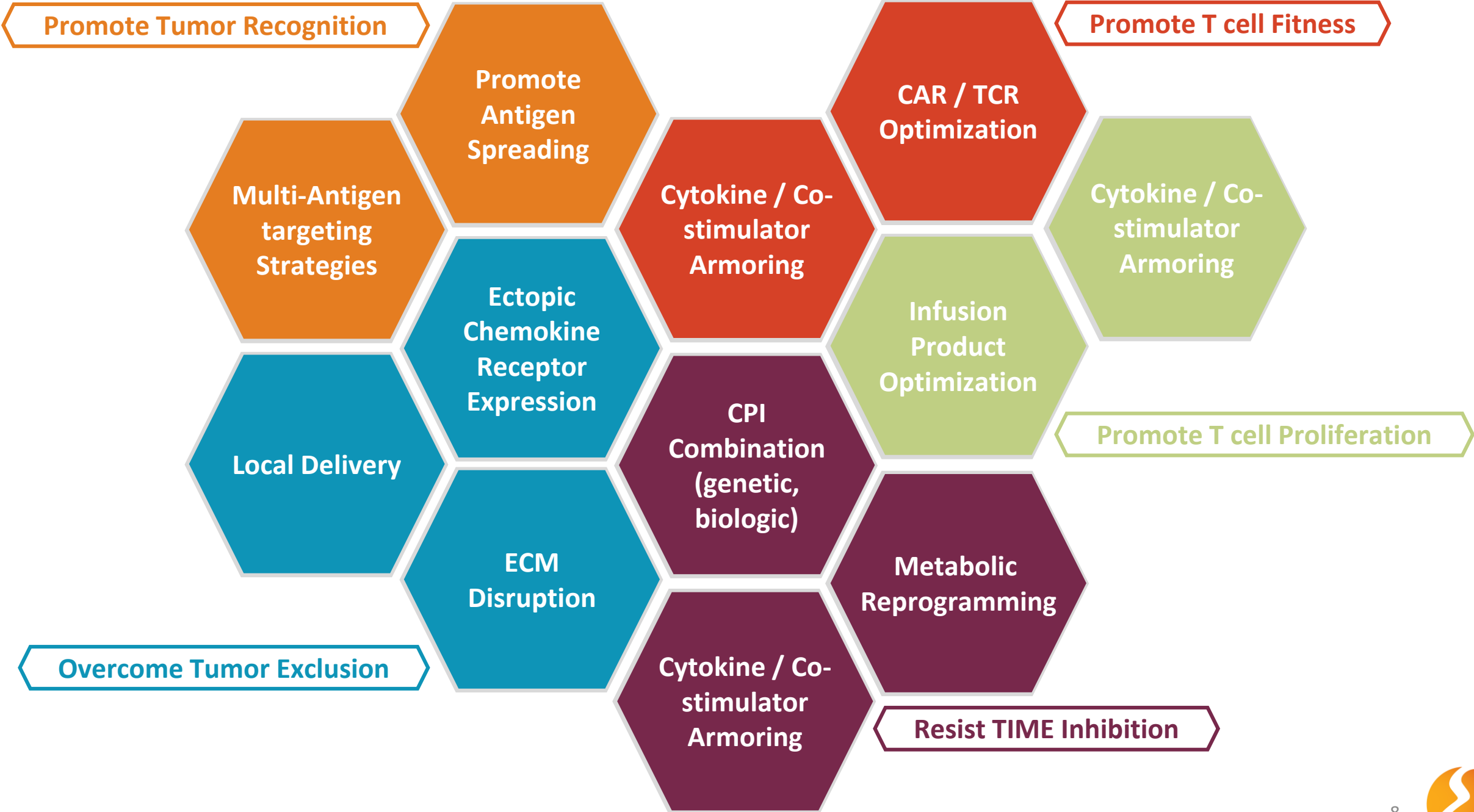
**Promote T cell Fitness**

**Promote T cell Proliferation**

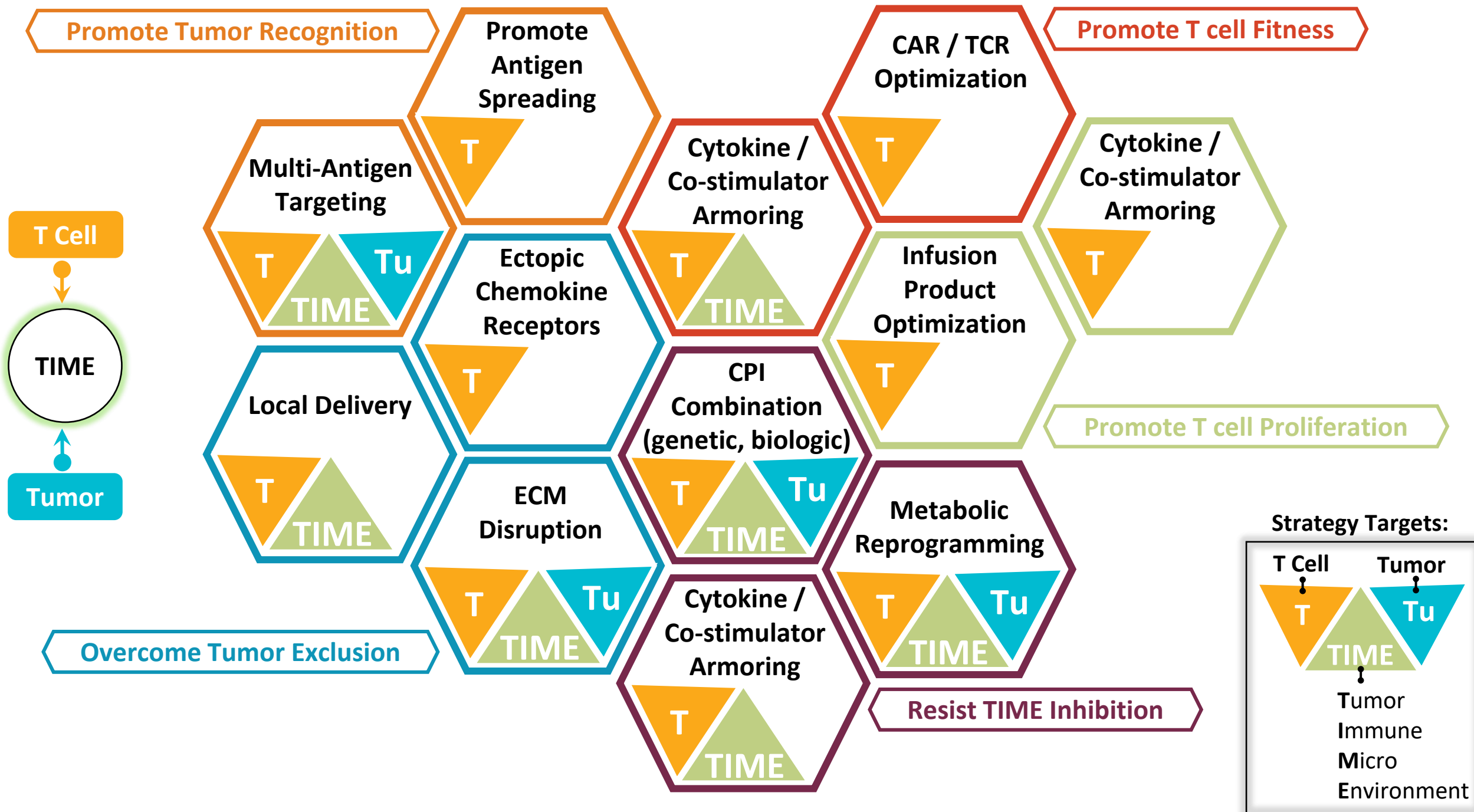
**Overcome Tumor Exclusion**

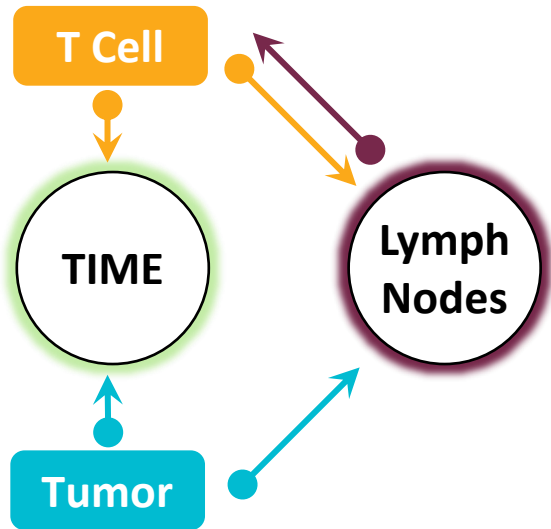
**Resist TIME Inhibition**









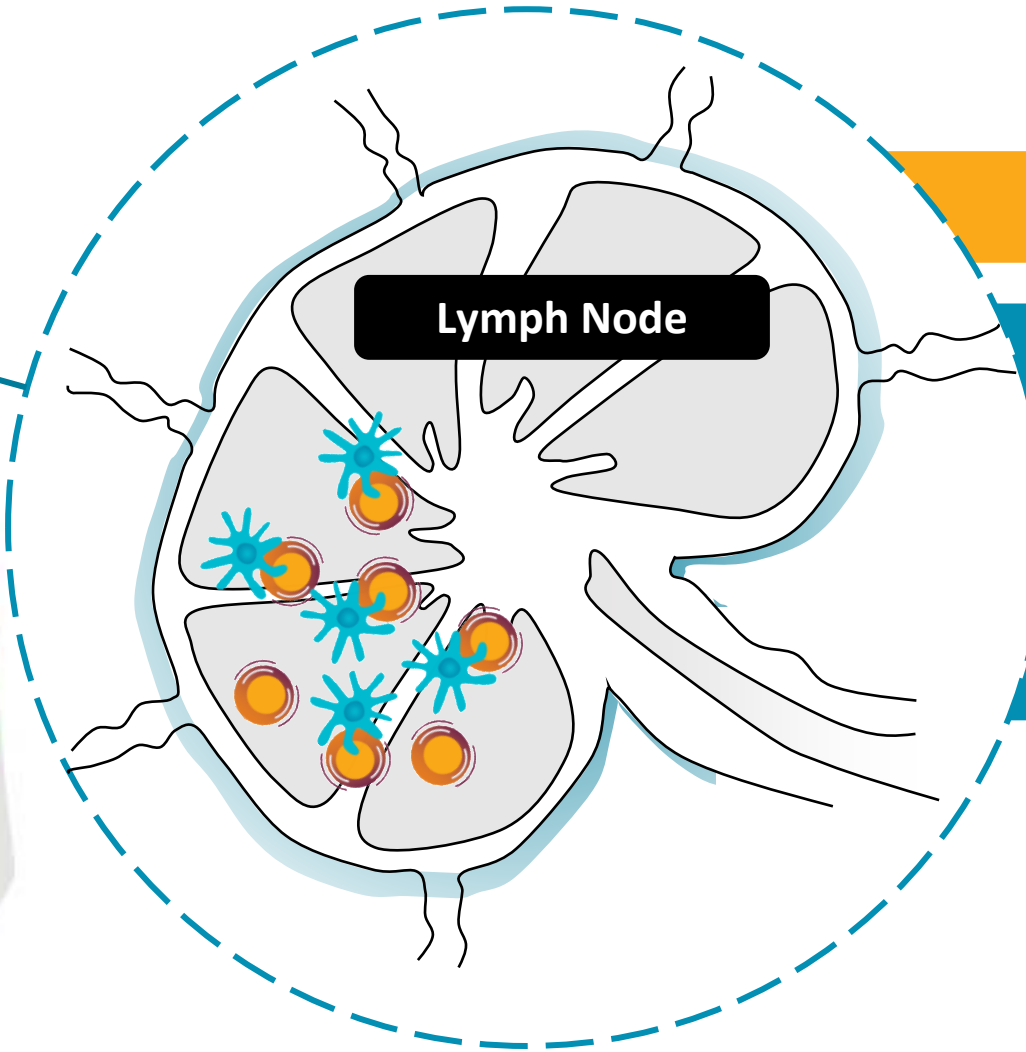
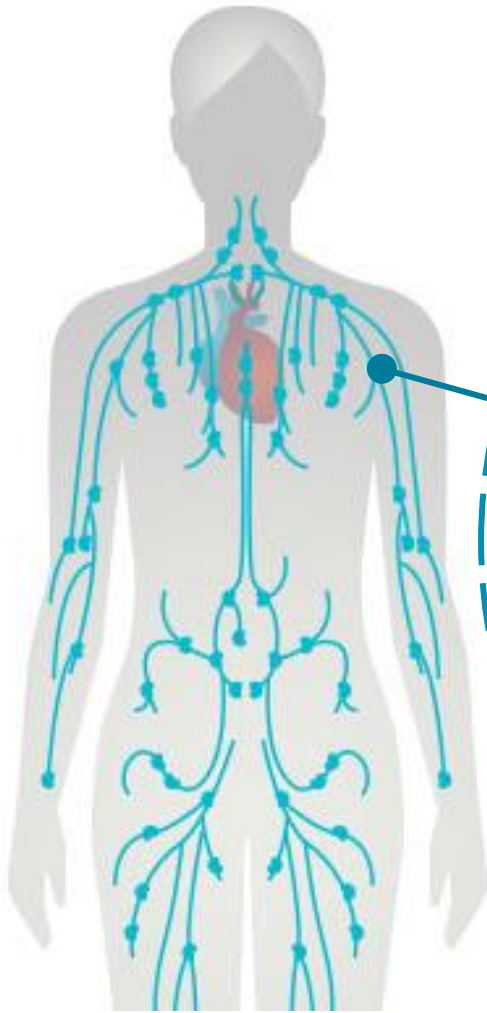


## What role can the lymphatics play in promoting T cell activity and therapeutic efficacy?

- 1) Designing a system to target immune agents to lymph nodes
- 2) Boosting **TCR-T Cell** therapeutic responses
- 3) Boosting **CAR-T Cell** therapeutic responses



# Lymph Nodes are Where the Immune Response is Orchestrated



**The Immune “School House”**

**Numerous Immune Cells**

**Response Coordination**

**APC : T Cell Interaction**

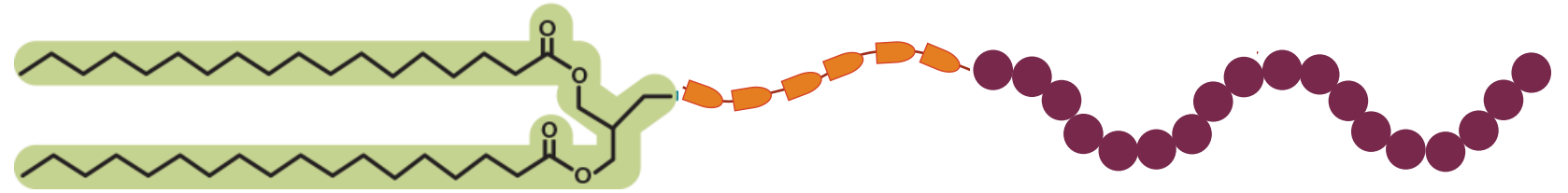
- **Expansion**
- **Persistence**
- **Solid tumor infiltration**
- **Anti-tumor effector function**
- **Antigen spreading**



# Designing a Lymph Node Targeted Booster for TCR-T Cell Therapy

## (1) AMP-Peptide Peptide Antigen

- Cognate peptide target for cancer antigen-specific TCR
- AMP modification gives >10-fold improved lymph node targeting



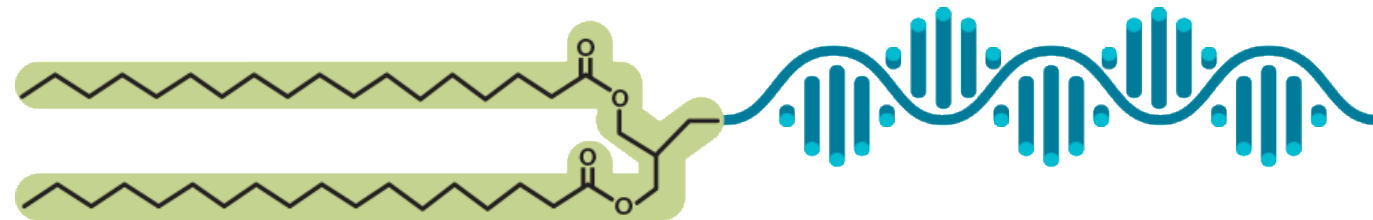
AMP Modification: Albumin Binding Lipid for Lymph Node Targeting

PEG Linker

Peptide: Cognate TCR Epitope

## (2) AMP-CpG Adjuvant

- Potent TLR-9 immuno-activator
- AMP modification gives >10-fold improved lymph node targeting

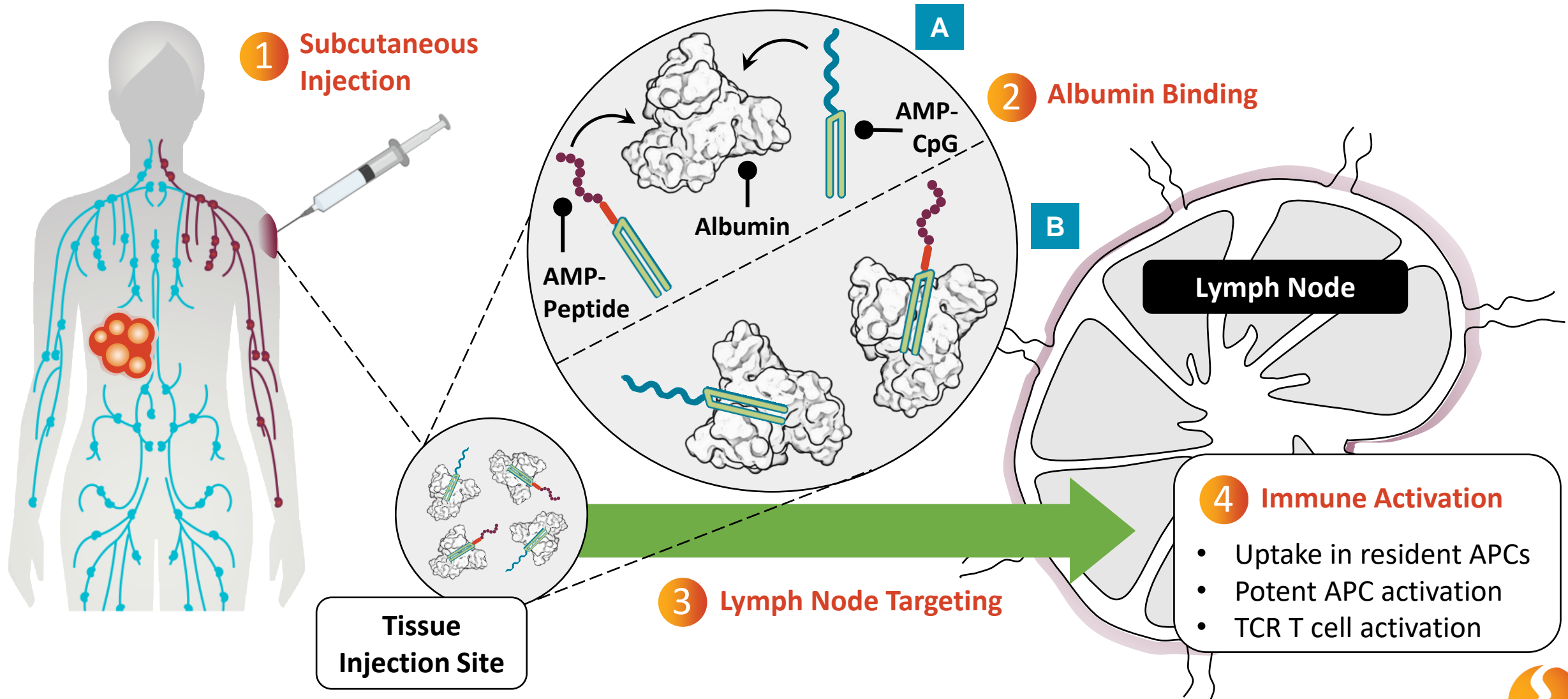


AMP Modification: Albumin Binding Lipid for Lymph Node Targeting

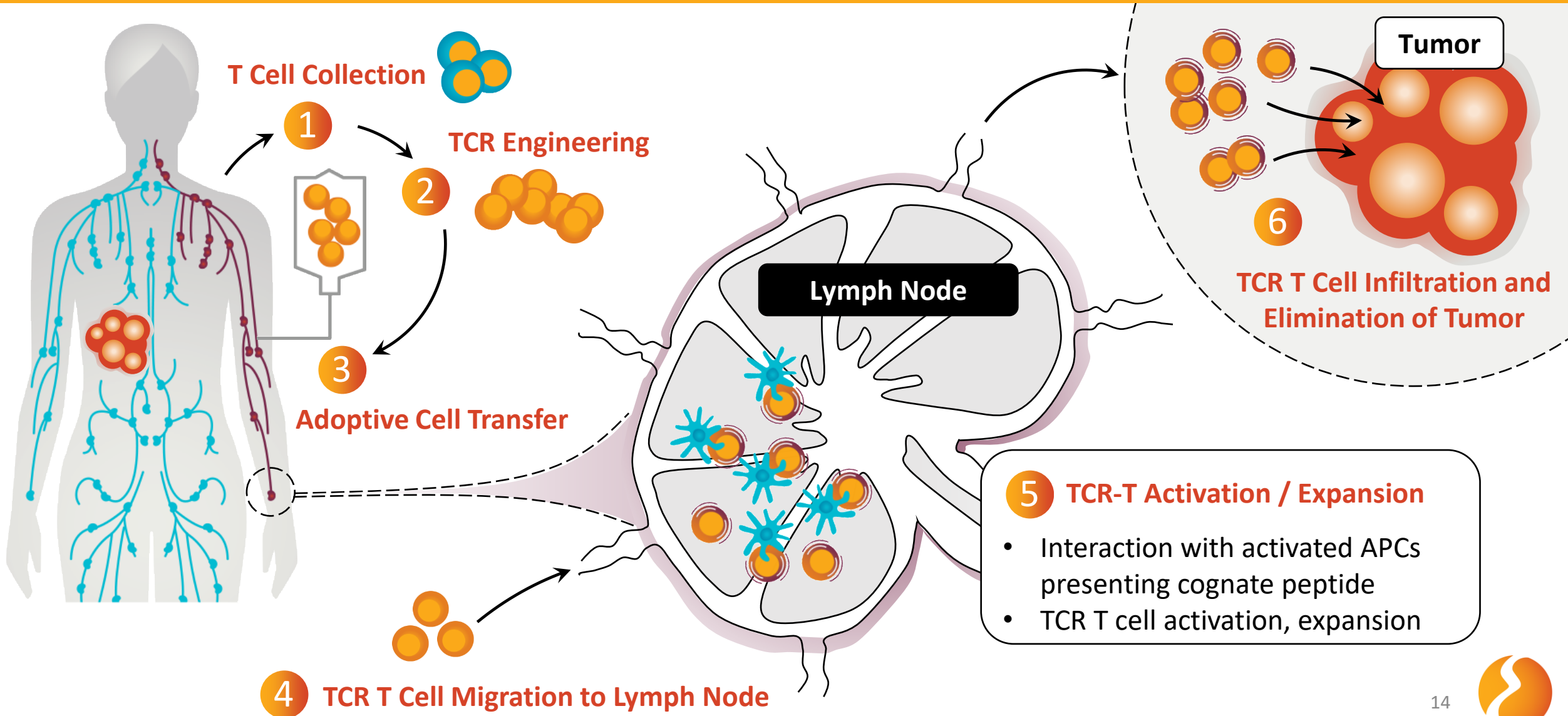
CpG DNA: TLR-9 Agonist



# Designing a Lymph Node Targeted Booster for TCR-T Cell Therapy



# Designing a Lymph Node Targeted Booster for TCR-T Cell Therapy



# AMP-Boosting Potently Enhances TCR-T Therapy to Eliminate Established Solid Tumors

## B16F10 Melanoma

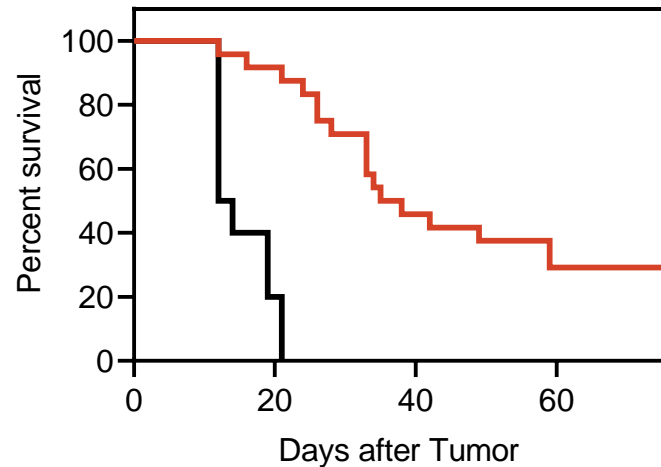
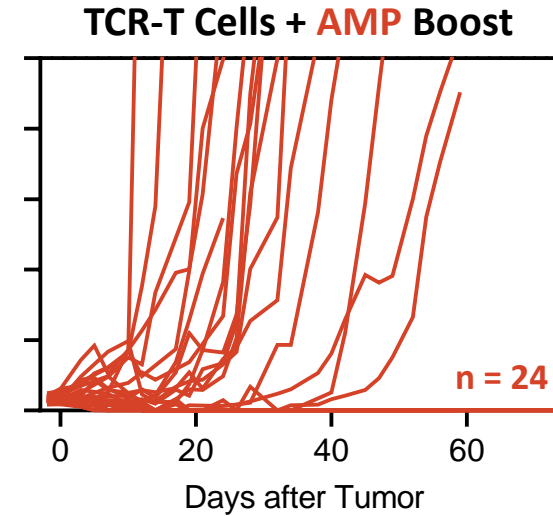
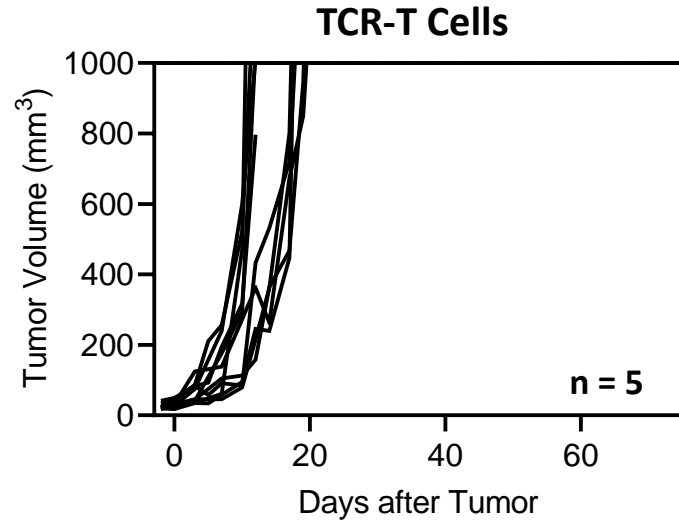


- day -10 Tumor Injection
- day -1 AMP-Boosting
- day 0 TCR-T Cells
- day 3 AMP-Boosting
- day 7 AMP-Boosting
- day 10 AMP-Boosting
- day 14 AMP-Boosting

$5 \times 10^6$  gp100-specific TCR-T Cells  
mCherry Transduced

### AMP- Boost:

10  $\mu$ g gp100 Peptide  
1 nmol CpG



TCR-T Cells + AMP Boost: **7/24 cured**

TCR-T Cells: **0/10 cured**



# AMP-Boosting Induces Durable TCR-T Cell Responses, Protection Against Secondary Tumor Challenge

## B16F10 Melanoma

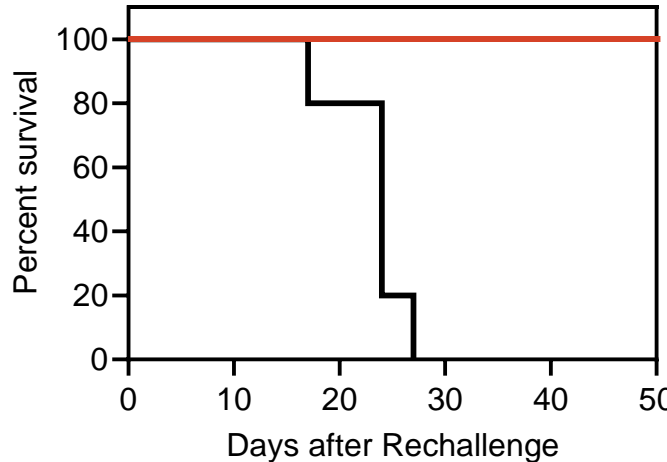
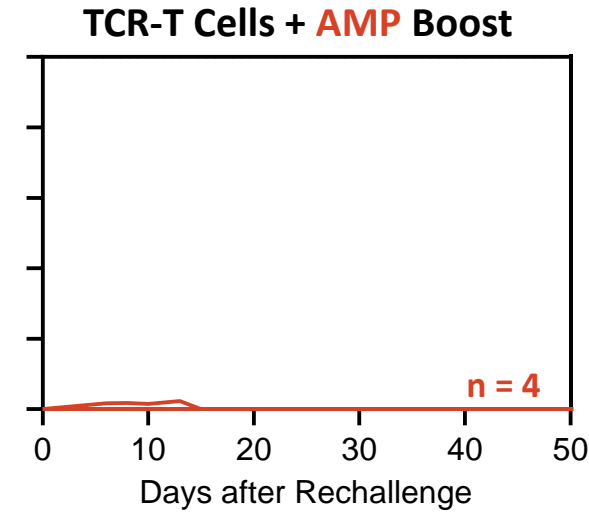
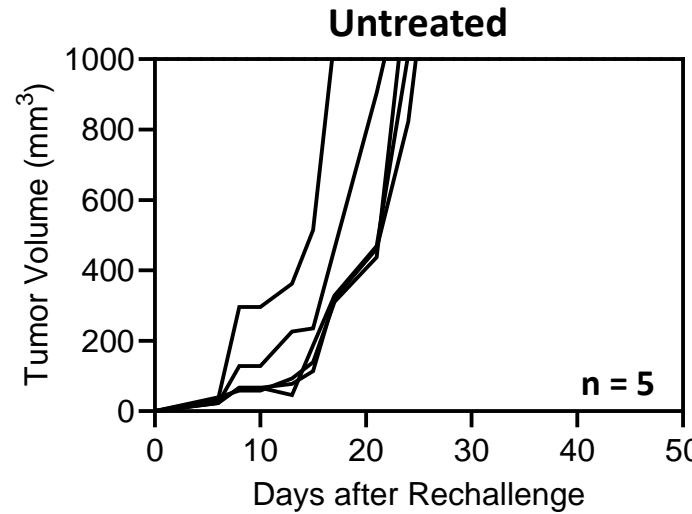
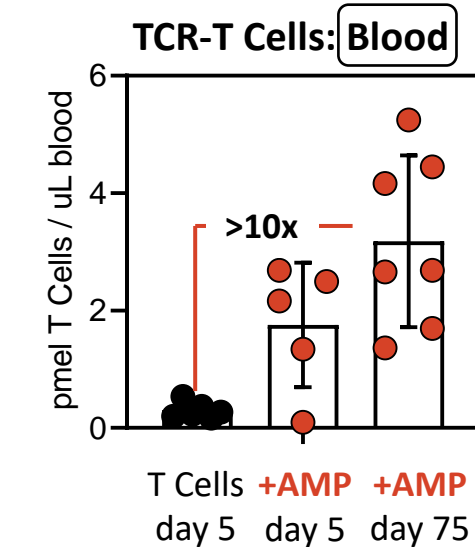


day -10 Tumor Injection  
 day -1 AMP-Boosting  
 day 0 TCR-T Cells  
 day 3 AMP-Boosting  
 day 7 AMP-Boosting  
 day 10 AMP-Boosting  
 day 14 AMP-Boosting  
 day 75 Tumor Injection

$5 \times 10^6$  gp100-specific TCR-T Cells  
 mCherry Transduced

### AMP-Boost:

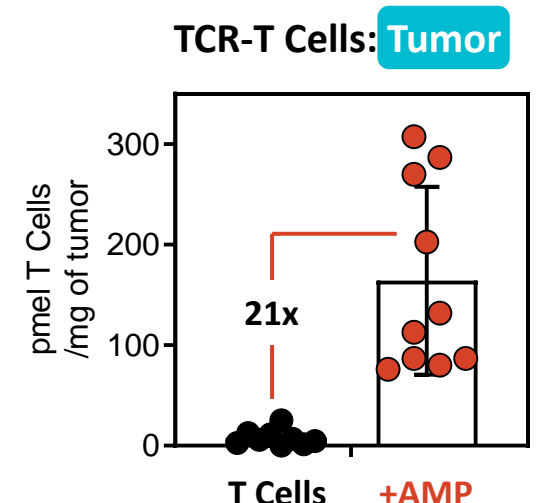
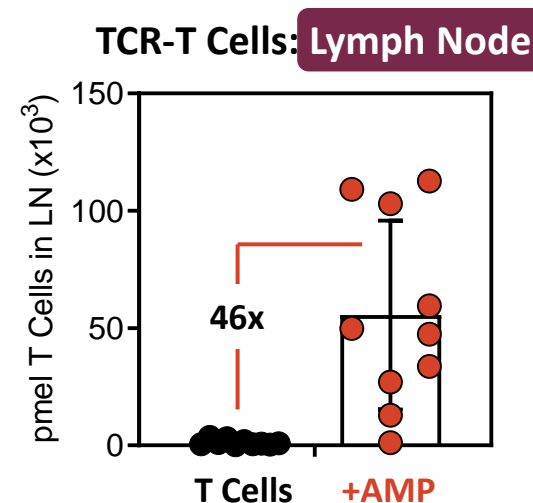
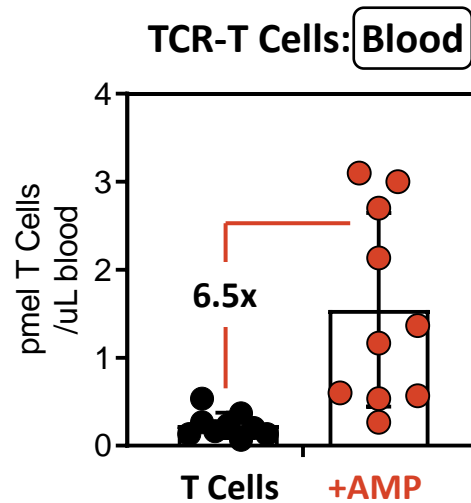
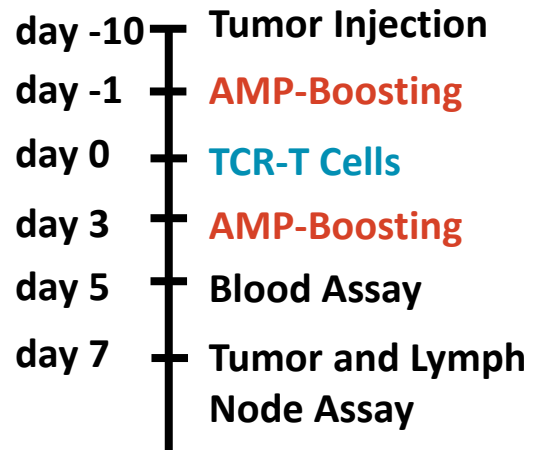
10  $\mu$ g gp100 Peptide  
 1 nmol CpG





# AMP-Boosting Expands TCR-T Cells in Blood, Lymph Nodes, and Enhances Tumor Infiltration

## B16F10 Melanoma



5x10<sup>6</sup> gp100-specific TCR-T Cells  
mCherry Transduced

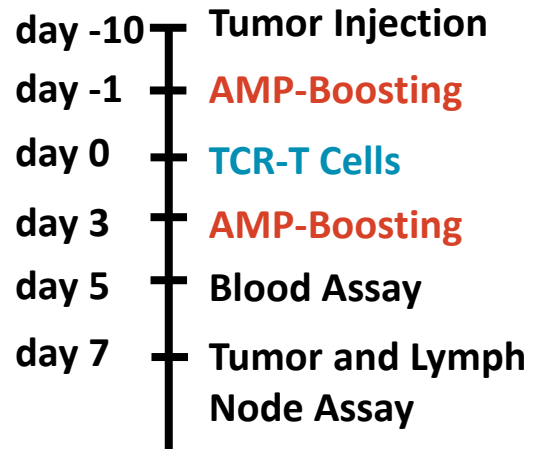
### AMP-Boost:

10 µg gp100 Peptide  
1 nmol CpG



# AMP-Boosting Enhances TCR-T Cell Tumor Infiltration, Functionality, and Proliferation

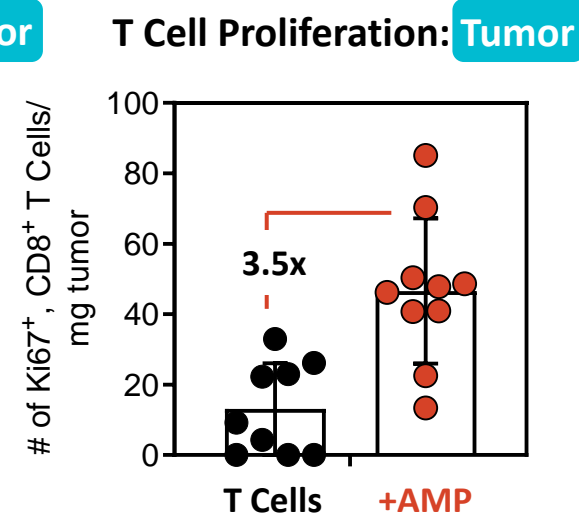
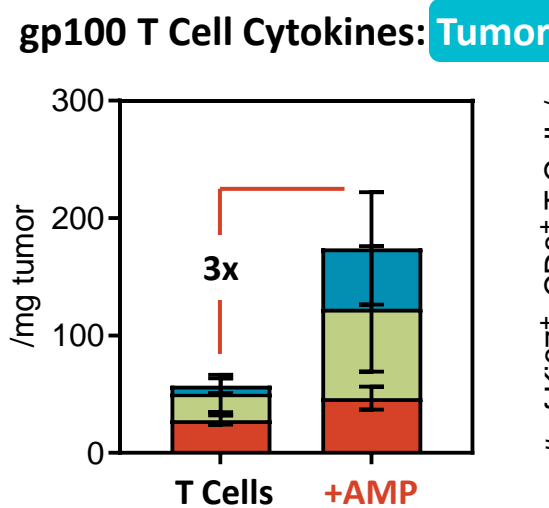
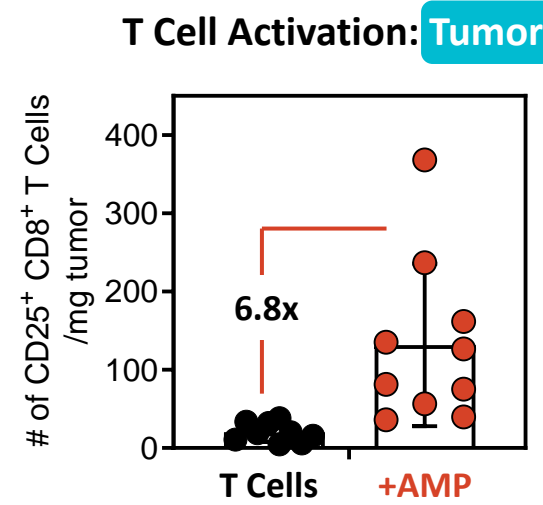
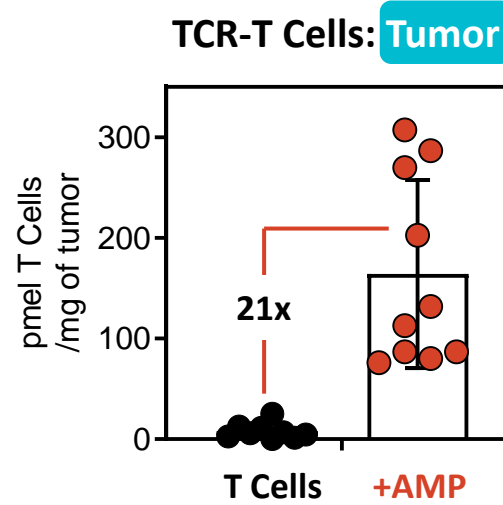
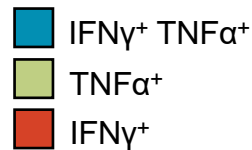
## B16F10 Melanoma



5x10<sup>6</sup> gp100-specific TCR-T Cells  
mCherry Transduced

### AMP-Boost:

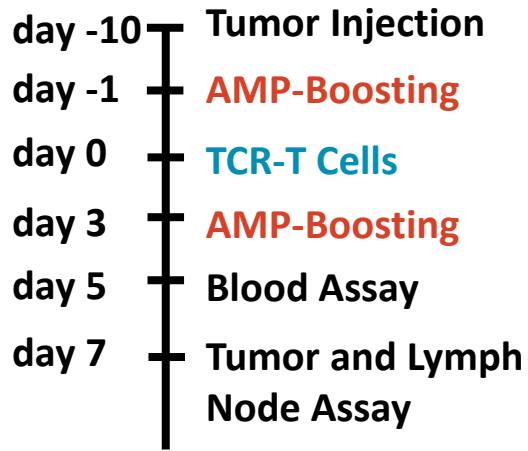
10 µg gp100 Peptide  
1 nmol CpG



# AMP-Boosting Induces Rapid and Potent Antigen Spreading Against Diverse Tumor Targets

B16F10 Melanoma

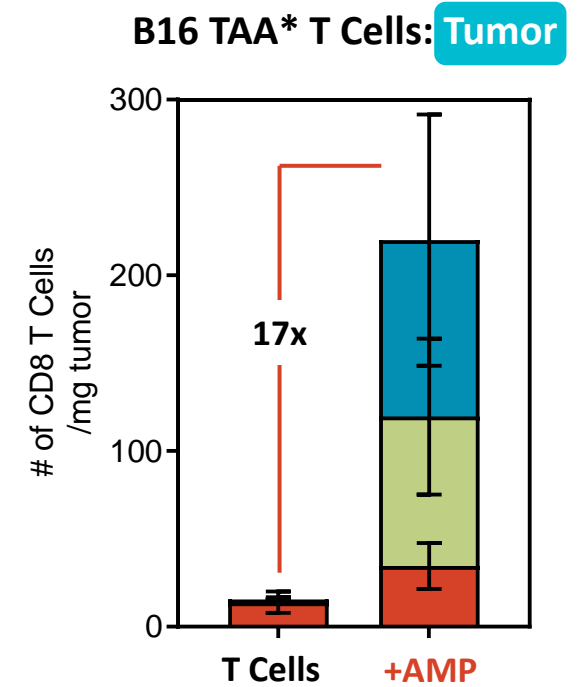
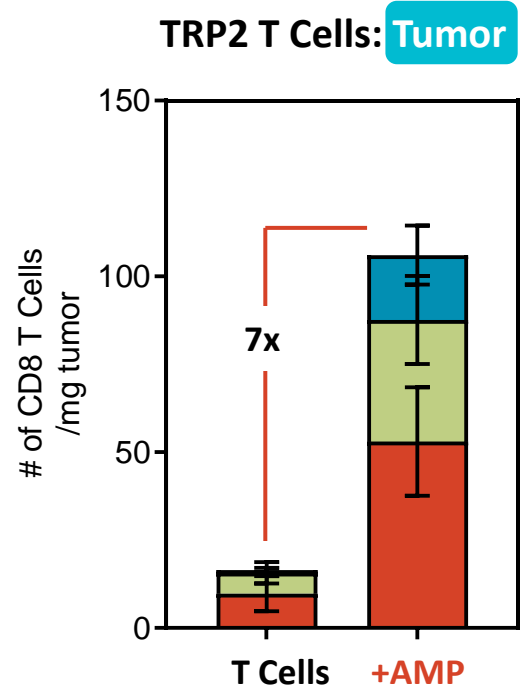
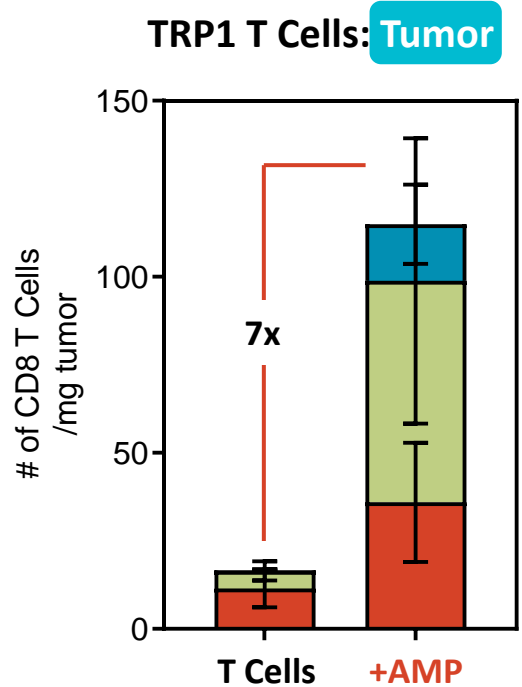
## B16F10 Melanoma NeoAg/TAA-specific T Cell Responses



5x10<sup>6</sup> gp100-specific TCR-T Cells  
mCherry Transduced

**AMP-Boost:**

10 μg gp100 Peptide  
1 nmol CpG

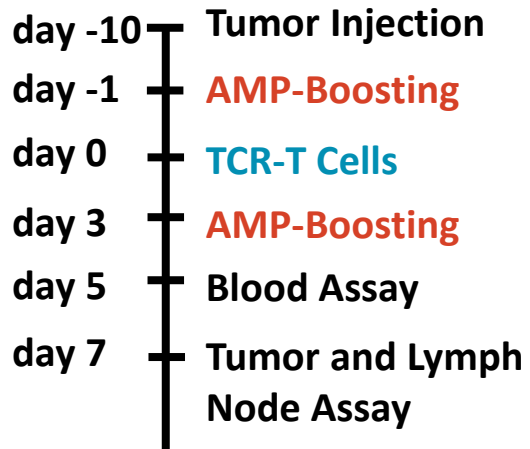


■ IFN $\gamma$ <sup>+</sup> TNF $\alpha$ <sup>+</sup>  
■ TNF $\alpha$ <sup>+</sup>  
■ IFN $\gamma$ <sup>+</sup>

\*M30, p15E, M27, M47, M48, Trp1

# AMP-Boosting Expands TCR-T Cells in Lymph Nodes and Induces Dendritic Cell Activation

## B16F10 Melanoma

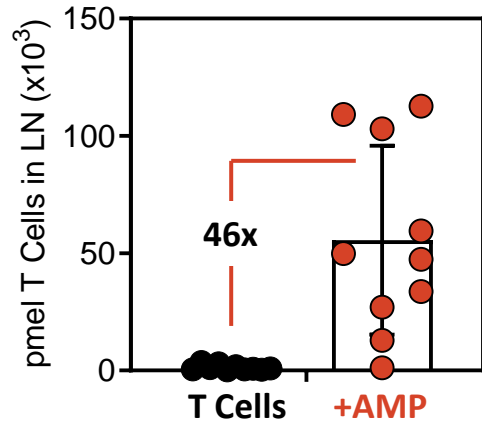


5x10<sup>6</sup> gp100-specific TCR-T Cells  
mCherry Transduced

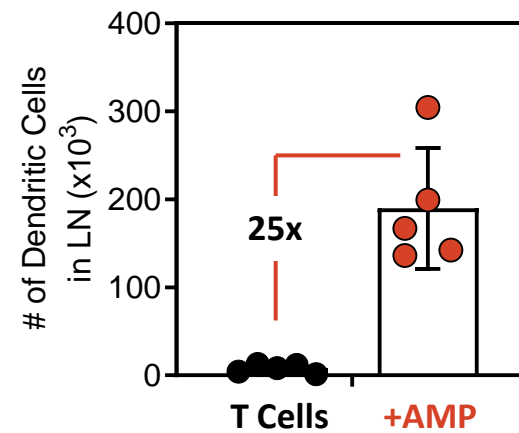
### AMP-Boost:

10 μg gp100 Peptide  
1 nmol CpG

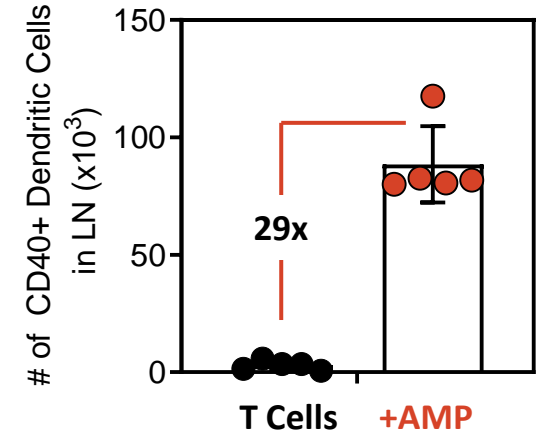
### TCR-T Cells: Lymph Node



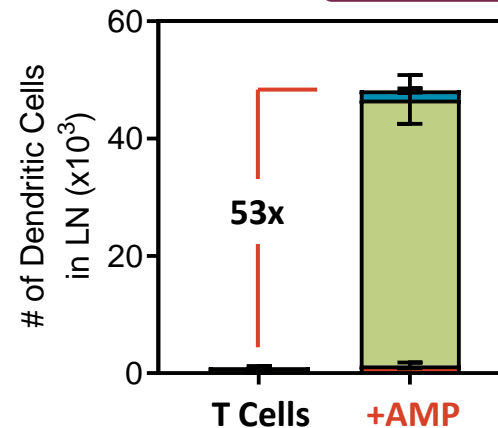
### DCs: Lymph Node



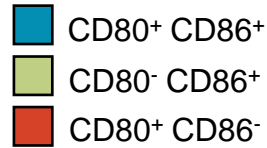
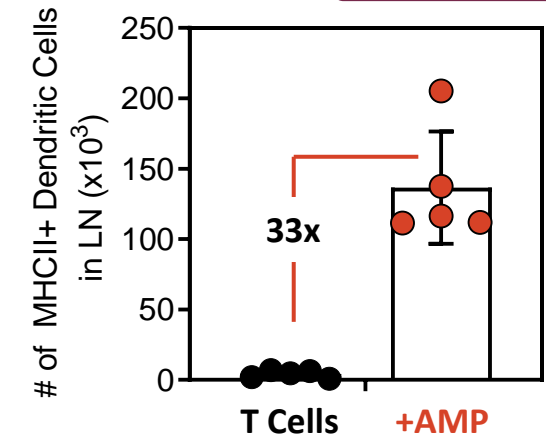
### DC CD40: Lymph Node



### DC CD80/86: Lymph Node

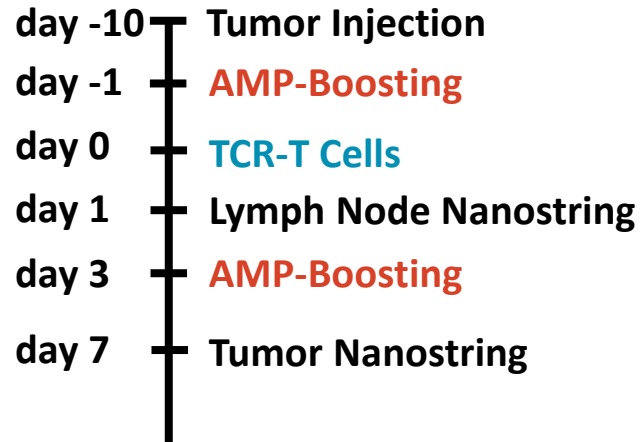


### DC MHCII: Lymph Node



# Evaluating Differential Gene Expression in Lymph Nodes and Tumors After TCR-T Therapy

## B16F10 Melanoma



## Lymph Node and Tumor Nanostring Gene Expression

Group	TCR-T	Treatment
1	5x10 <sup>6</sup> TCR-T Cells	Mock
2	Mock	AMP-gp100 / AMP-CpG
3	5x10 <sup>6</sup> TCR-T Cells	AMP-gp100 / AMP-CpG

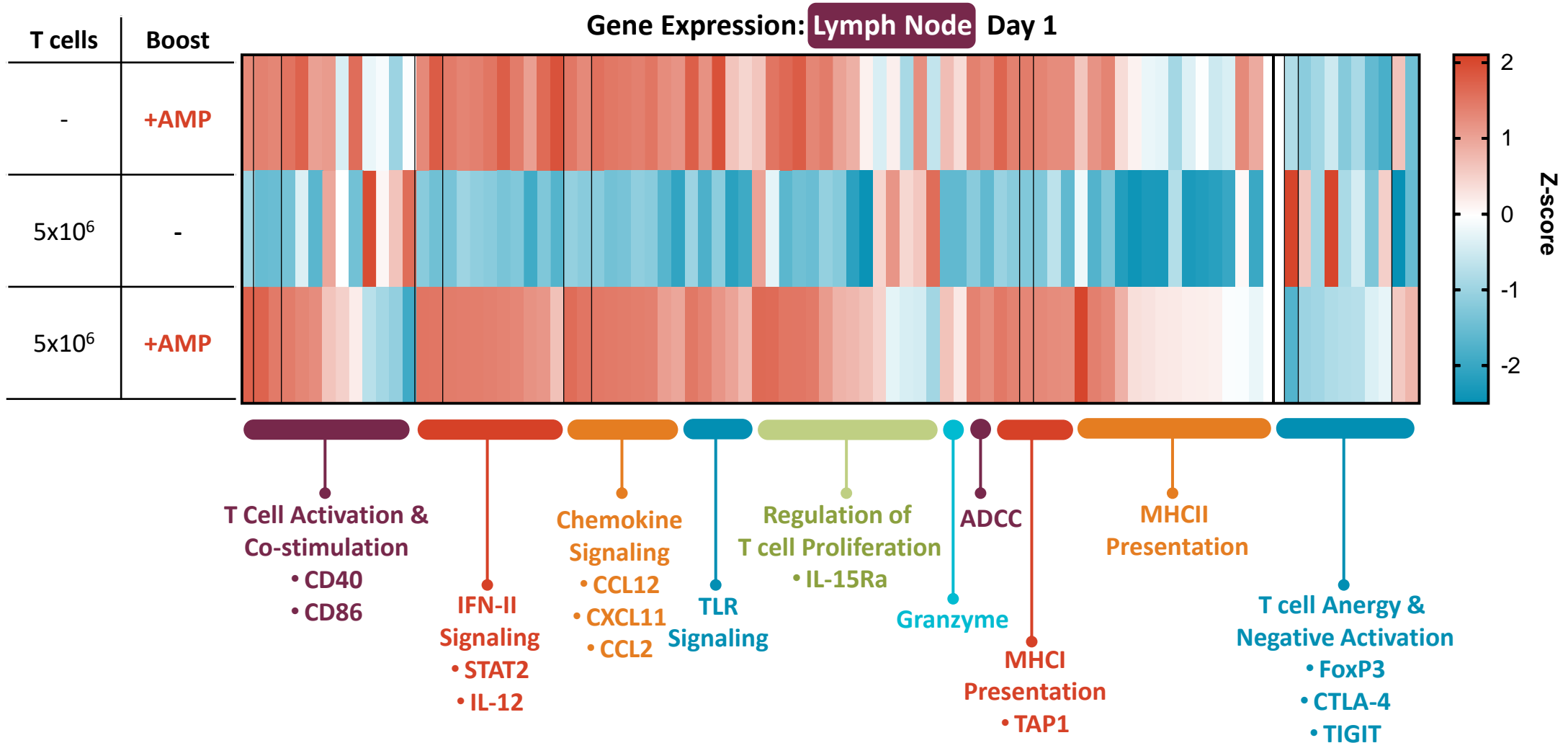
5x10<sup>6</sup> gp100-specific TCR-T Cells  
mCherry Transduced

### AMP-Boost:

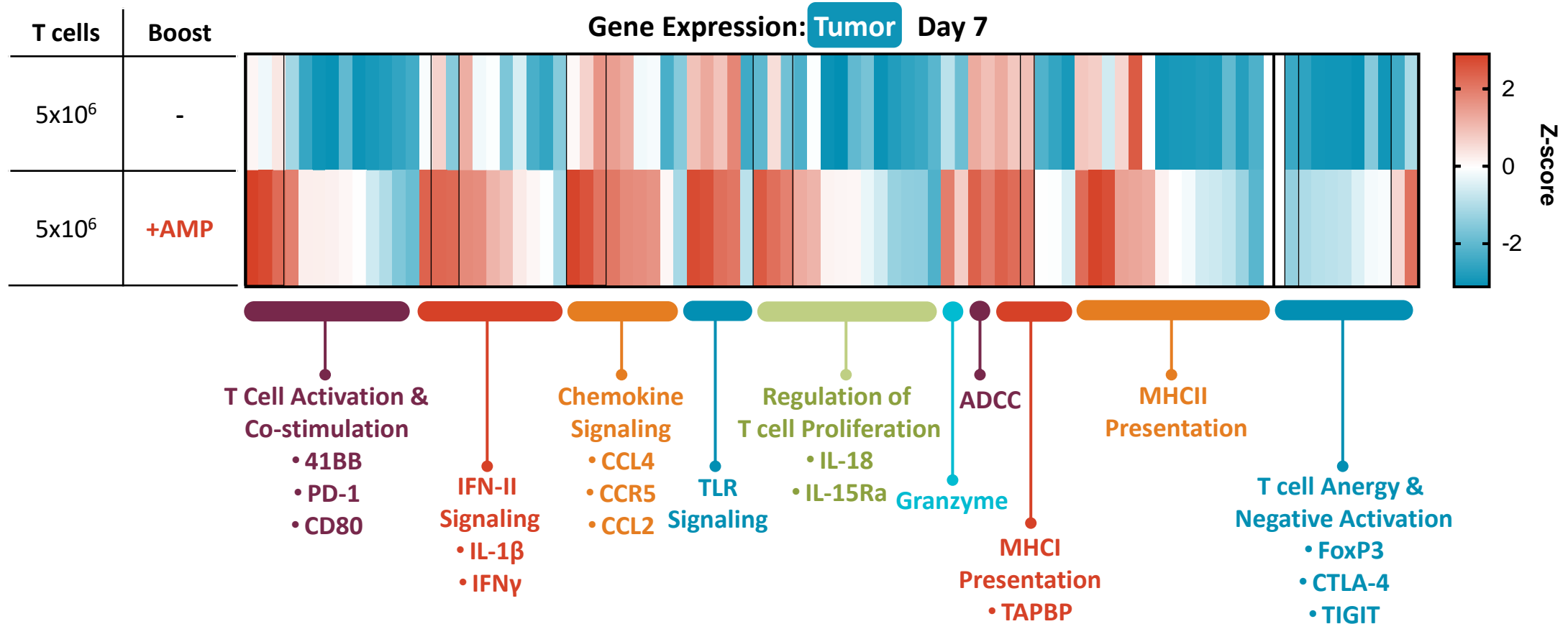
10 µg gp100 Peptide  
1 nmol CpG

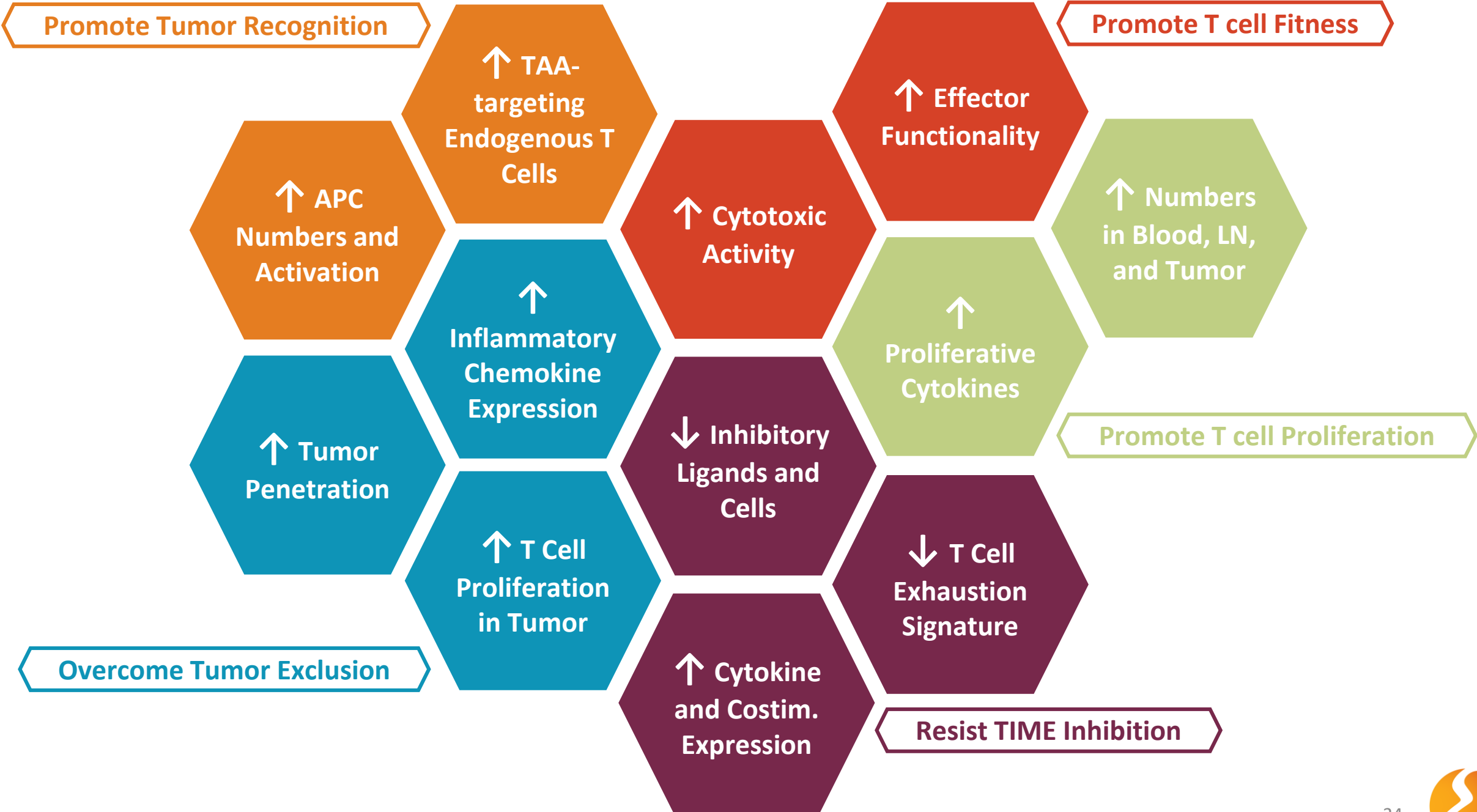


# AMP-boosting Induces Coordinated Inflammation in Lymph Nodes

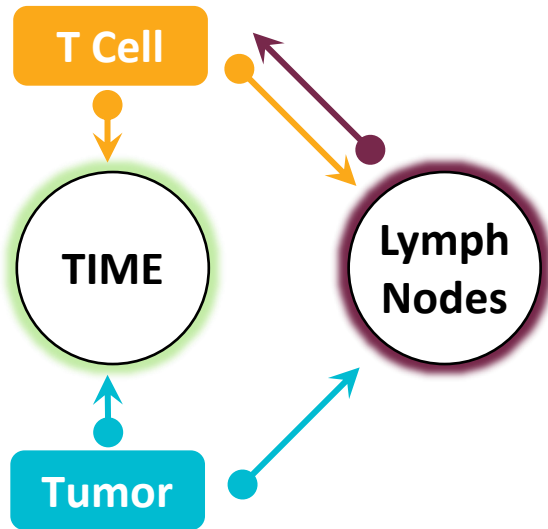


# AMP-boosting Enhances Multiple Axes of Immune Activation in the Tumor Microenvironment









## What role can the lymphatics play in promoting T cell activity and therapeutic efficacy?

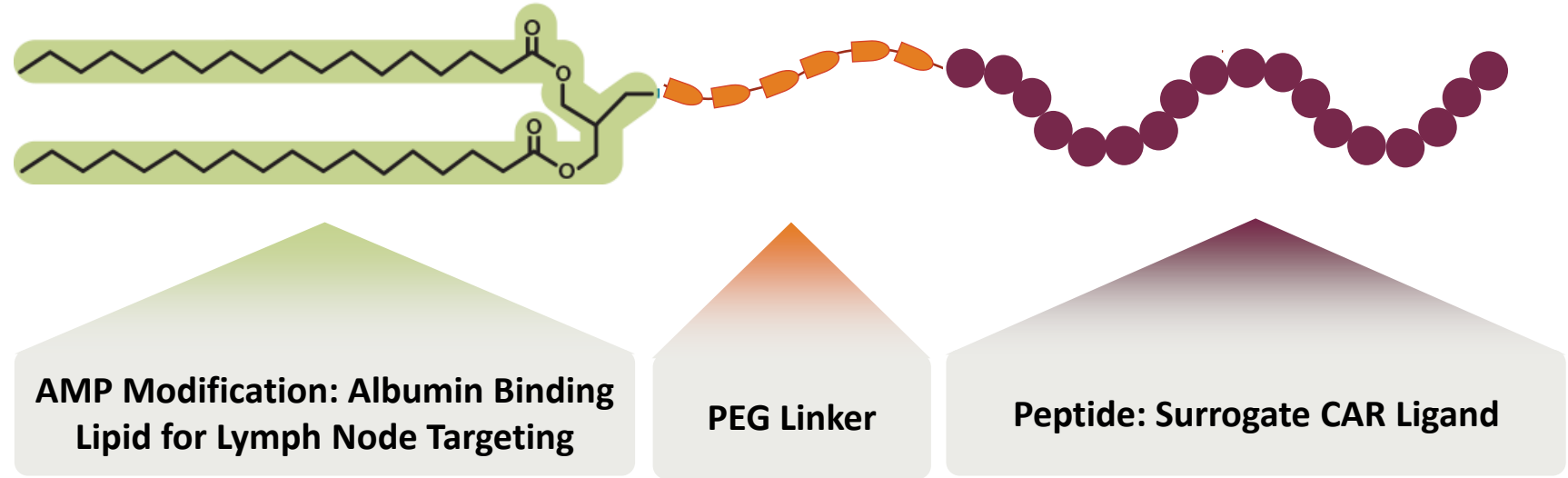
- 1) Designing a system to target immune agents to lymph nodes
- 2) Boosting **TCR-T Cell** therapeutic responses
- 3) Boosting **CAR-T Cell** therapeutic responses



# Designing a Lymph Node Targeted AMPlifier for CAR-T Cell Therapy

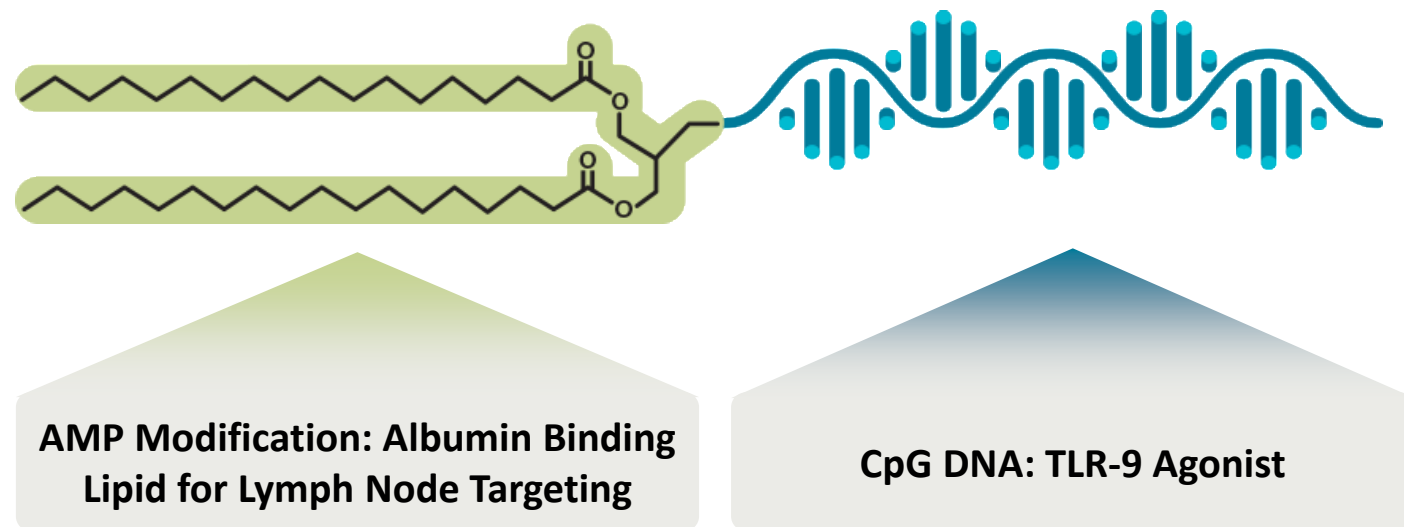
## (1) AMP-Peptide Peptide Surrogate Ligand

- Surrogate peptide ligand (mimotope) for CAR antigen binding domain
- AMP modification gives >10-fold improved lymph node targeting



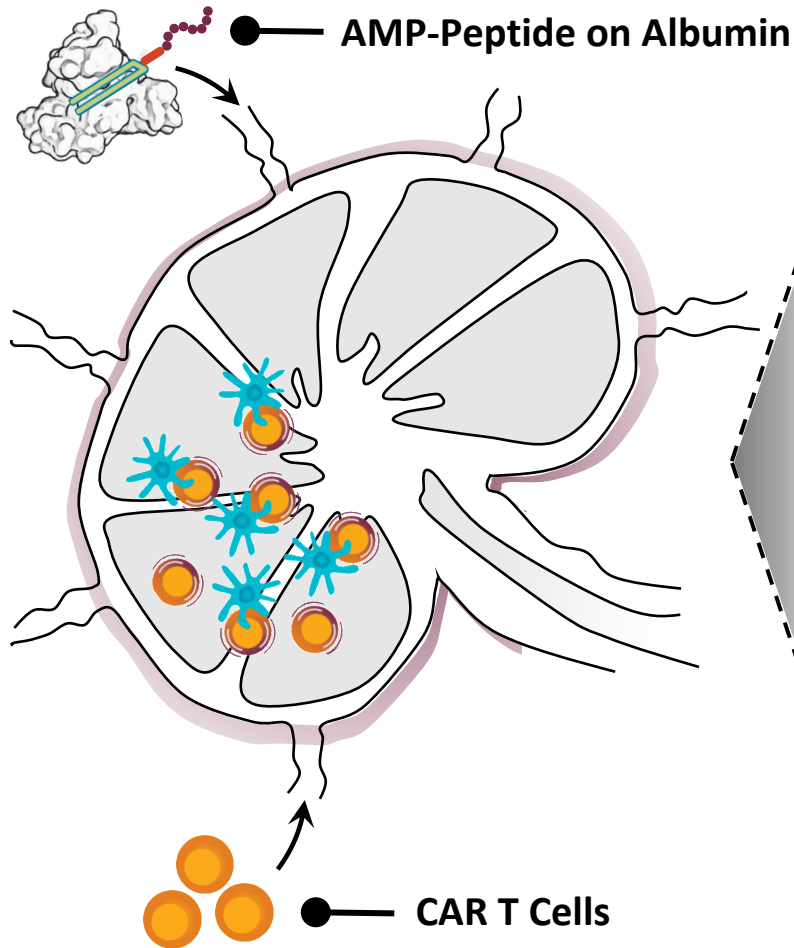
## (2) AMP-CpG Adjuvant

- Potent TLR-9 immuno-activator
- AMP modification gives >10-fold improved lymph node targeting

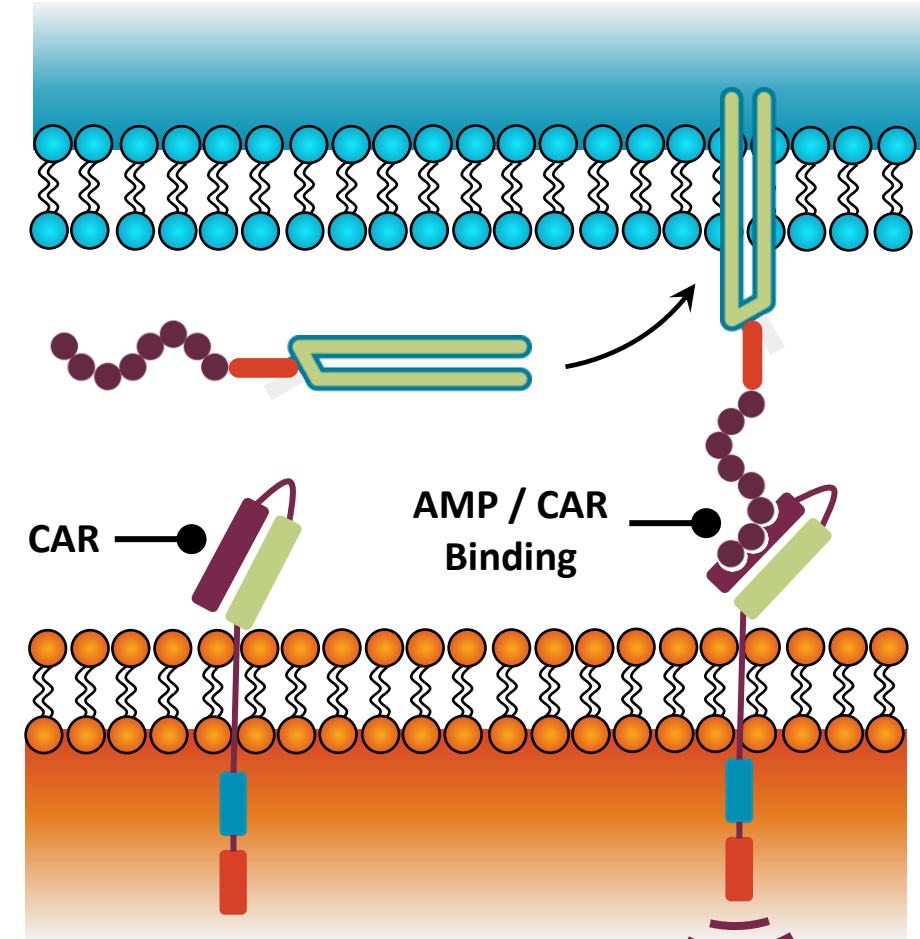
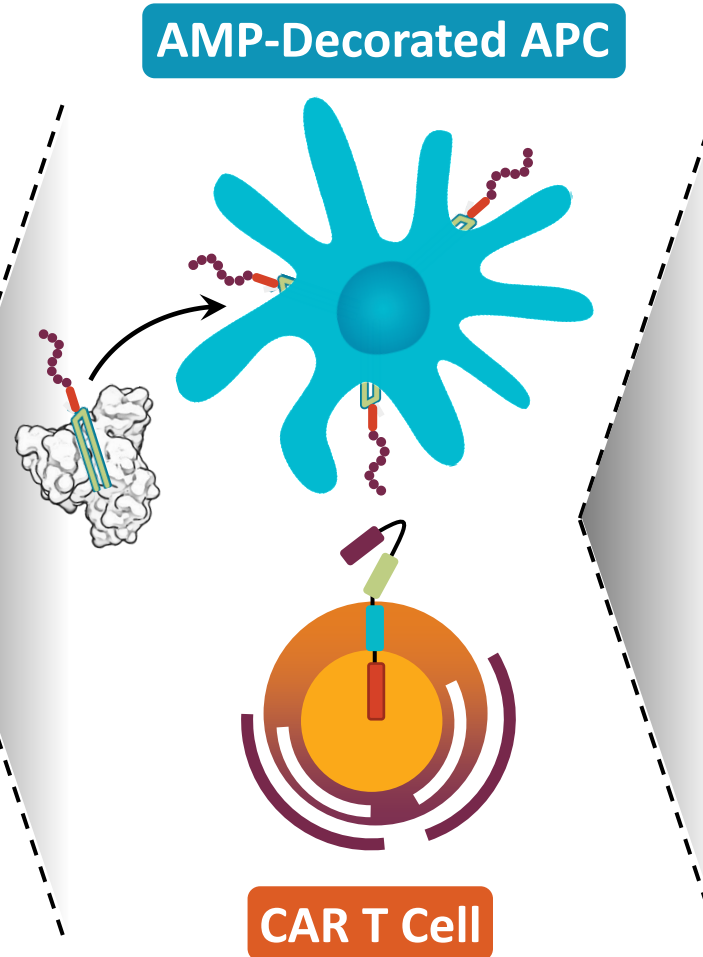


# Designing a Lymph Node Targeted Booster for CAR-T Cell Therapy

## Surrogate Peptide as CAR Ligands



## AMP-Decorated APC



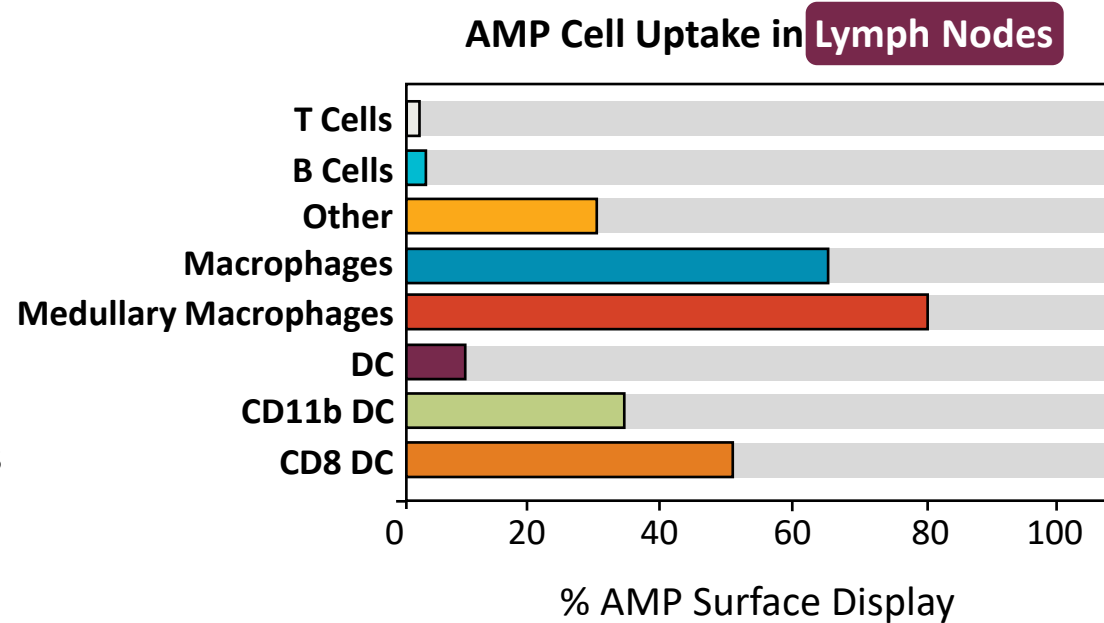
# AMP-FITC Decorates Lymph Node APCs to Stimulate CAR T Cell Activation



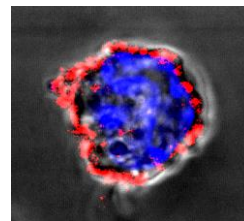
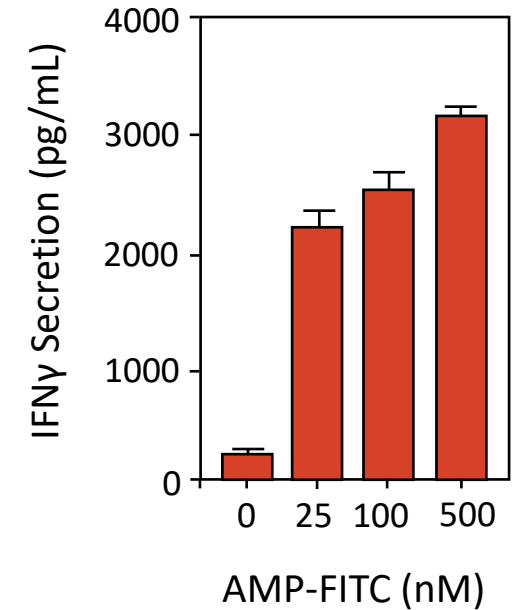
day 0 AMP-Boosting  
day 1 Analyze Lymph Node Uptake

FITC Retargetable CAR-T Cells

AMP-Boost  
AMP-FITC  
CDN



### CAR-T Cytokine Production



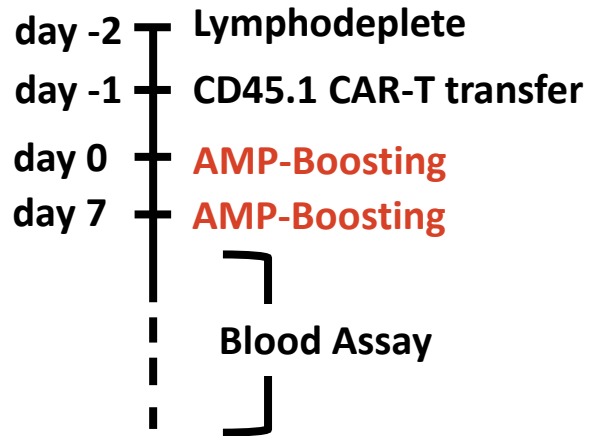
anti-FITC Ab on sorted CD11c<sup>+</sup> cells:



# AMP-Boosting Significantly Expands CAR-T Cells *In Vivo*



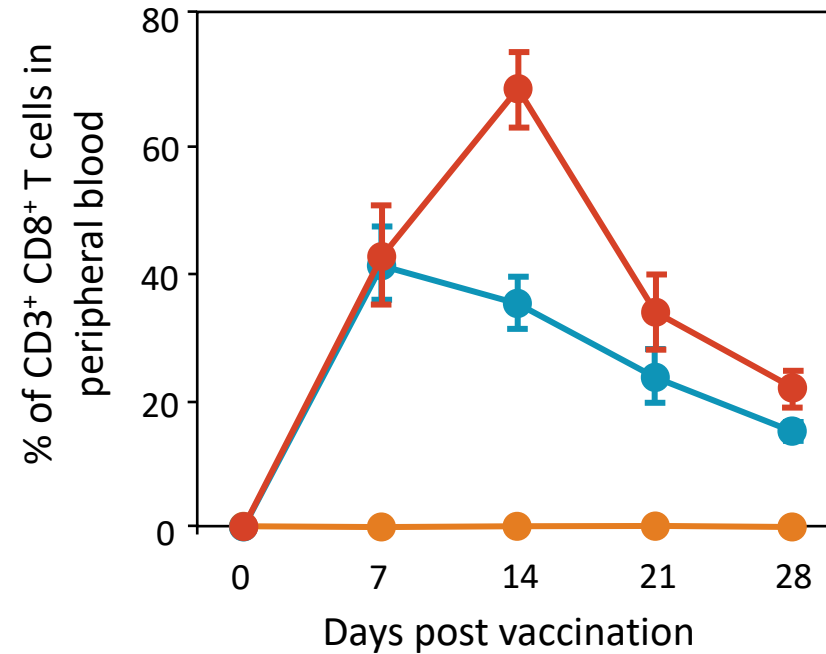
CD45.2 Recipient Mice



FITC Retargetable CAR-T Cells

AMP-Boost  
AMP-FITC  
CDN

Expansion of CAR-T Cells: Blood

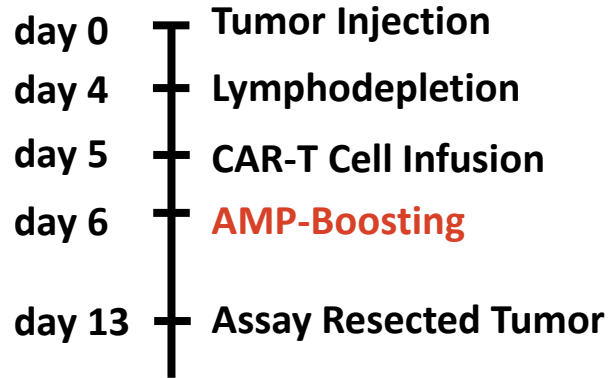


- 10x10<sup>6</sup> CAR T
- 50x10<sup>3</sup> CAR T + AMP-Boosting
- 50x10<sup>3</sup> CAR T



# AMP-CAR-T Activators Enhance CAR T Cell Tumor Infiltration and Effector Function

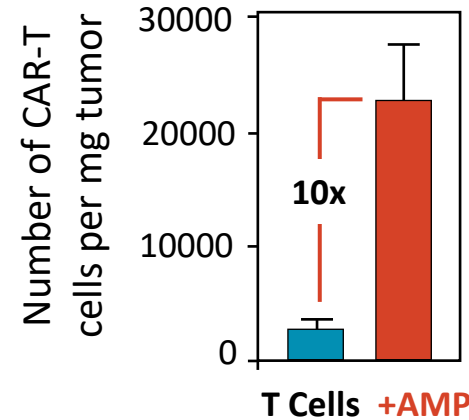
EGFRvIII+ CT-2A Glioma



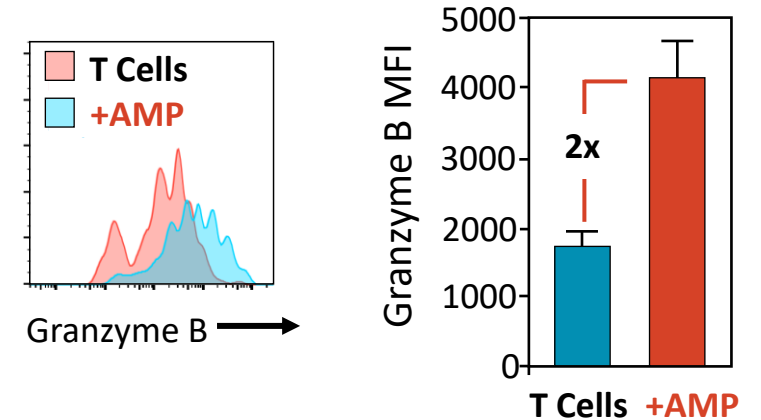
EGFRvIII-specific CAR-T Cells

**AMP-Boost:**  
AMP-EGFRvIII  
CDN

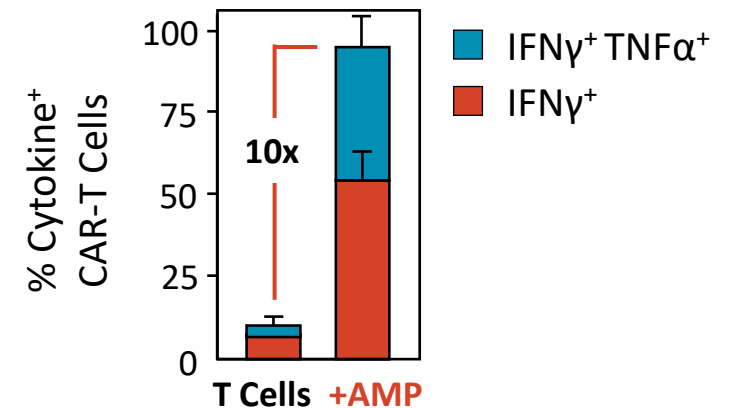
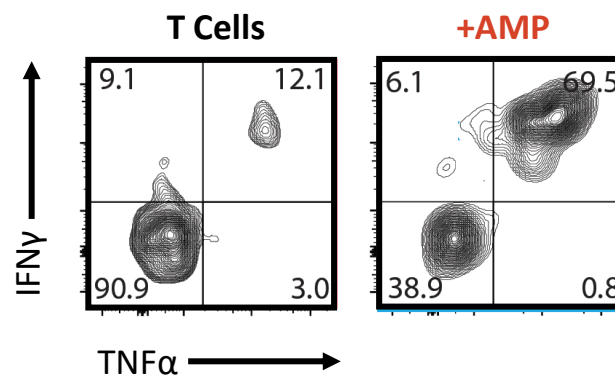
CAR T Cells: Tumor



CAR T Cell Granzyme: Tumor



CAR T Cell Cytokines: Tumor



# AMP-CAR-T Activators Potently Drive CAR-T Cells to Eradicate Large Established Tumors

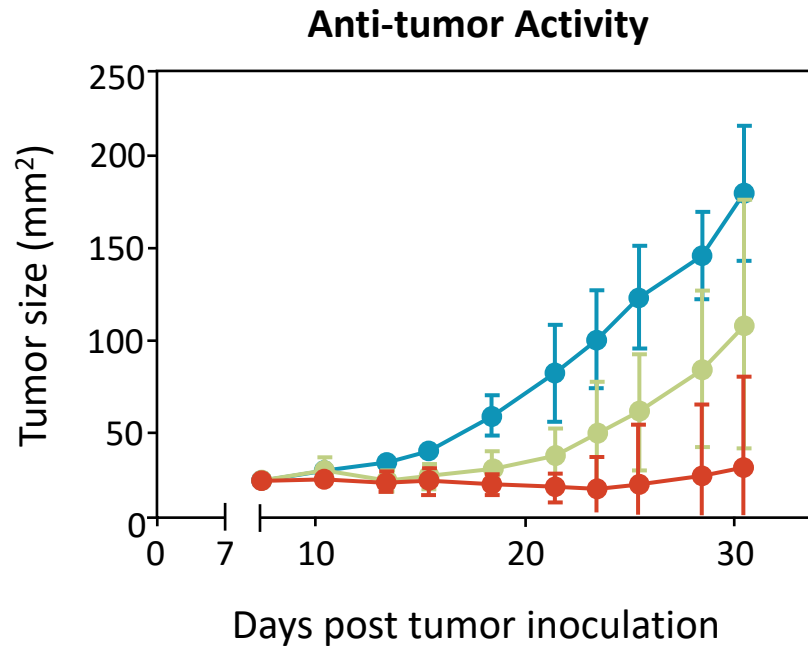
EGFRvIII+ CT-2A Glioma



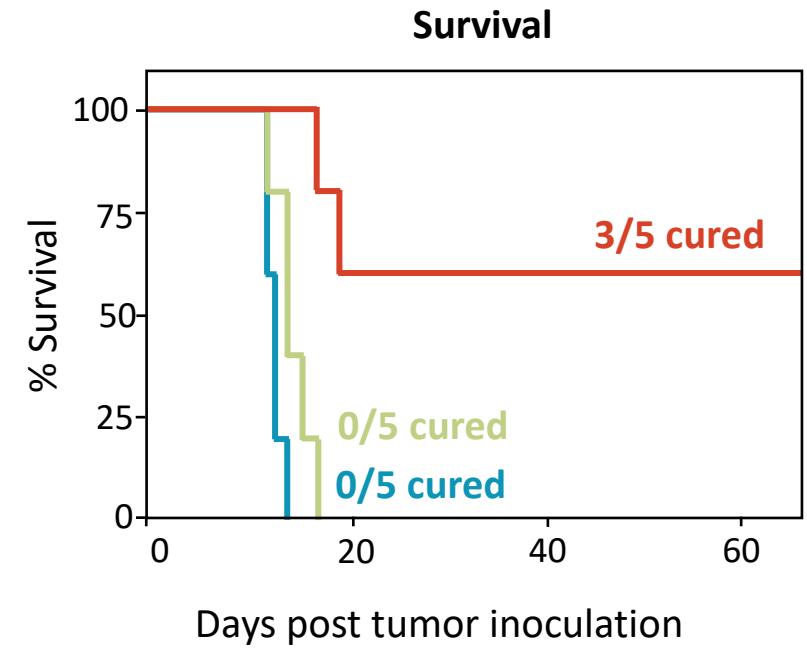
day 0 — Tumor Injection  
 day 7 — Lymphodepletion  
 day 8 — CAR-T Cell Infusion  
 day 9 — AMP-Boosting  
 day 16 — AMP-Boosting  
 day 23 — AMP-Boosting

EGFRvIII-specific CAR-T Cells

**AMP-Boost:**  
 AMP-EGFRvIII  
 CDN

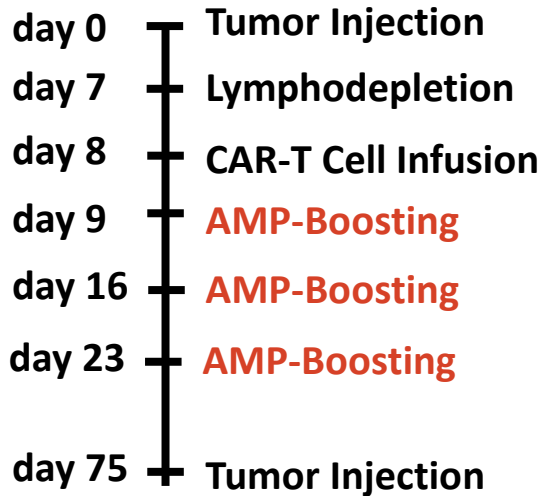


- 10x10<sup>6</sup> Control CAR T
- 1x10<sup>6</sup> CAR T
- 1x10<sup>6</sup> CAR T + AMP Boost



# AMP Boosted Responses Provide Durable Protection From Relapse and Antigen-loss

## EGFRvIII+ CT-2A Glioma

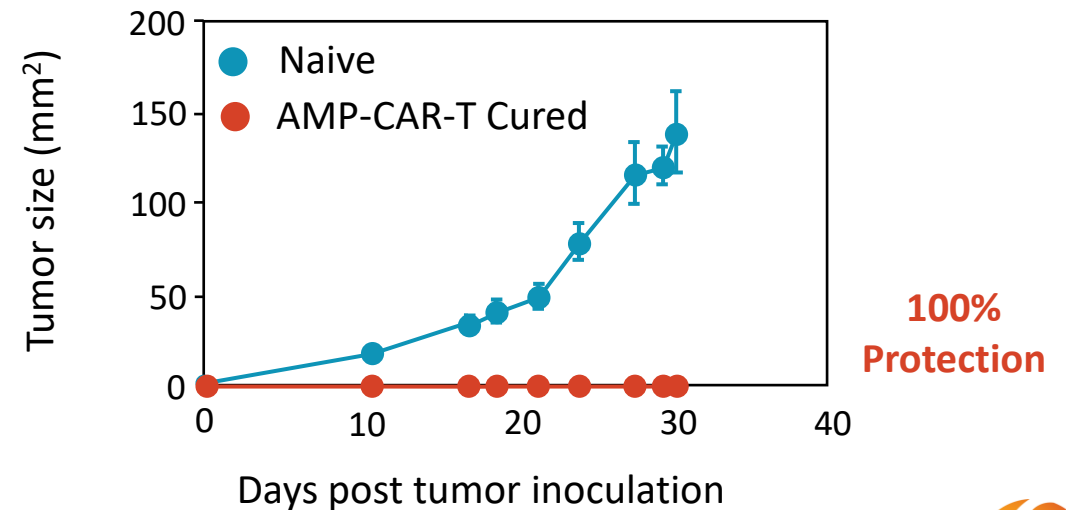
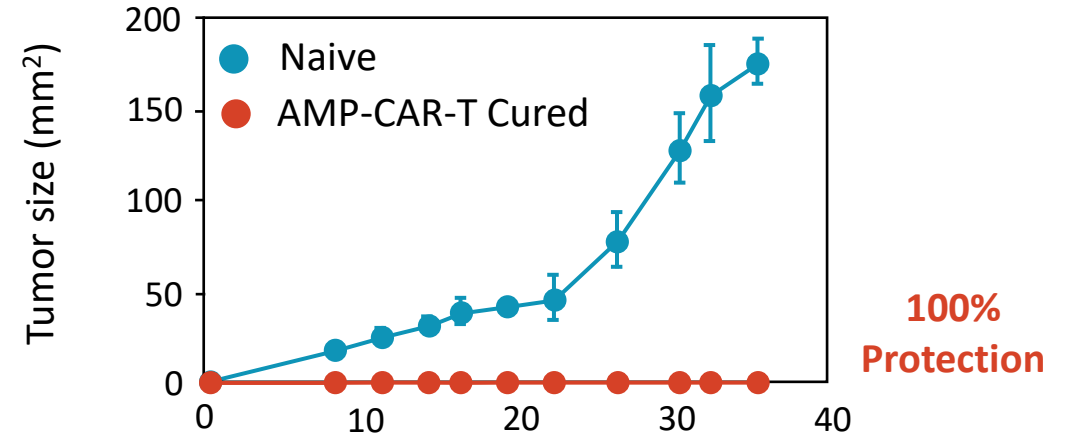


EGFRvIII-specific CAR-T Cells

**AMP-Boost:**  
AMP-EGFRvIII  
CDN

Challenged with  
EGFRvIII-expressing  
tumor  
↓  
Mediated by Memory  
EGFRvIII-CAR-T Response

Challenged with  
EGFRvIII-negative tumor  
↓  
Mediated by  
Endogenous Memory T  
cell Response



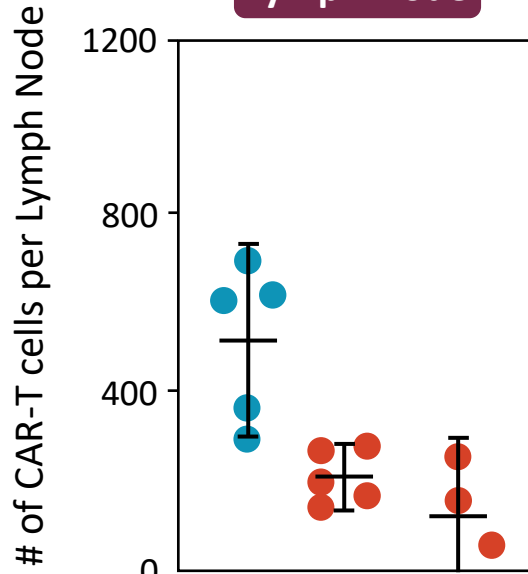


# Lymph Node APC Co-stimulation Drives CAR T Cell Expansion and Effector Function Enhancements

## APC Depletion

### CAR-T Expansion:

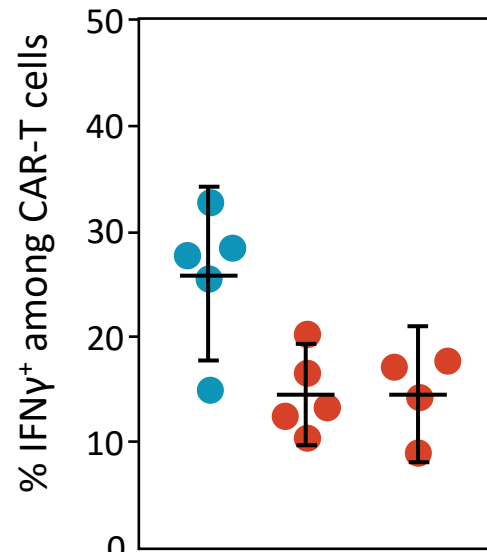
#### Lymph Node



CART	+	+	+
AMP	+	+	+
Depletion	-	Mφ	DC

### CAR-T Function:

#### Lymph Node

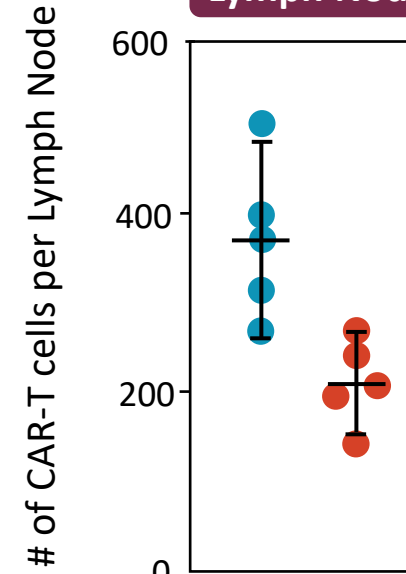


CART	+	+	+
AMP	+	+	+
Depletion	-	Mφ	DC

## Co-stimulatory Signal Blockade

### CAR-T Expansion:

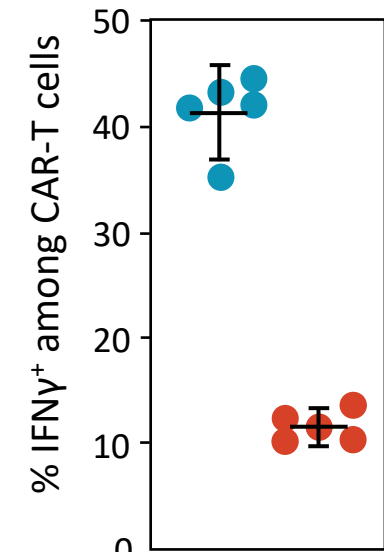
#### Lymph Node



CART	+	+
AMP	+	+
Blockade	-	Costim

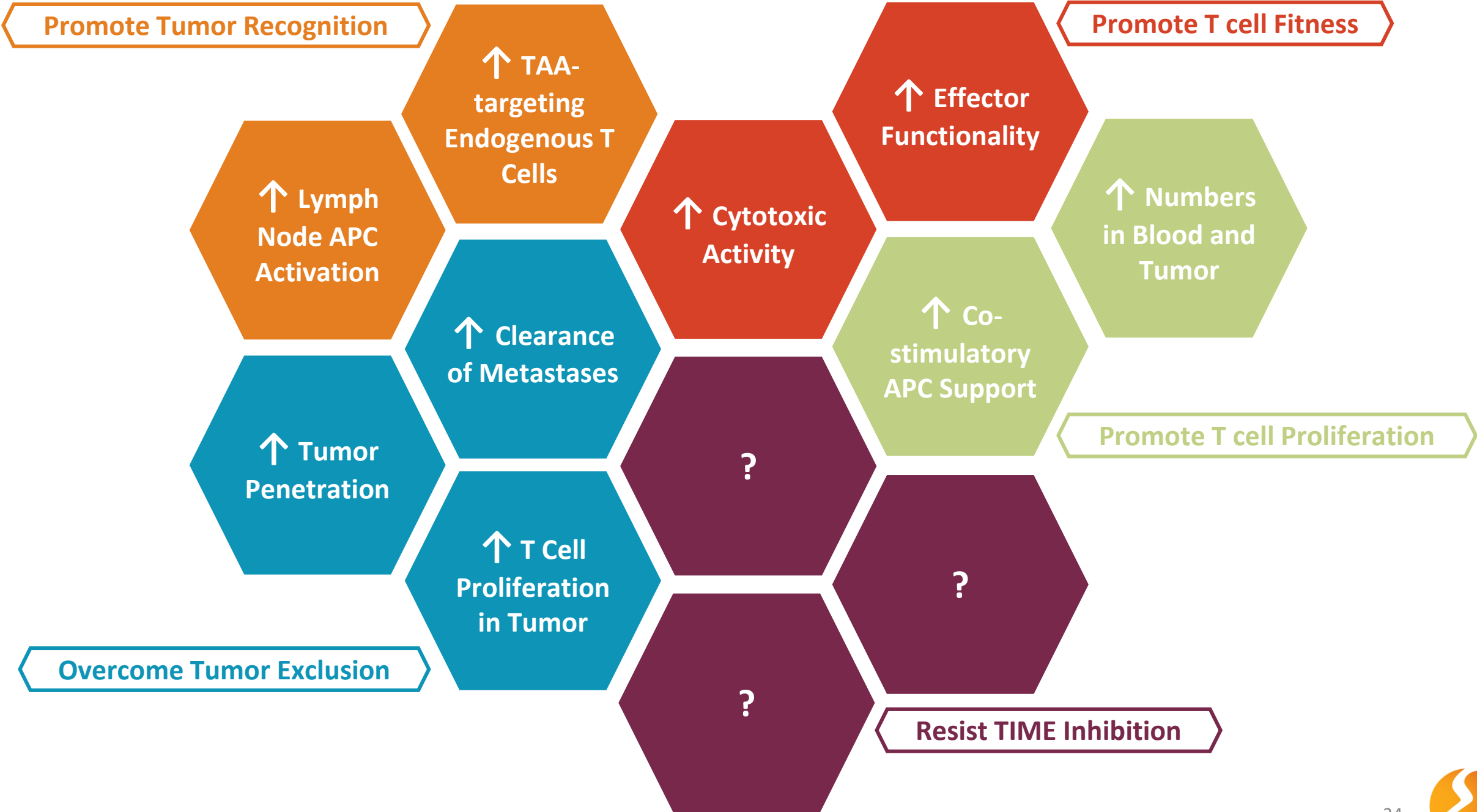
### CAR-T Function:

#### Lymph Node

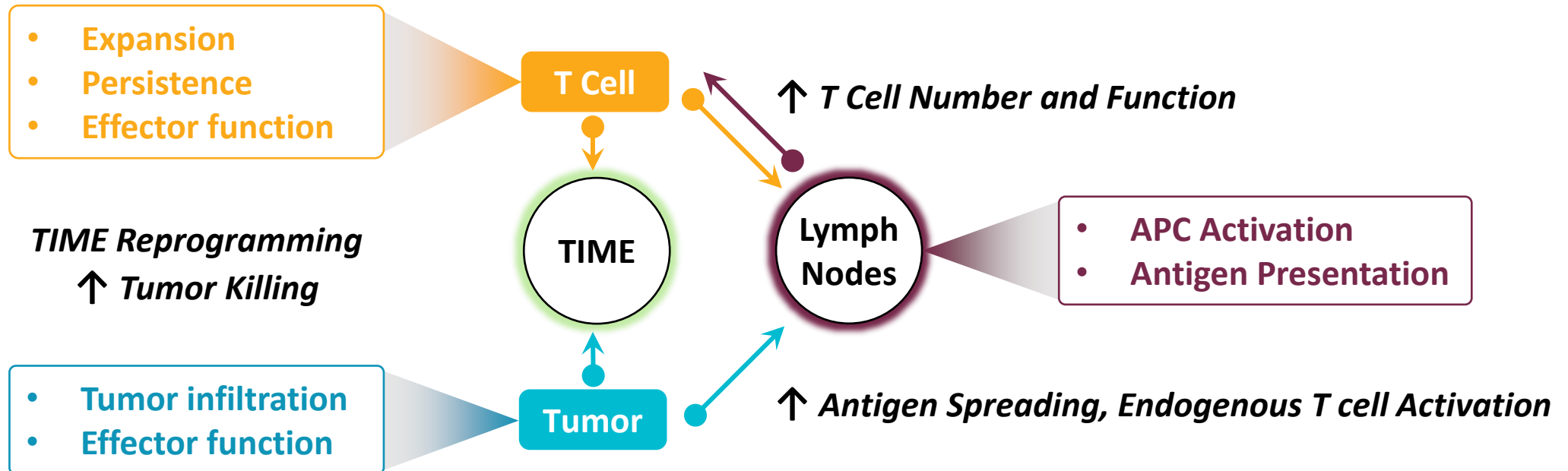


CART	+	+
AMP	+	+
Blockade	-	Costim





# What lessons can we learn? How should this inform our strategy?





**Dylan Drakes PhD, Abdul Abbas MS, Jackie Shields, Chris Haqq MD PhD**



**Darrell Irvine PhD, Leyuan Ma PhD**

