# An all-in-one adjuvanted therapeutic cancer vaccine approach targeting dendritic cells to induce potent tumor killing cellular immune responses

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# A Novel Built-in Adjuvanted Vaccine Platform Inducing Robust Cross Presentation

## X-presentation required:

# Vaccines that should induce stronger cellular immunity

**Tuberculosis**
**Viral infections**
**Cancer**
**Therapeutic vaccines**

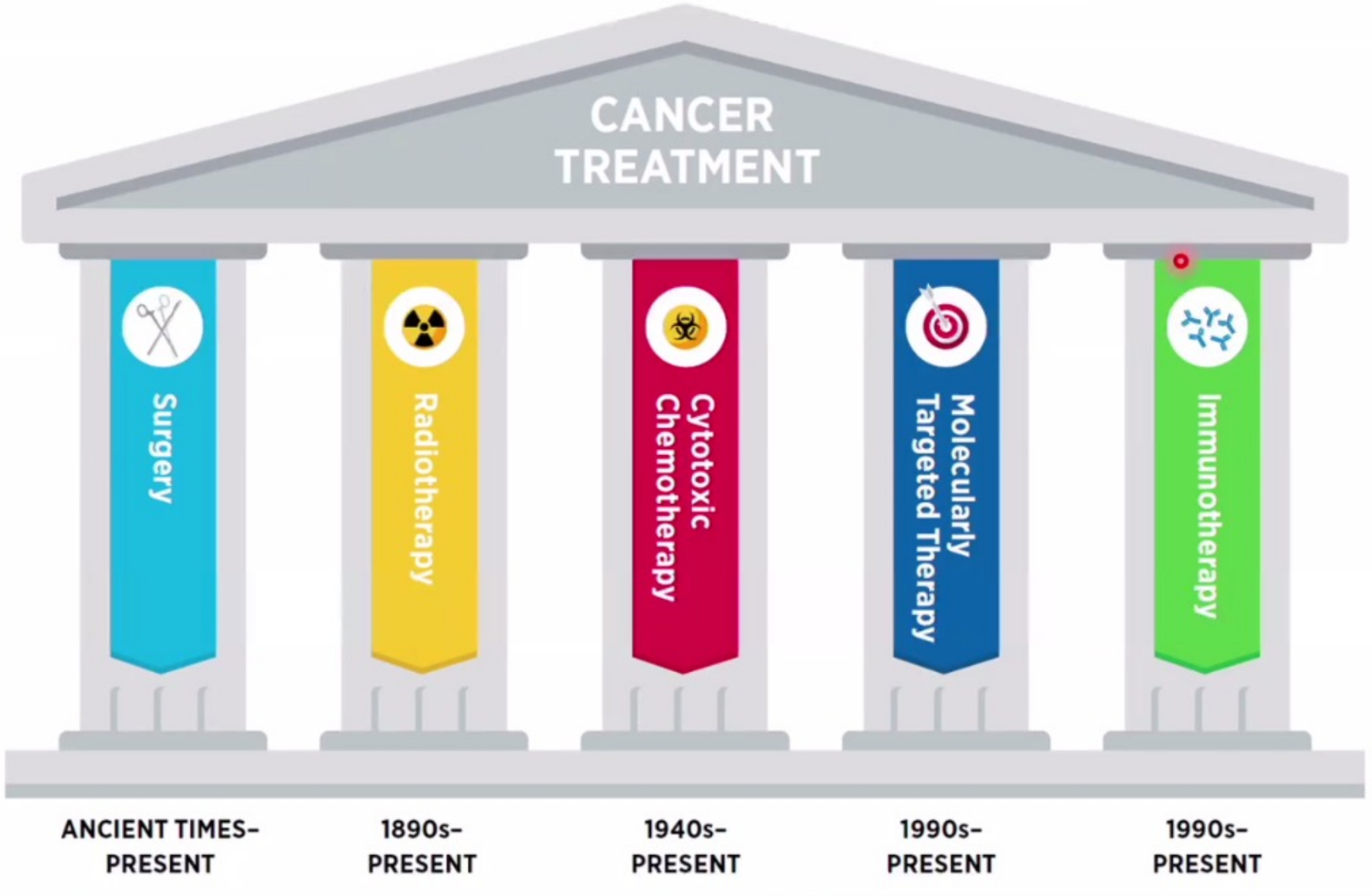
# TCV: ALL-in-ONE Vaccine Platform


D: DC-Targeting/Internalization
X: Antigen
F: Flagellin Adjuvant

**Engineered vaccine: get THREE birds with ONE stone?**

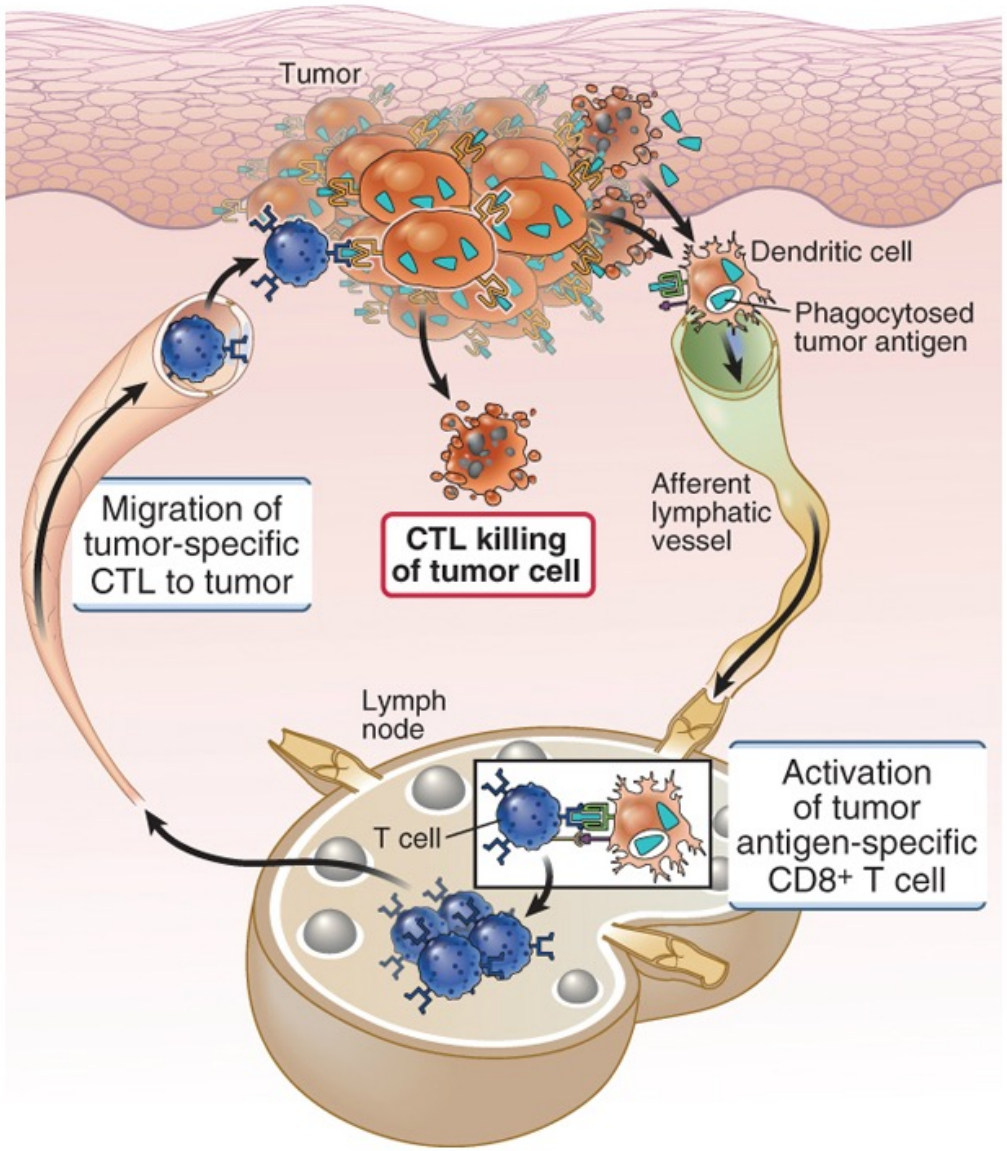
1. APC Targeting/Activation
2. Cross presentation
3. Built-in adjuvanted vaccine

# Era of Cancer Immunotherapy: One of the Pillars for Cancer Treatment



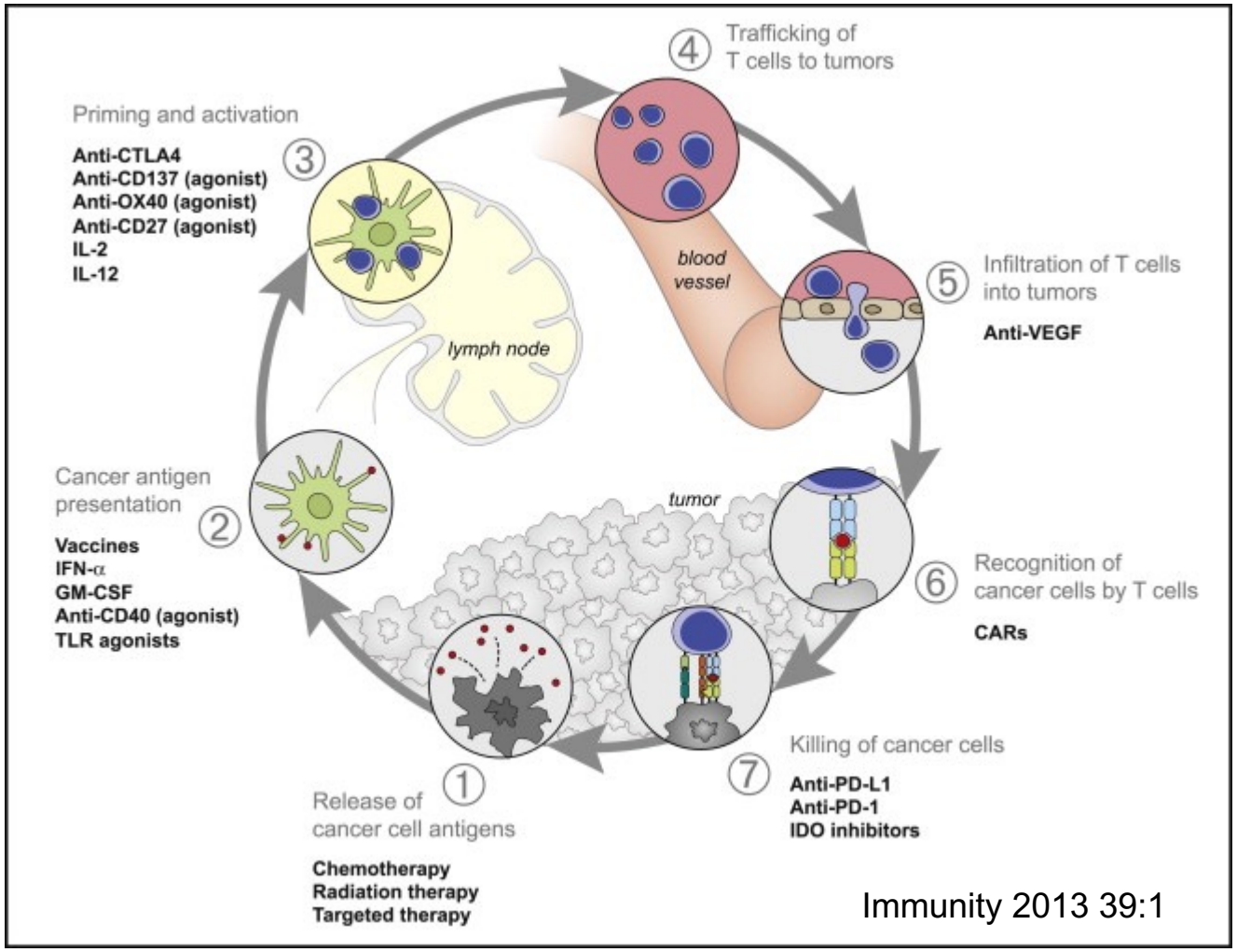

AACR Progress Report 2022

# Immune Responses against Tumors



# Tumor Immunotherapy

## Therapies that Might Affect the Cancer-Immunity Cycle




Immunity 2013 39:1

## Current and future of tumor immunotherapy

### 1. Immunomodulators

- ICI
- Co-stimulating agonists
- Cytokines
- Inhibitors of immunosuppressors (Treg cells and MDSC etc.)

### 2. Immune cell therapy

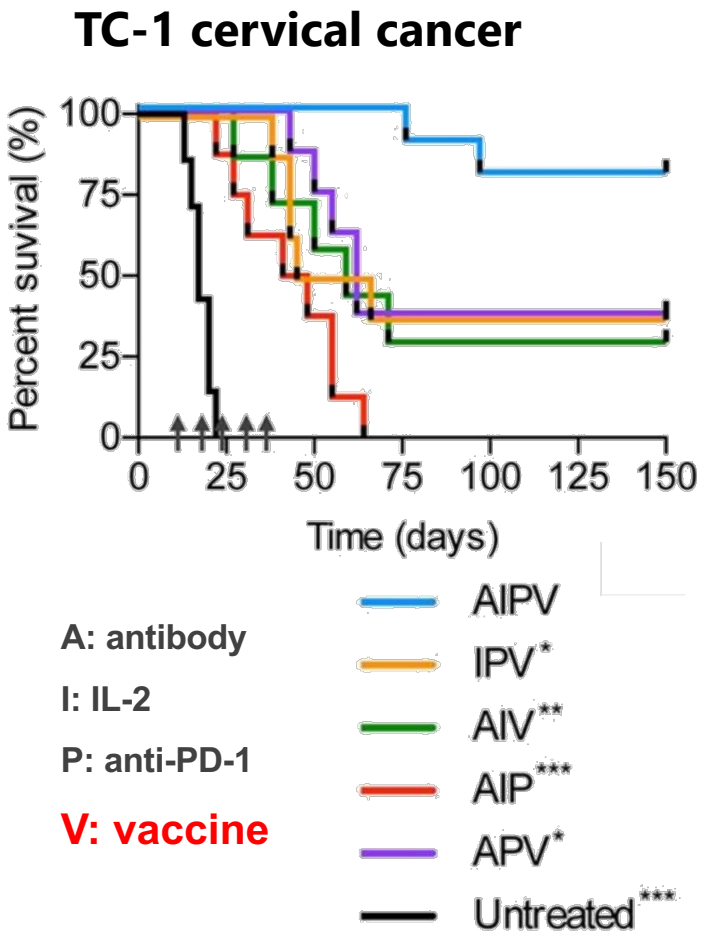
- CAR-T cells
- TCR-transduced cells
- NK cells

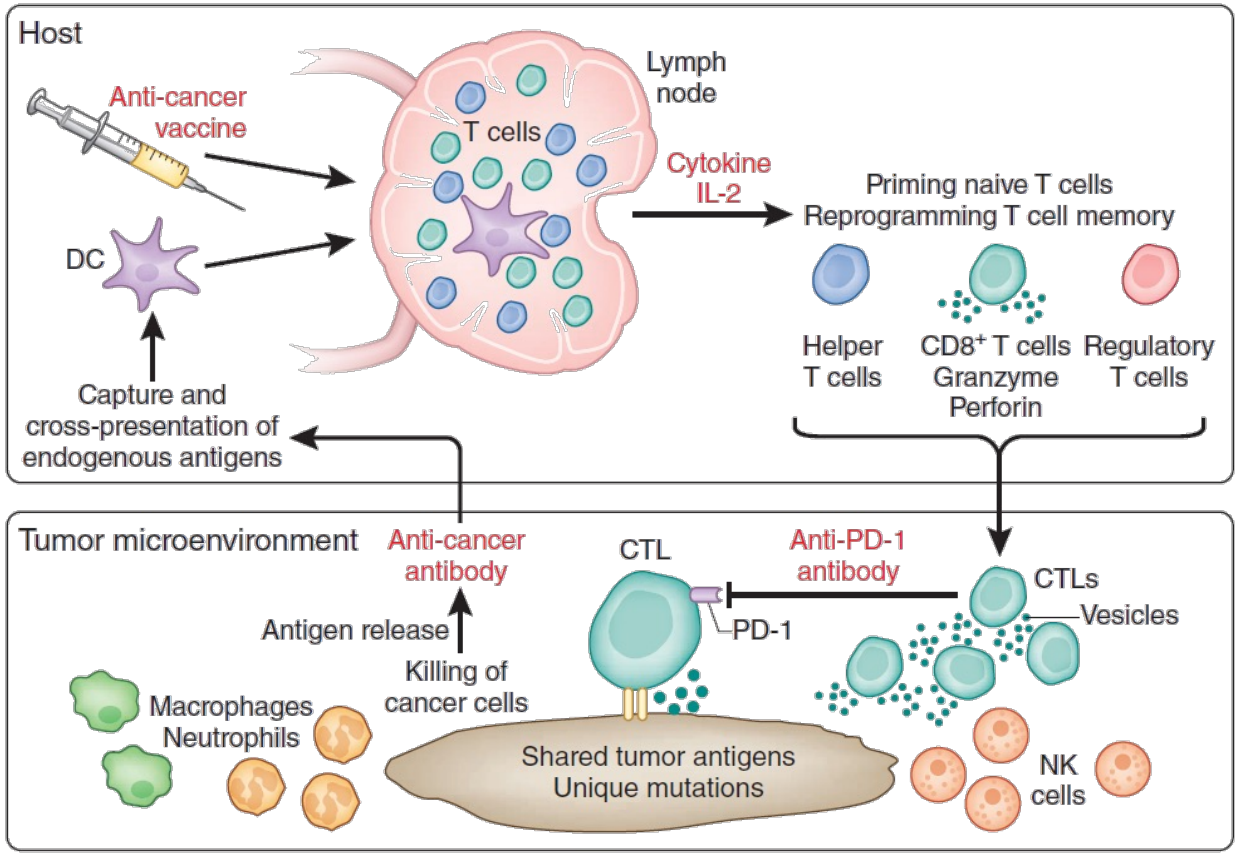
### 3. Cancer vaccine

- Neoepitope-based cancer vaccine

# Combination therapy to launch curative immunity: Cancer vaccine

## Cancer Combination Therapy – Two Keys for Success

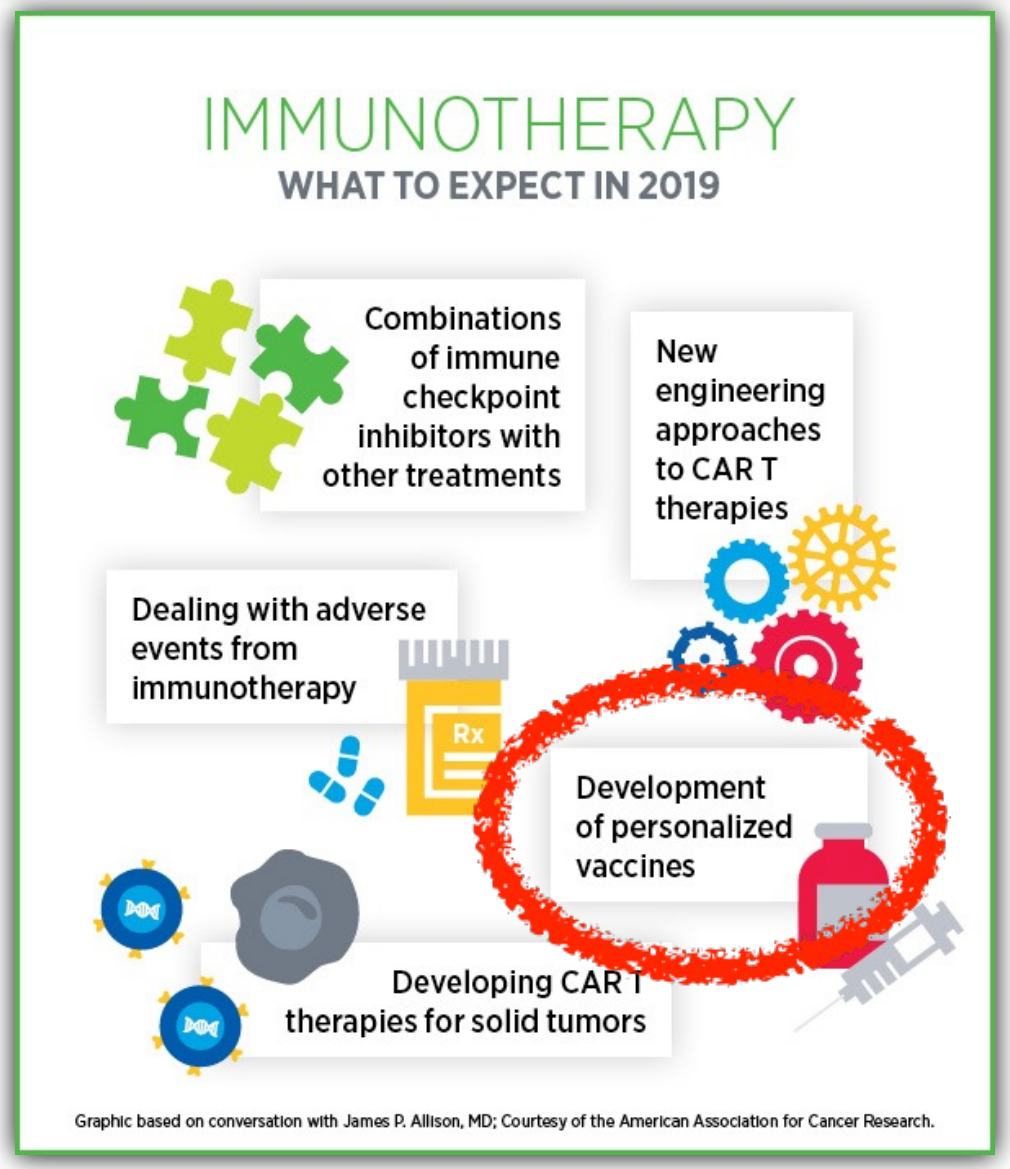


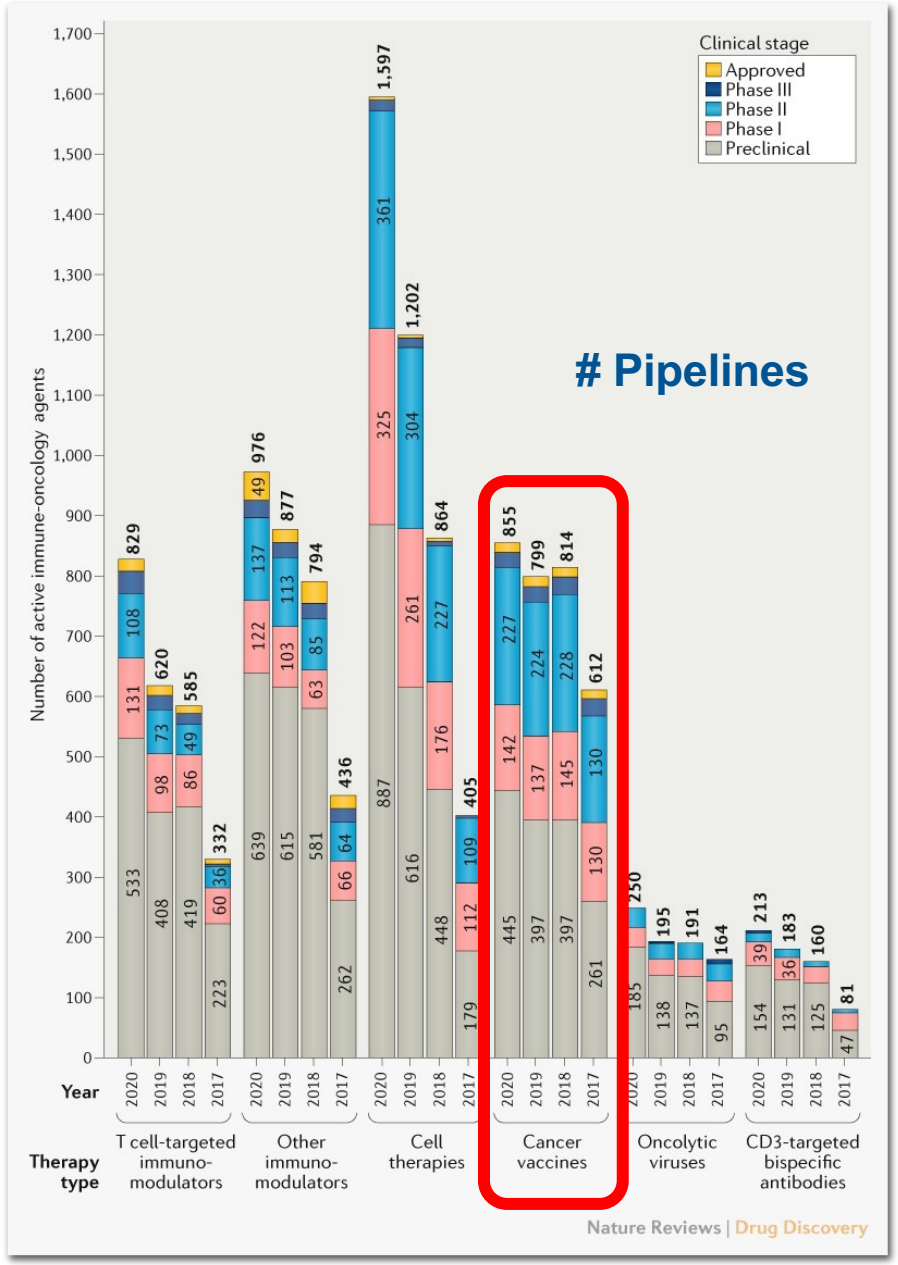

Nat Med 22; 1390, 2016

**1. Tumor-specific immune responses / 2. Tumor microenvironment modulation**

# Trends in Tumor Immunotherapy (2019, AACR)



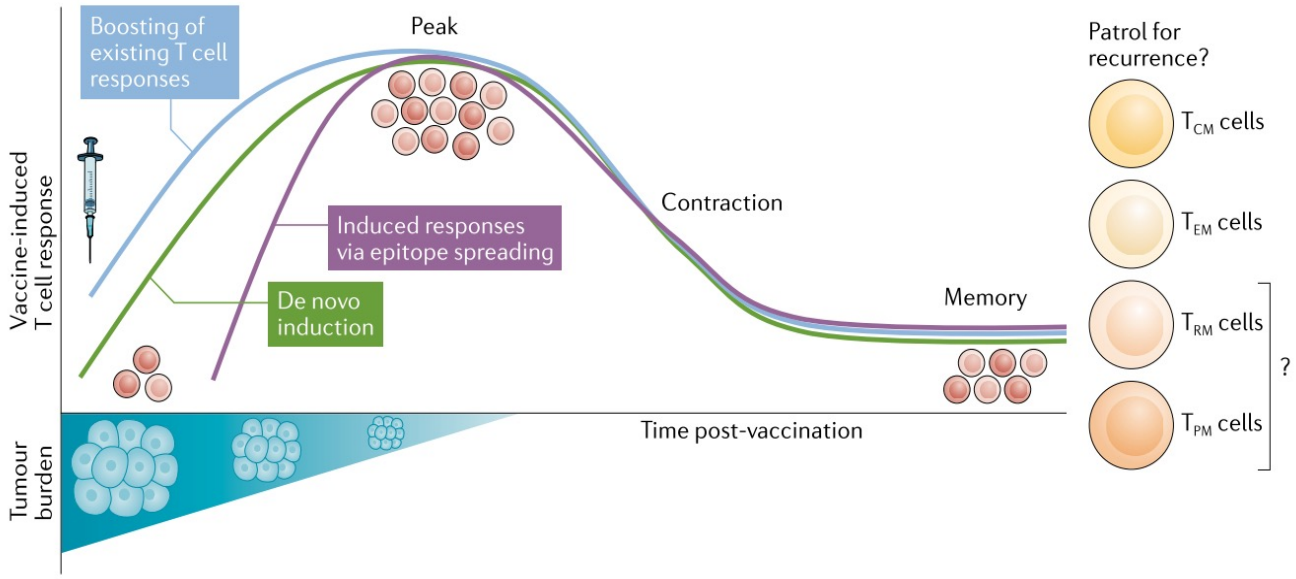
# Global I-O landscaping by modality




Nature Reviews Drug Discovery 2020 September

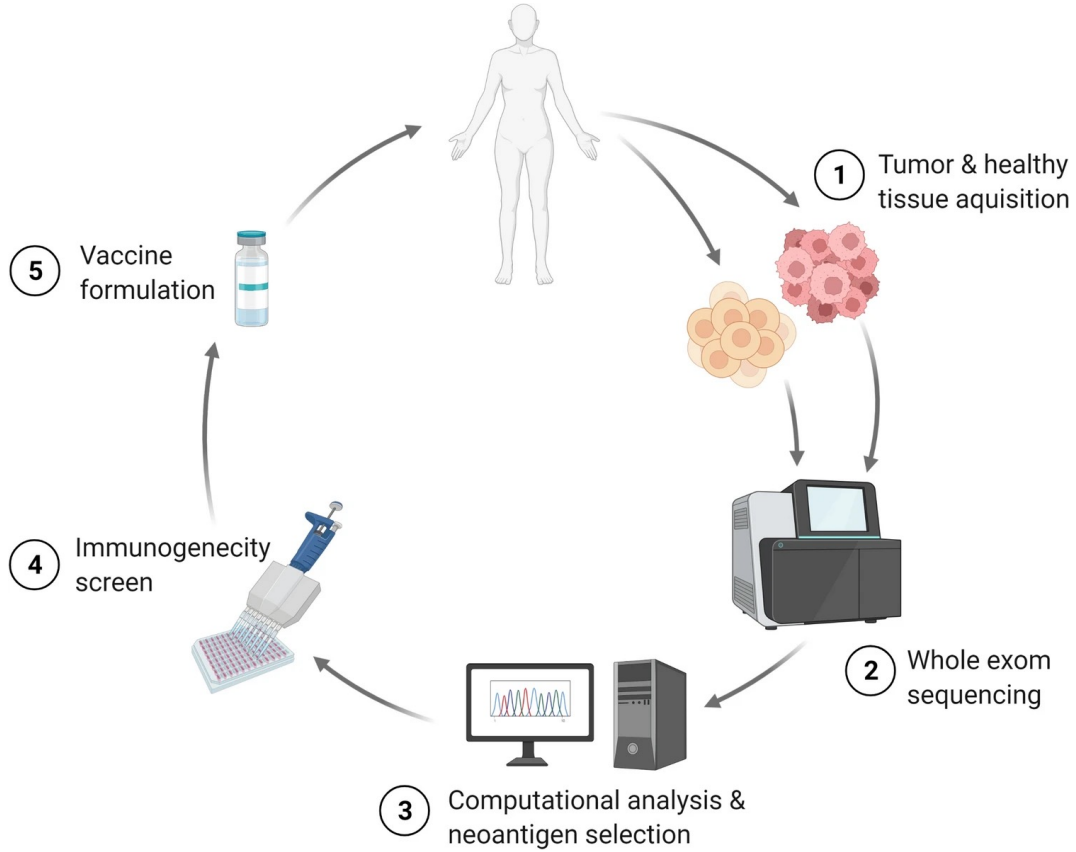
# Therapeutic Cancer Vaccine (TCV)

## Potential to induce long-lasting tumor-specific memory T cell populations



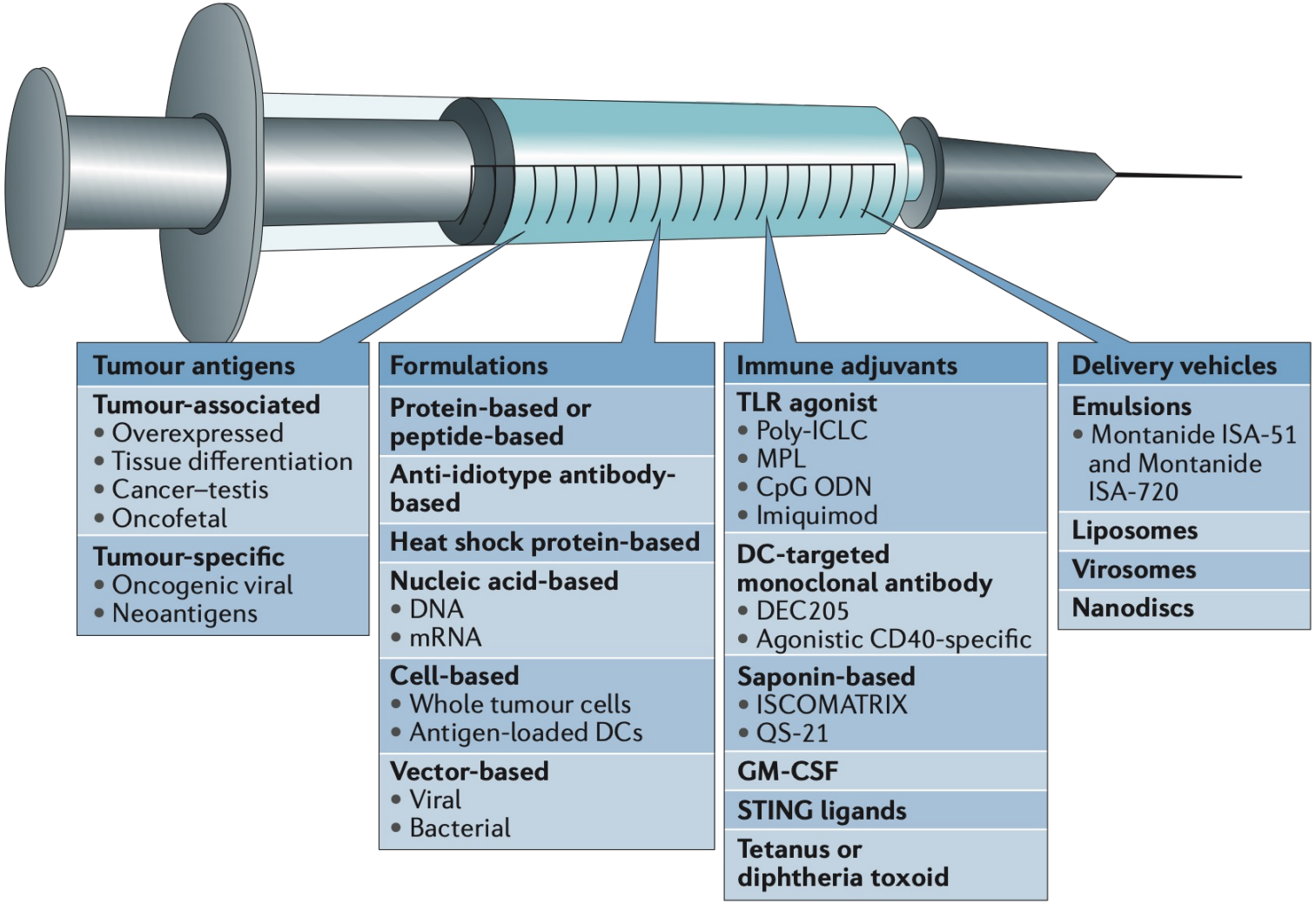

Nat Rev Cancer 2021; 21(6):360

## Neoantigen-based personalized cancer vaccine




Discover Oncology (2022) 13:31

# Four key components of cancer vaccines

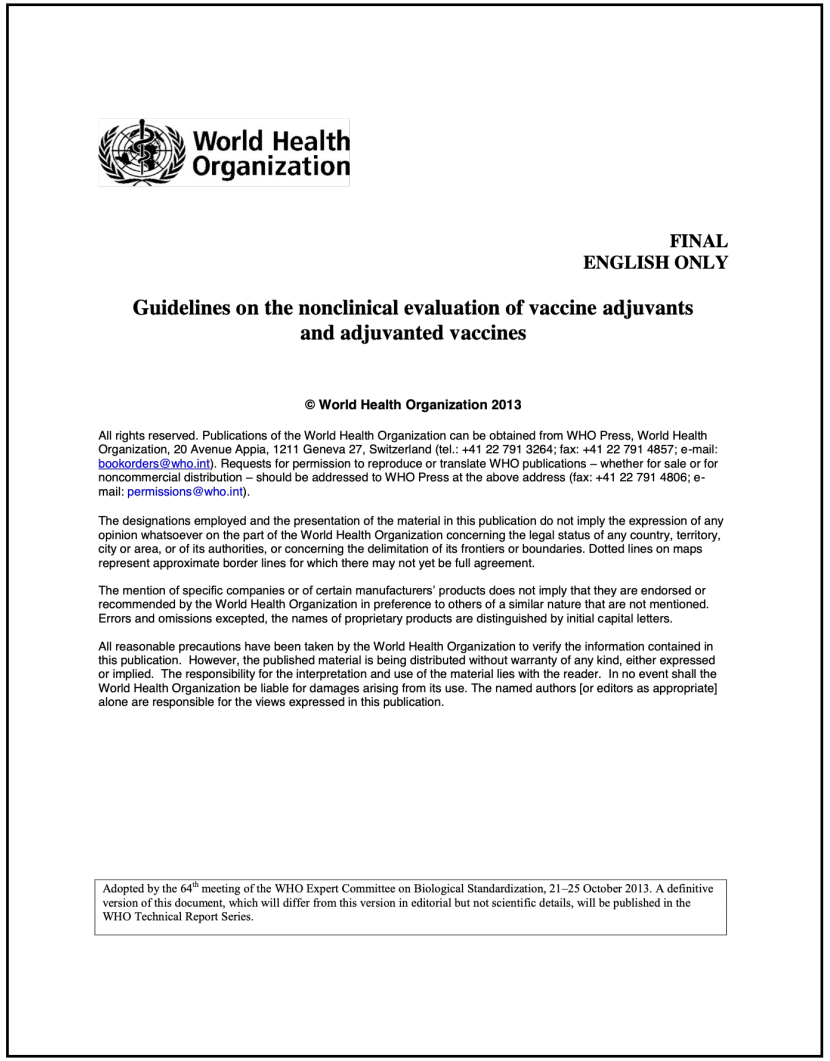



Nature Reviews Immunology. 2018 Mar;18(3):168

# 21c Vaccine and Adjuvant

## Classification of Adjuvants

- ✓**Mineral salts or gels**
  - – aluminum salts or calcium phosphate
- ✓**Oil-in-water and water-in-oil emulsions, amphiphilic molecules and surfactant-base formulations**
  - – MF59, QS-21, AS03, and Montanide
- ✓**Particulate adjuvants**
  - – liposomes, virosomes, DC Chol, ISCOMS, Iscomatrix, biopolymers such as PLGA, etc
- ✓**PAMPs (natural and synthetic)**
  - – low-toxicity LPS or lipid A (MPL, MPLA, OM-174), CpG, flagellin, nontoxic bacterial toxins (mLT and CTB), Poly IC, Poly ICLC, imiquimod/resiquimod (R837/R848), etc
- ✓**Endogenous human immunostimulators**
  - – cytokines (hGM-CSF and hIL-12) administered as proteins or as plasmid preparations
- ✓**Inert vehicles** – gold particles
- ✓**Adjuvants derived from inulin** – delta inulin (Advax)
- ✓**Combination adjuvants or adjuvant systems**
  - – AS01, AS03, AS04, AS15, glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE), CAF01, etc



World Health Organization

FINAL
ENGLISH ONLY

**Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines**

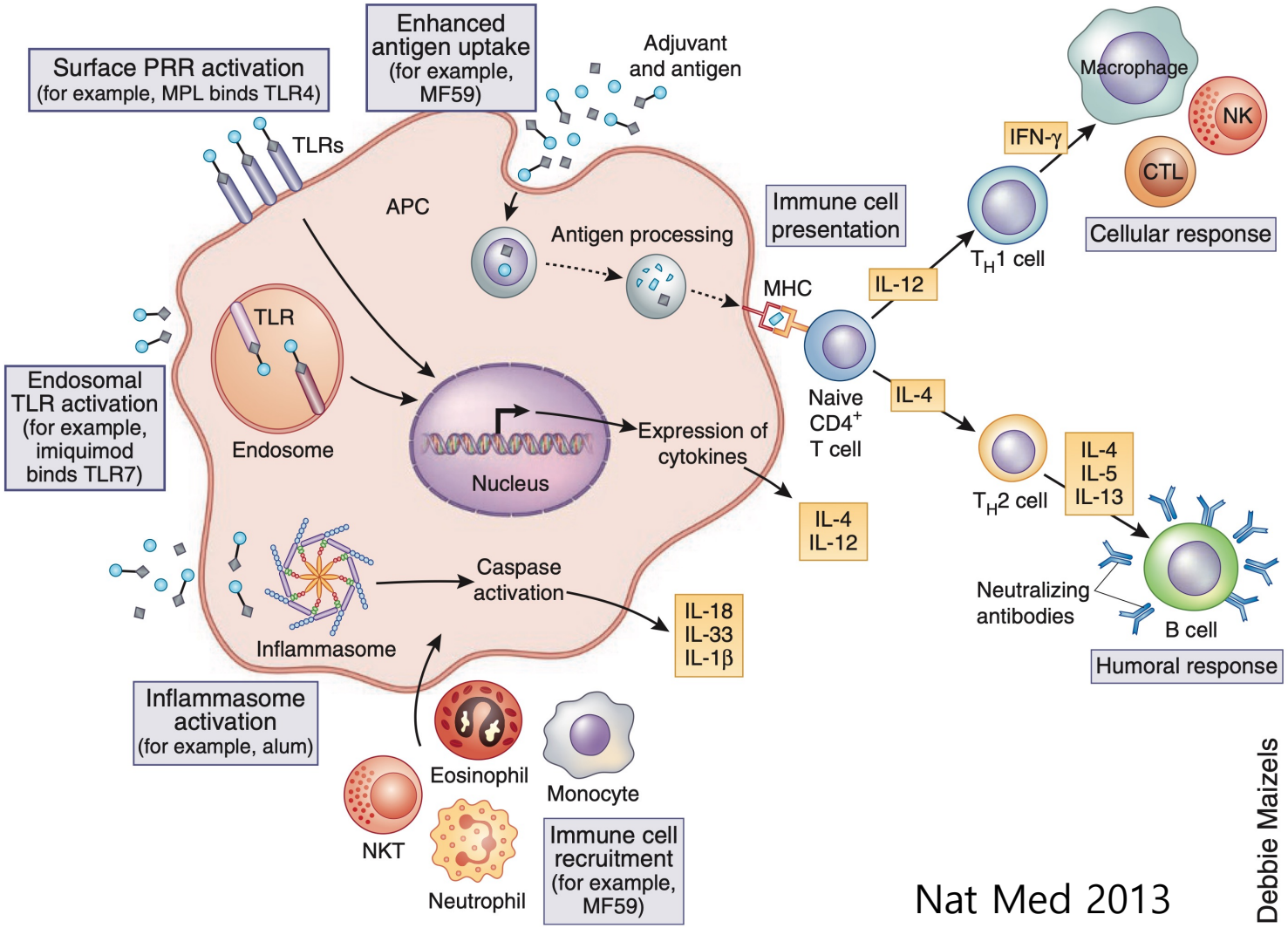


Adopted by the 64th meeting of the WHO Expert Committee on Biological Standardization, 21–25 October 2013. A definitive version of this document, which will differ from this version in editorial but not scientific details, will be published in the WHO Technical Report Series.

***WHO guideline, 2013***

# Adjuvant: putative mechanism of action and DC

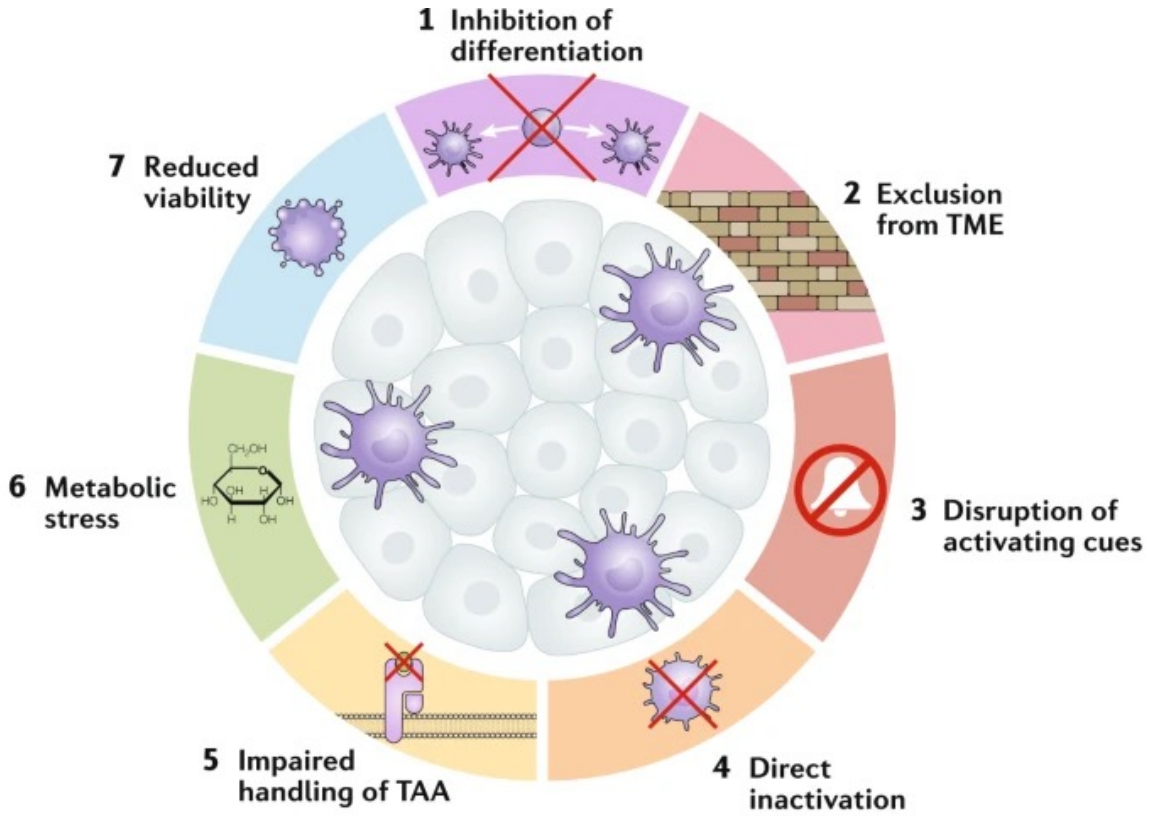
## APC: Antigen Presenting Cell

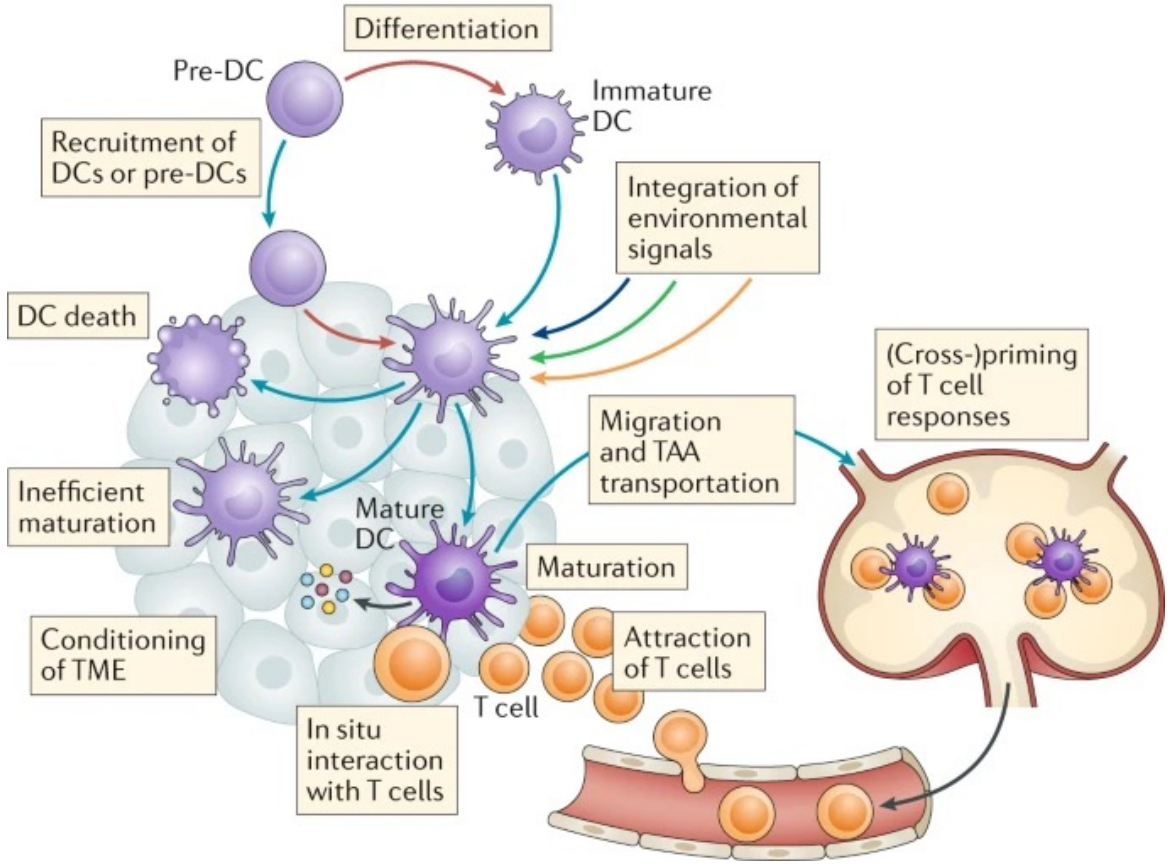



Nat Med 2013

Debbie Maizels

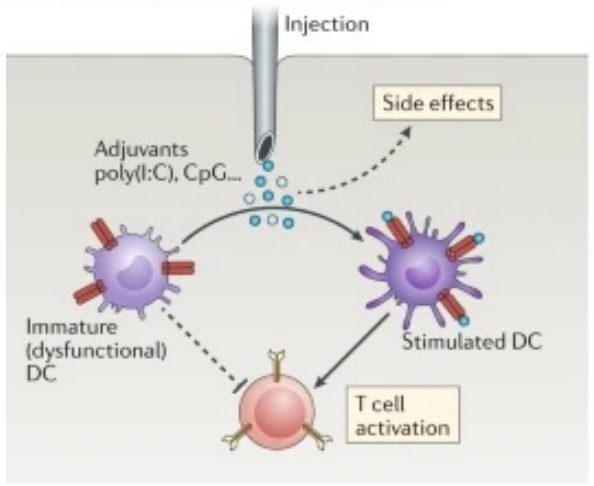
# DC: Impaired in tumor patients



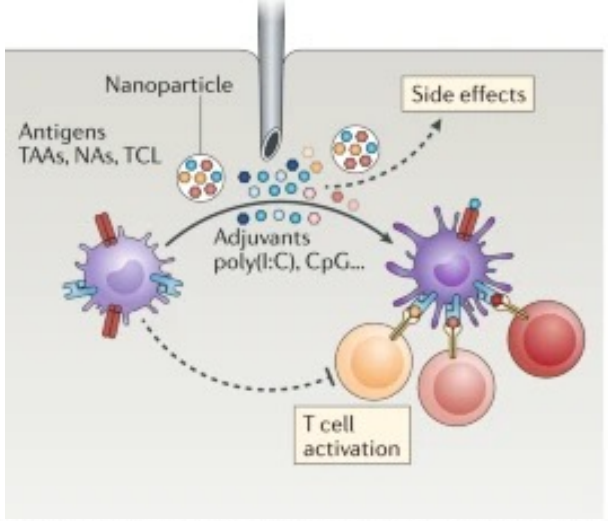



# Therapeutic approaches targeting DCs

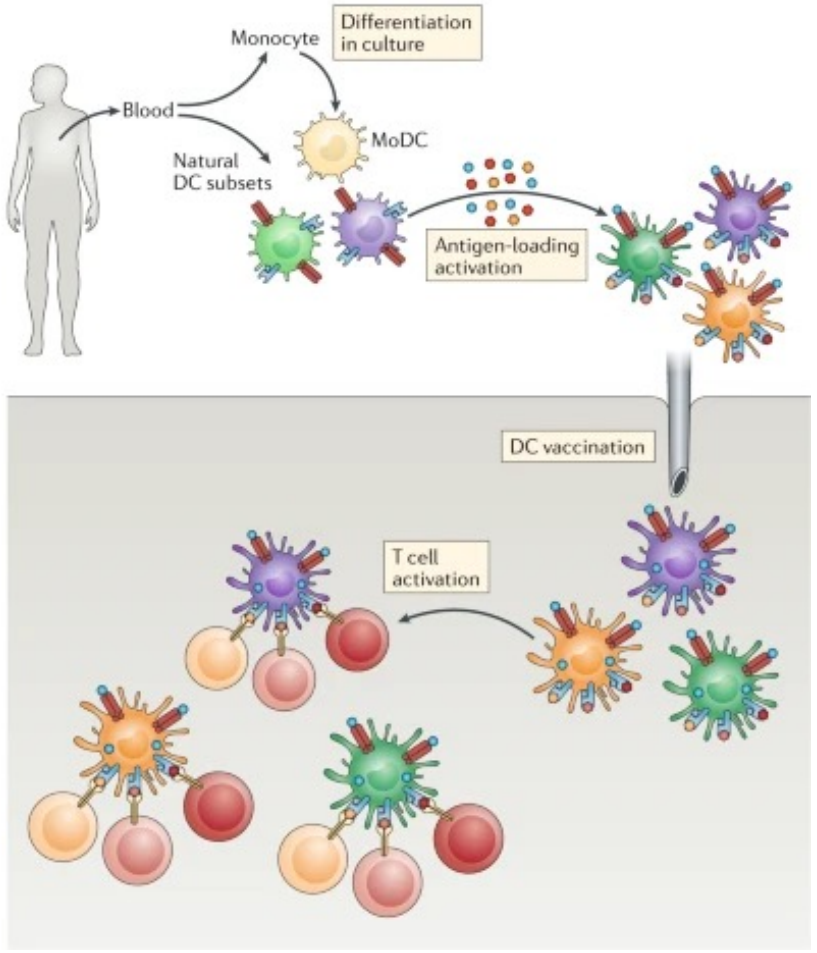
**Administration of DC-activating factor**

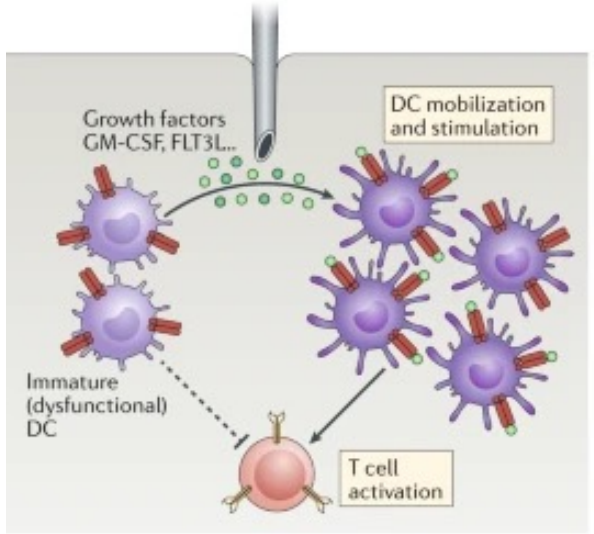


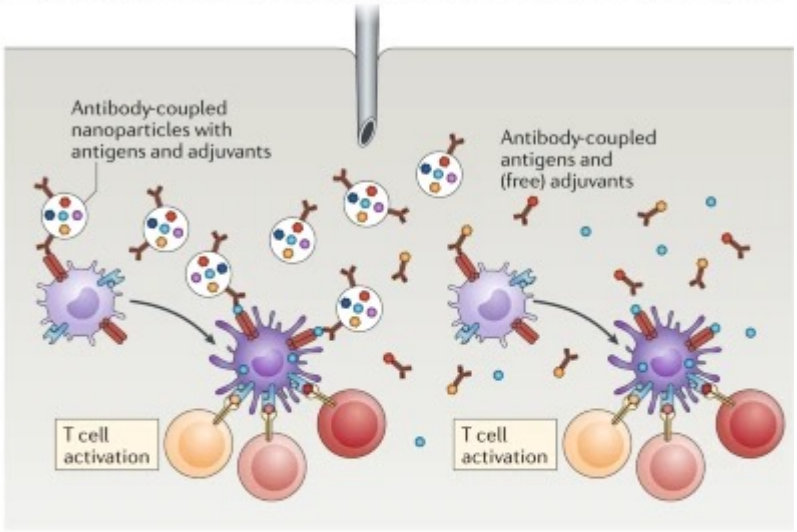

**Administration of antigens and adjuvants (carriers)**




**Adoptive transfer of autologous, antigen-loaded and activated DCs**



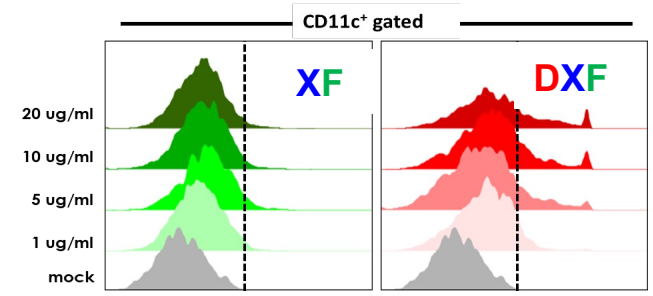

**Administration of DC-mobilizing agents**

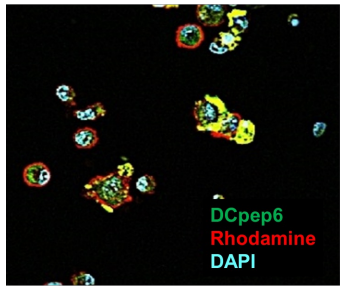
**Using DC (subset)-specific Abs to deliver Ag/Adjuvant or nanoparticles**

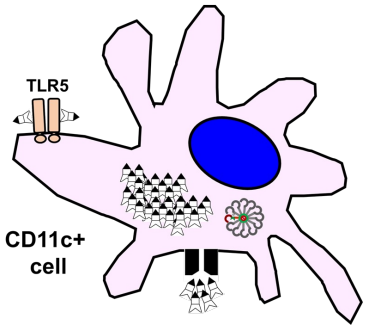
# STRATEGY: All-in-One vaccine

D X F

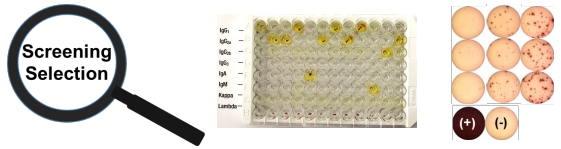
## D: DC-targeting / internalization / carry cargo / upgradable

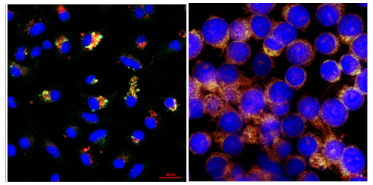


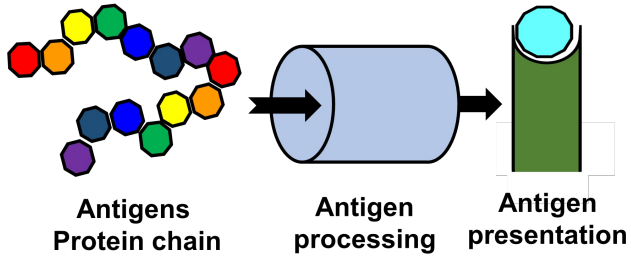



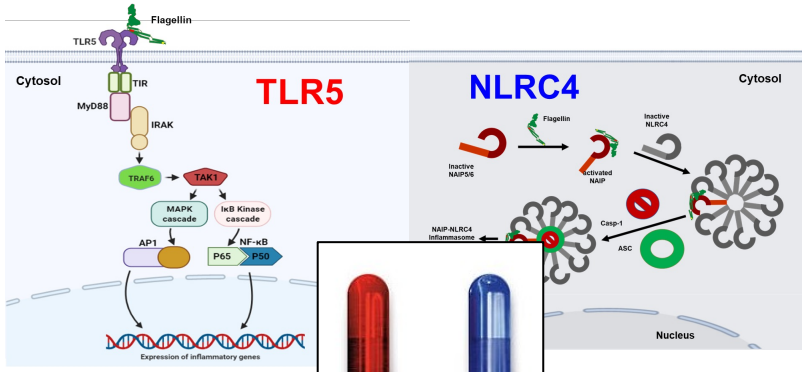

## X: Antigen / Evaluation / Optimization / Antigen processing & presentation

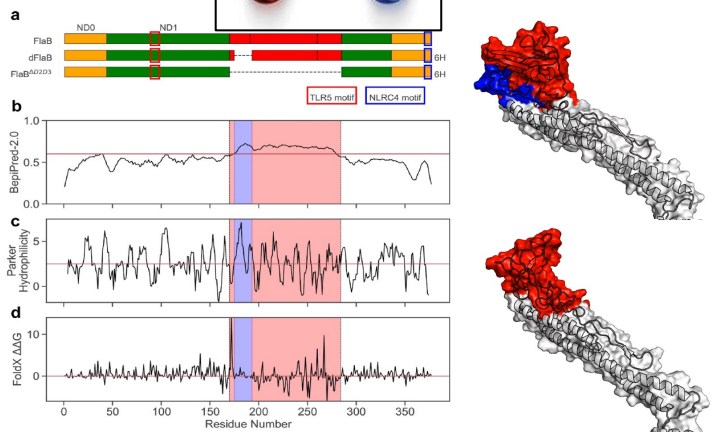




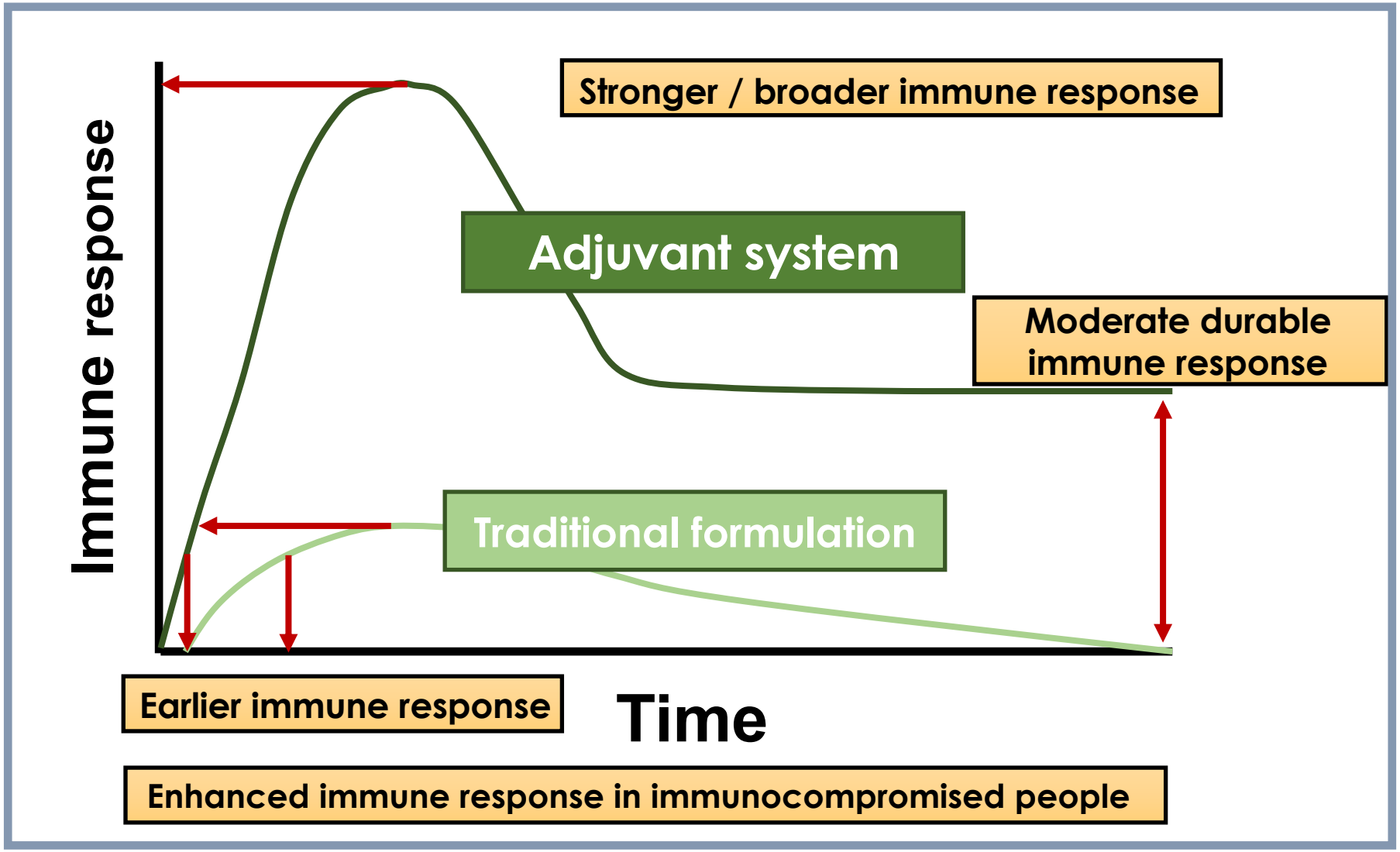

## F: Adjuvant PAMP signaling / TLR5 and/or NLRC4 / engineerable




**Platform Technology / Ready-to-Use / Versatile Application / Addressing Unmet Needs**

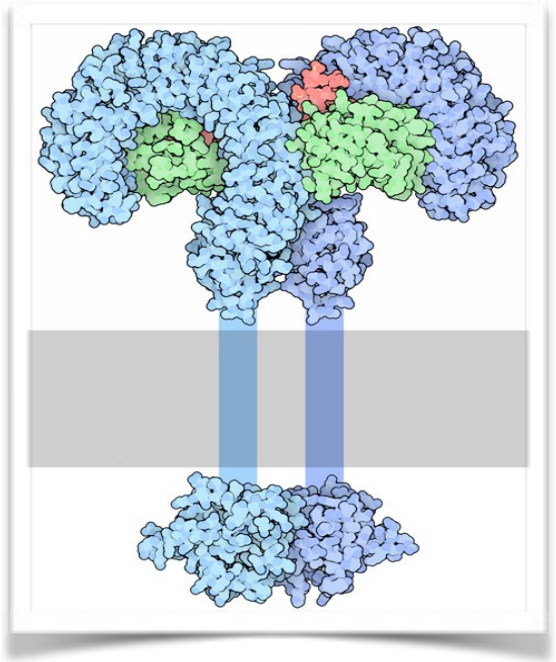
# Adjuvant paradigm

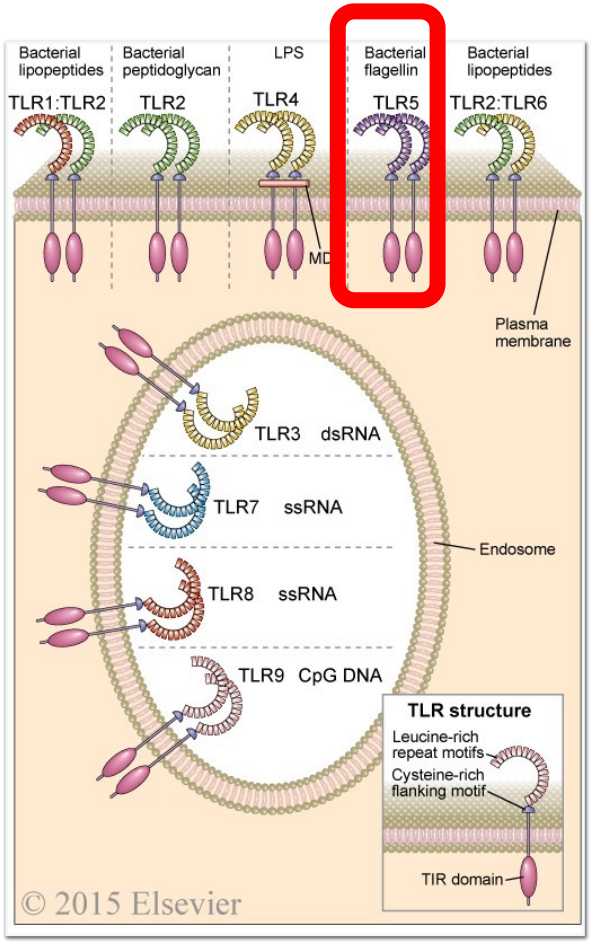


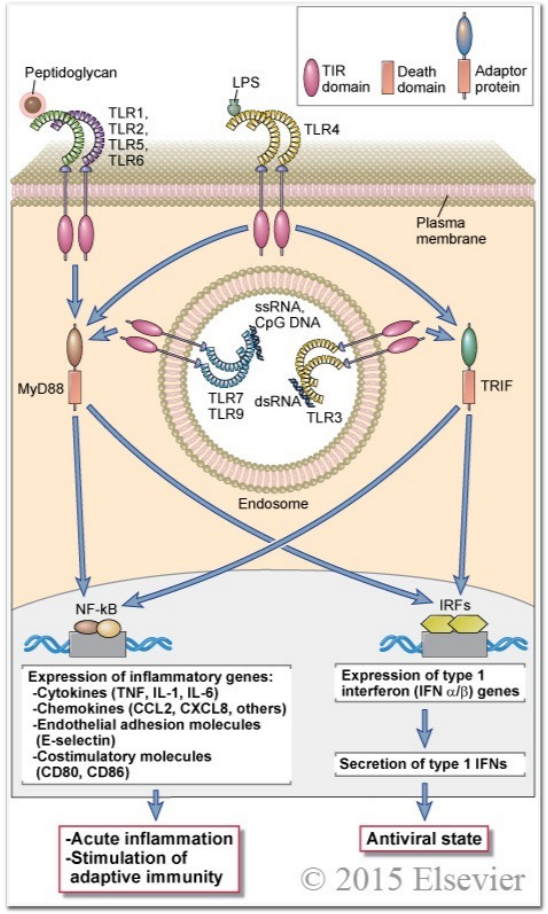

Tom-Heineman, GSK

# TLR ligands: immune modulators

## TLR signaling serves linker between innate and adaptive immunity

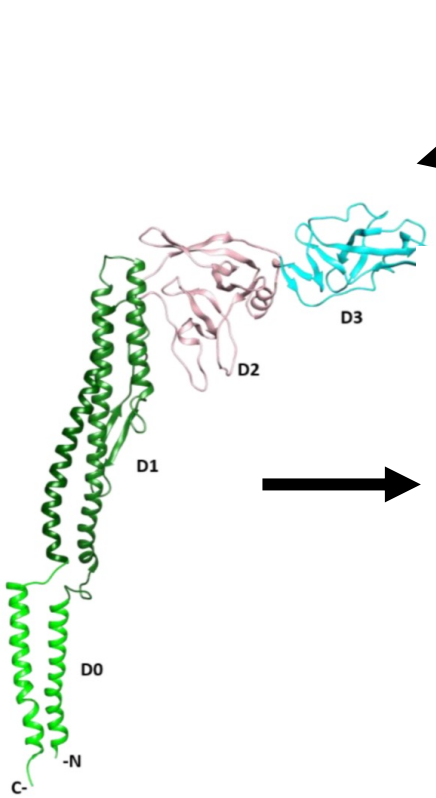


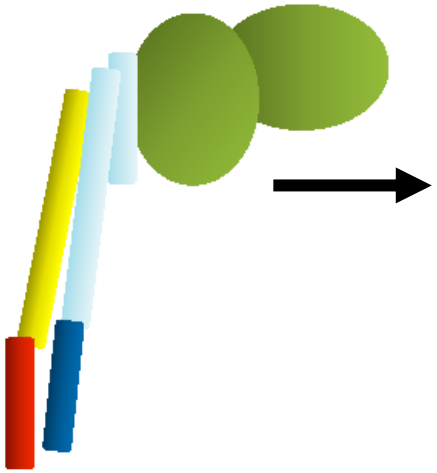


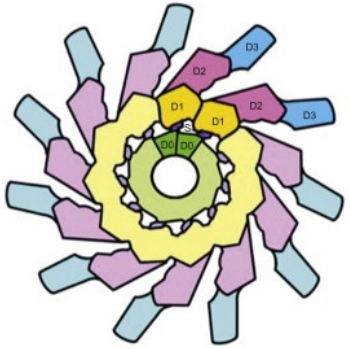



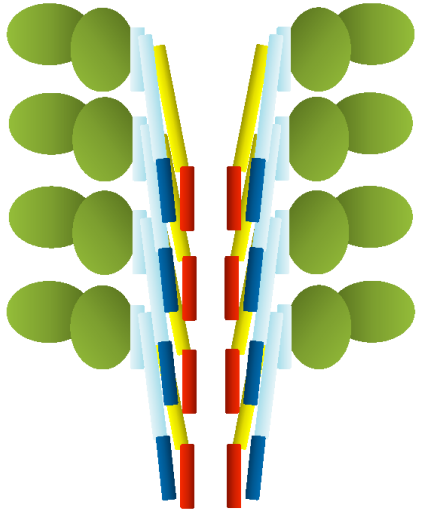

**TLR ligands are considered as an attractive adjuvant candidate in vaccine development**

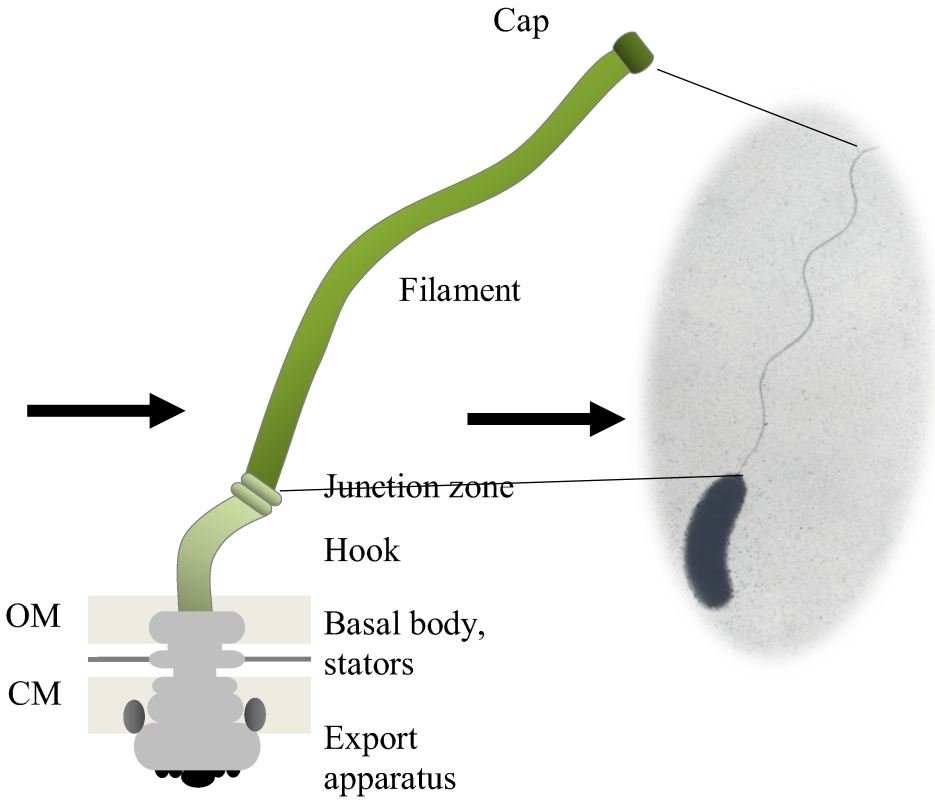
# Flagella & Flagellin



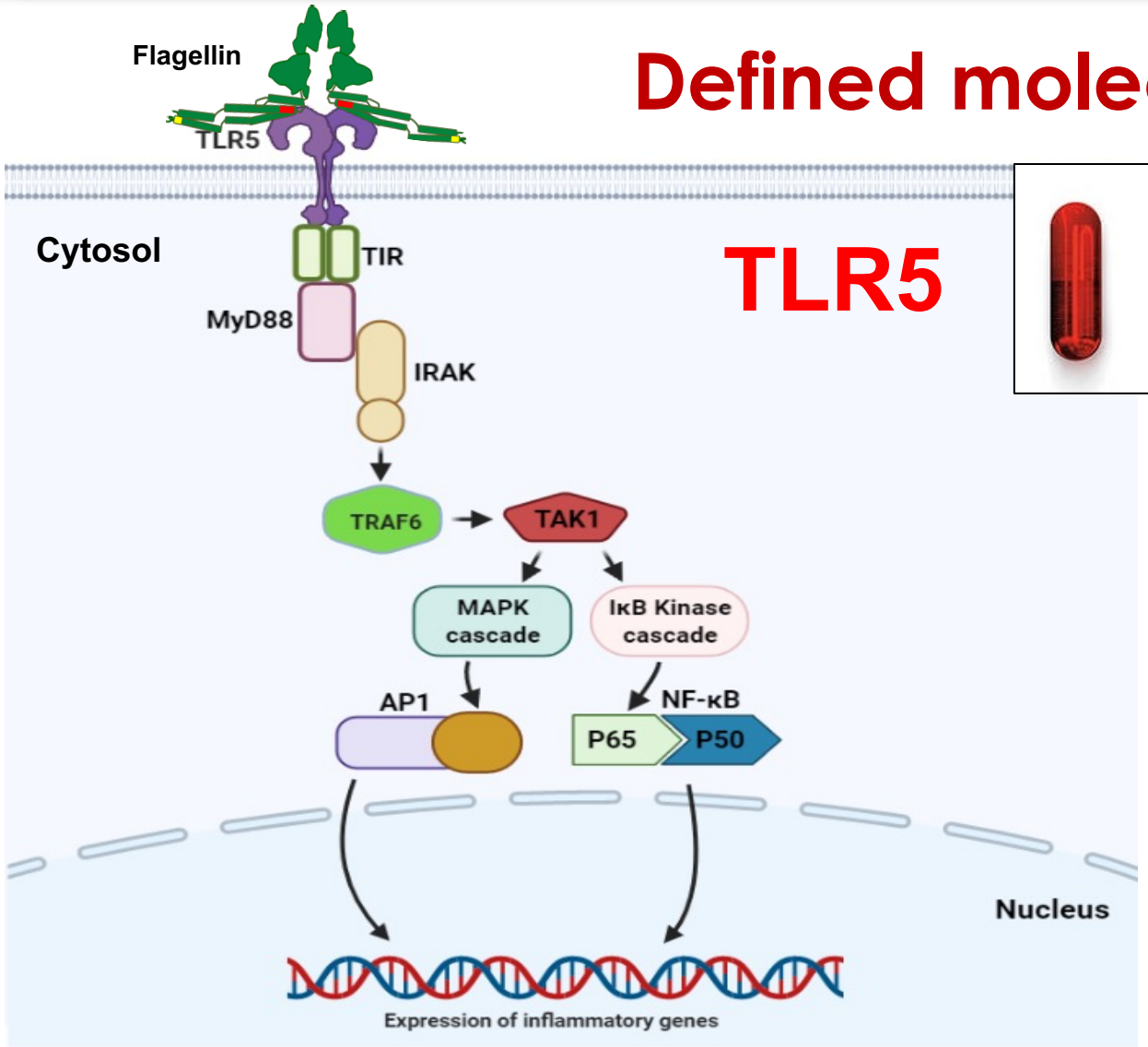





# Flagellin Adjuvant: Uniqueness

D X F

## Defined molecular targets




TLR5

NLRC4

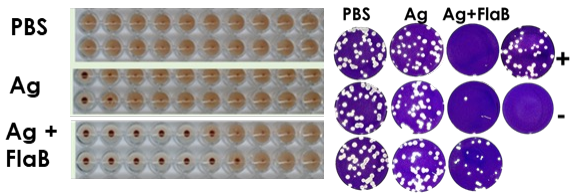


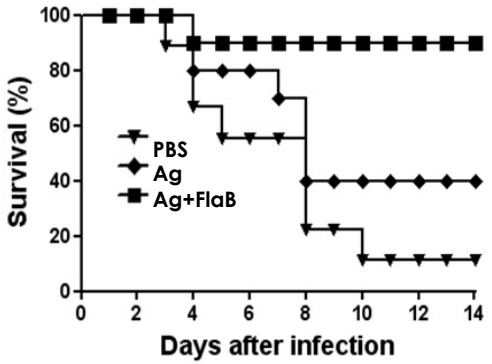

**Flagellin: ligand for both TLR5 and cytosolic PRR (NLRC4)**

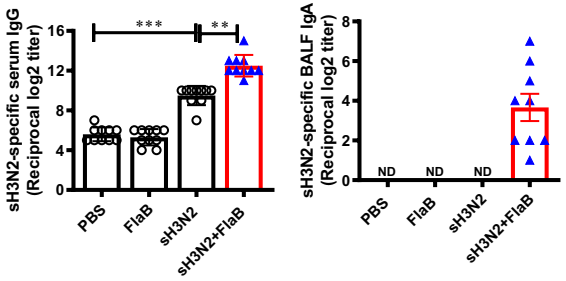
# Our Previous Studies: Flagellin Adjuvant (Efficacy)

D X F

## Mucosal adjuvant

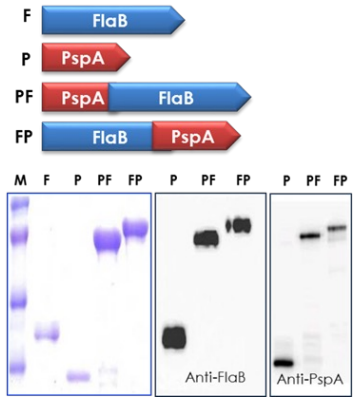


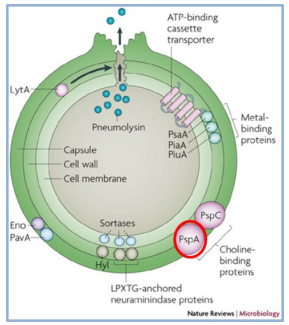



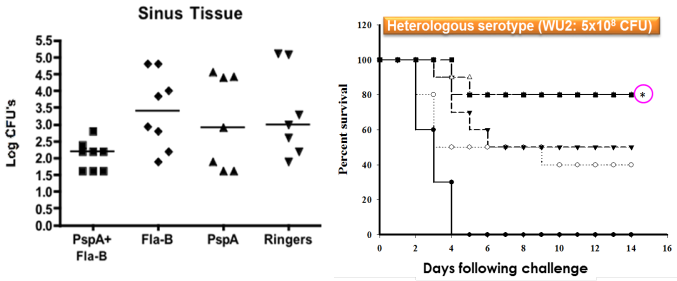

*Lee SE et. al. Infect. Immun. (2006)*
*Verma V et. al. J. Transl. Med. (2016)*
*Hwang HS et. al. Human Vaccin. Immunother. (2018)*
*Puth S et. al. Human Vaccin. Immunother. (2017)*

## Built-in adjuvant

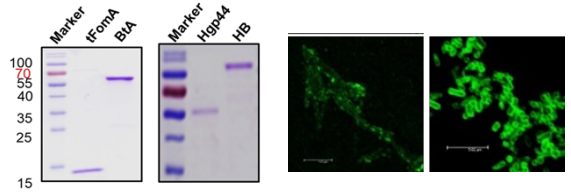




*By David E. Briles of UAB*

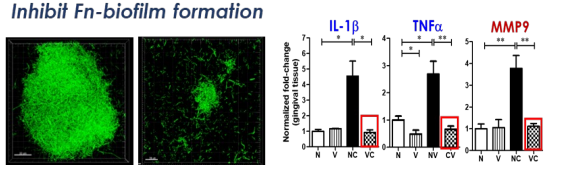



*Nguyen CT et. al. Vaccine (2011)*
*Lim JS et. al. Aging Cell (2015)*
*Lee SE et. al. Clin Exp Vaccin Res 92015)*
*Puth S et. al. Mucosal Immunol. (2019)*
*Puth S et.al. Biomaterials (2022)*

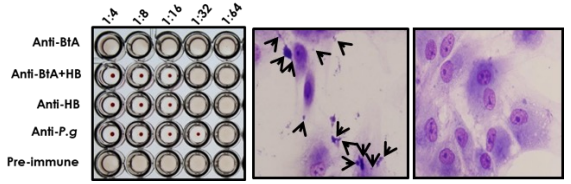
## Multivalent vaccine for dysbiotic disease



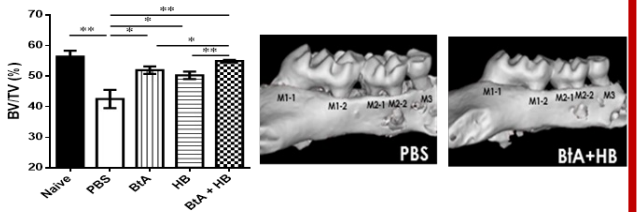

*Inhibit Fn-biofilm formation*




*Inhibit Host-Pg interaction: HA / Adhesion*

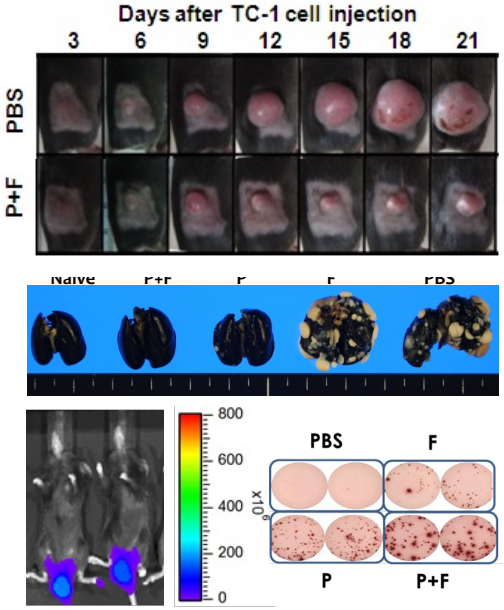



*Inhibit Fn-Pg-induced alveolar bone loss*

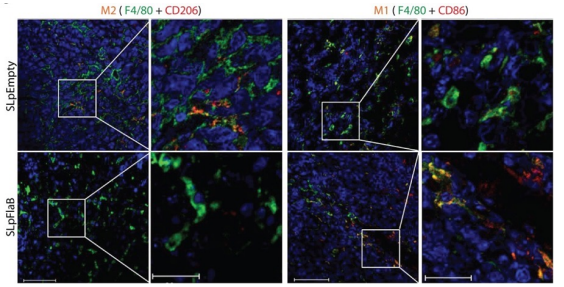



*Puth S et. al. Mucosal Immunol. (2019)*
*Puth S et. al. Human Vaccin. Immunther. (2017)*
Jeong K et. al. Int. J. oral Biol. (2017)

## Cancer vaccine



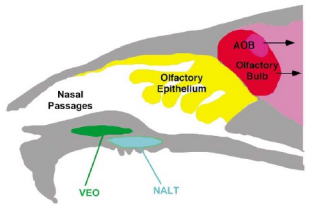

## TME modulator




*Nguyen CT et. al. Vaccine (2013)*
*Lee SE et. al. OncoImmunol. (2016)*
*Zheng JH et. al. Sci. Transl. Med. (2017)*
*Puth S et.al. Biomaterials (2022)*

# Our Previous Studies : Flagellin Adjuvant (Safety & Biodistribution)

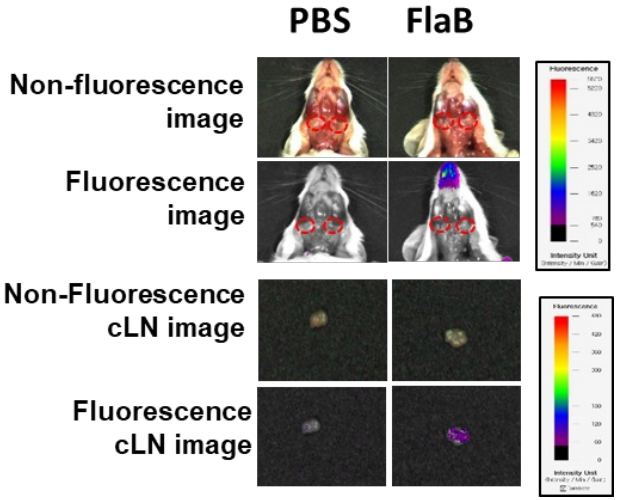
- **Flagellin: preclinical trial (Korea)**
- **CNS accumulation?**

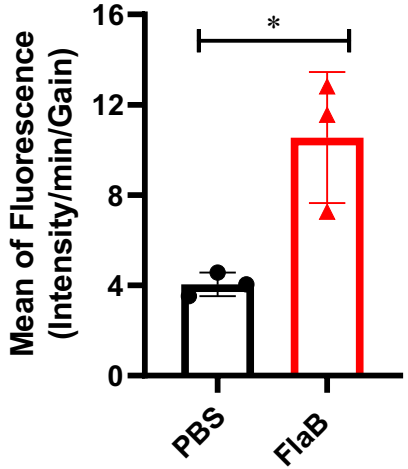



**Safety issue!**
**CNS accumulation**

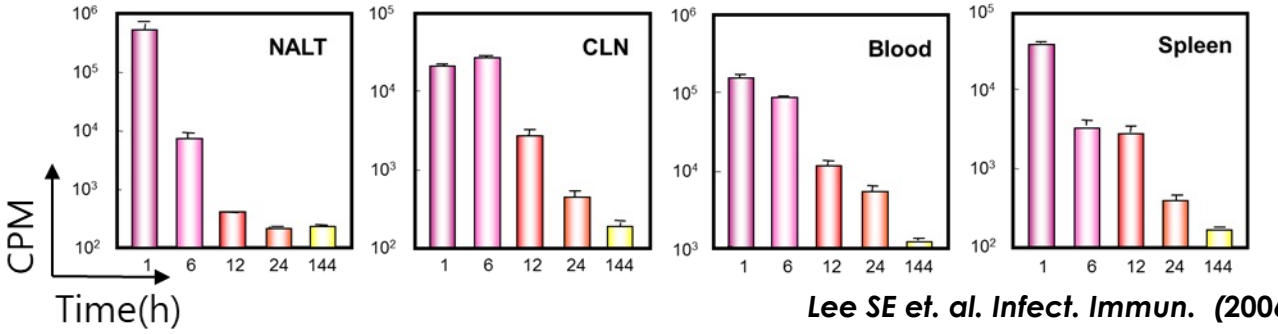
*Lee SE et. al. Infect. Immun. (2006)*

| Tissue | Treatment: 6.0 μg CT | Treatment: 5.0 μg Vv-FlaB | *p** |
|---|---|---|---|
| ON/E | 1.26 ± 0.57 | 0.29 ± 0.05 | <0.01 |
| OB | 1.28 ± 0.24 | 0.20 ± 0.02 | <0.01 |



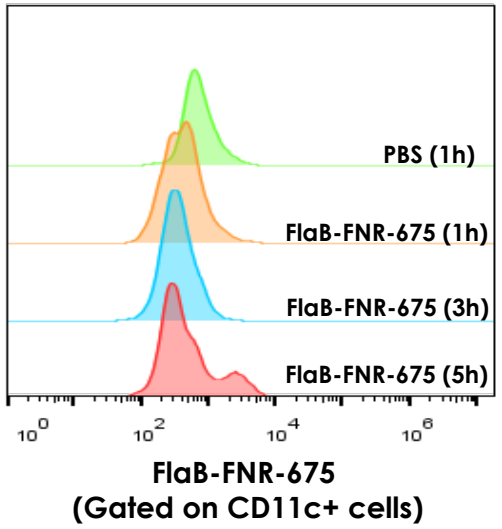



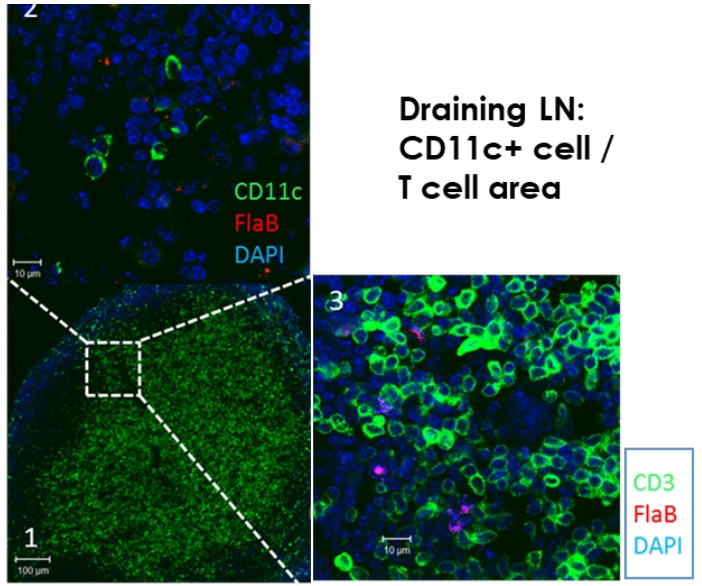

## Intranasally administered $^{131}$I-Vv-FlaB: retain in dLNS




*Lee SE et. al. Infect. Immun. (2006)*

## Intravaginal administration: retain in dLNs CD11c⁺ cells

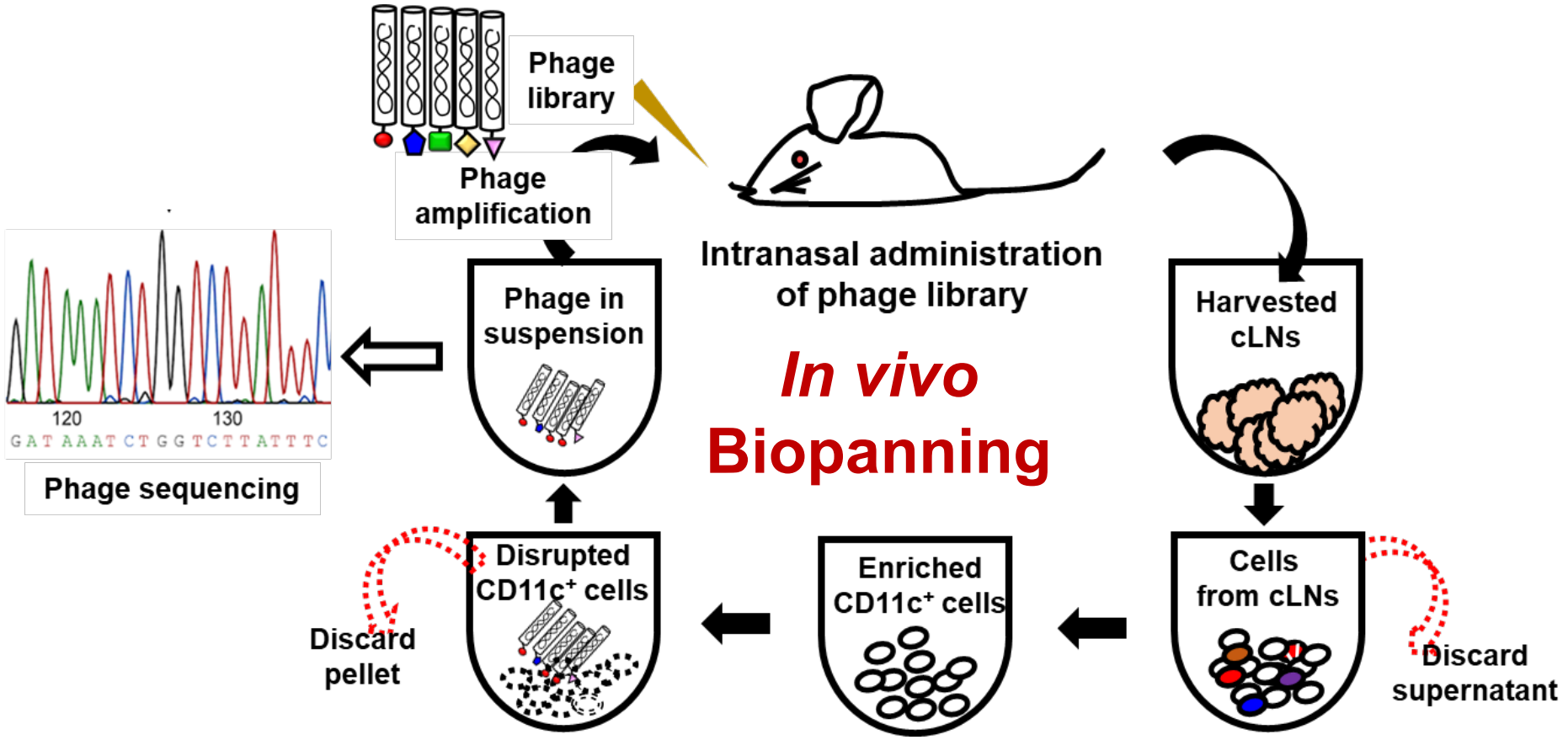



**Draining LN:**
**CD11c+ cell / T cell area**

# A Novel Approach

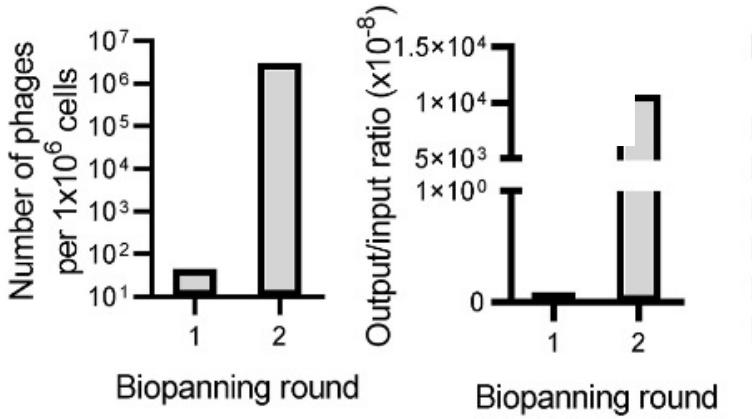
## Phage display library screening

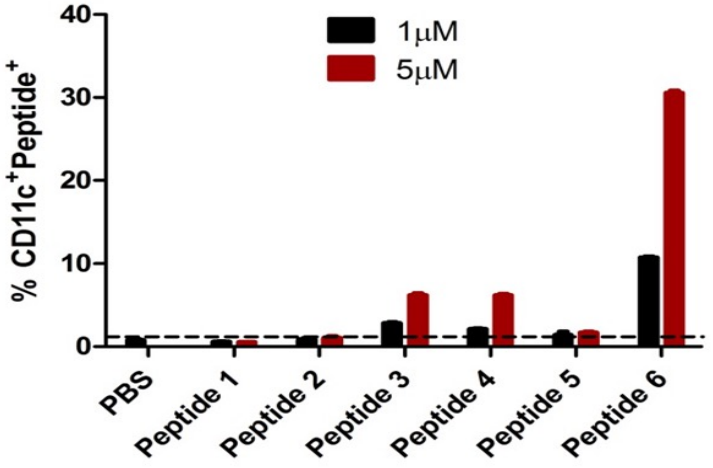


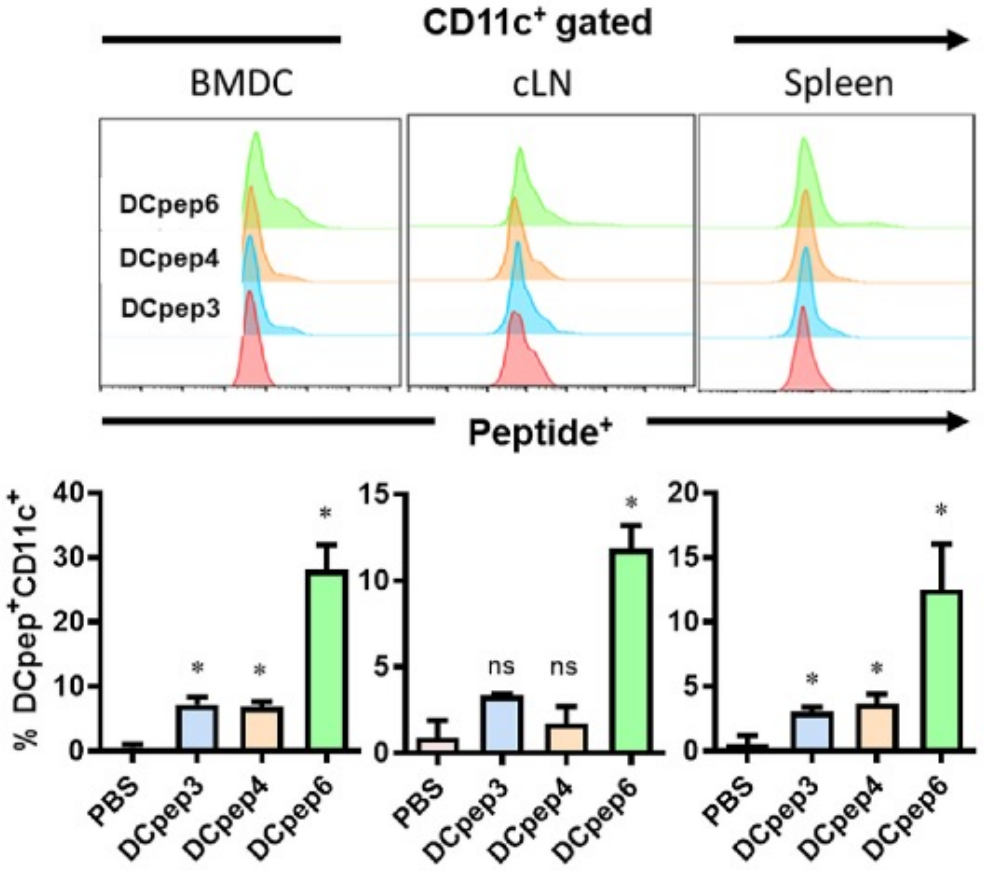

## *In vivo* CD11c⁺ Cell Targeting / Intracellular Localization

# In vivo biopanning

## *In vivo* CD11c⁺ Cell Targeting / Intracellular Localization



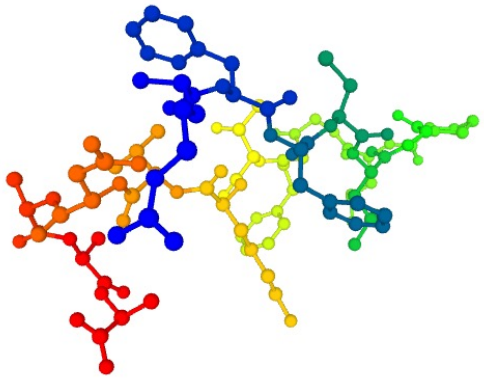



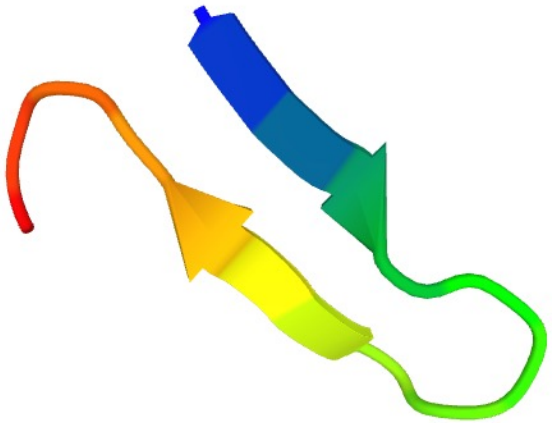




**DCpep6 : highest specific signals in DCs from various sources**

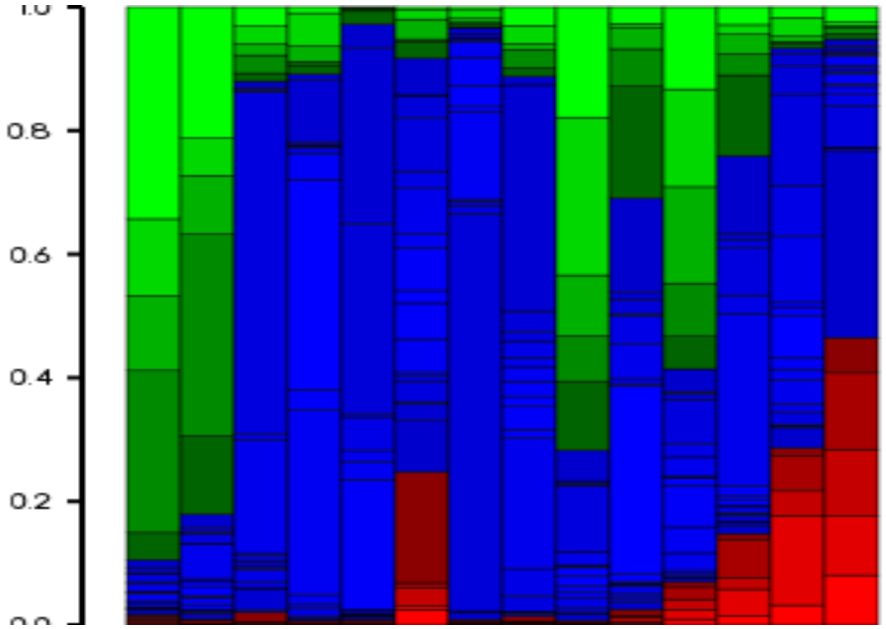
# Intracellular DC-Targeting Peptide

## DCpep6



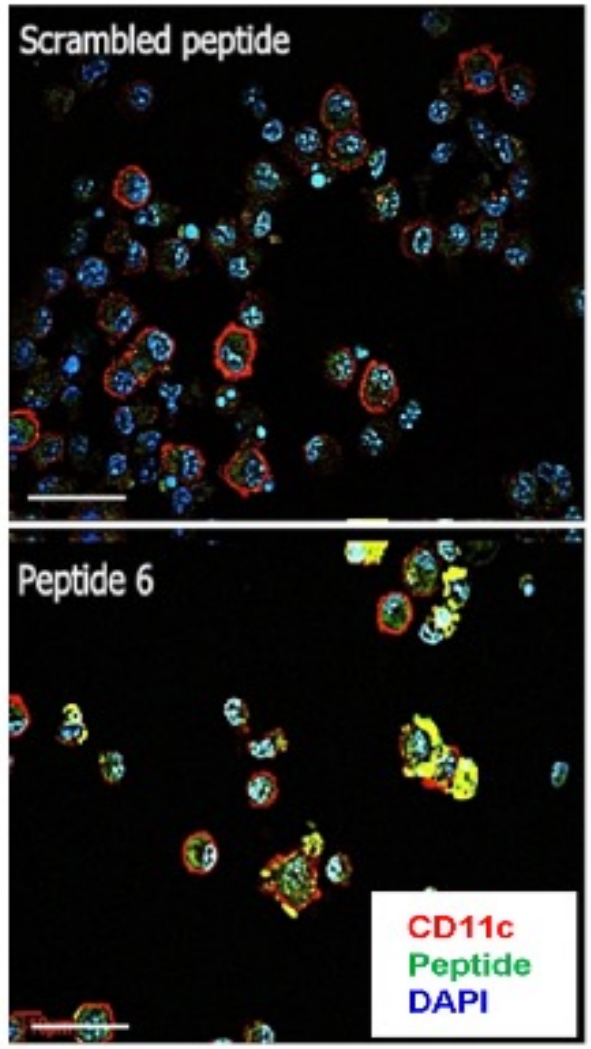


**Structural alphabet (SA) prediction profile**
**(After the addition of AAA)**




https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/#Concepts

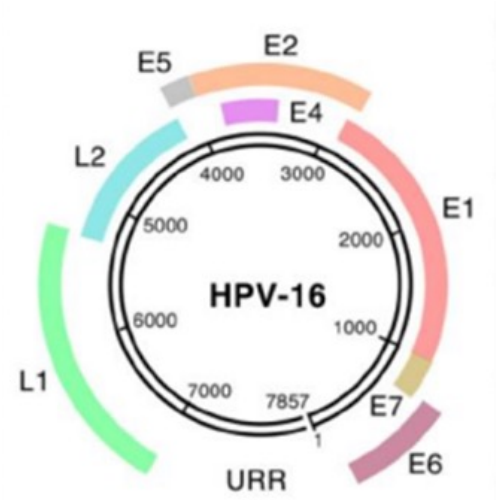
helical / coil / extended

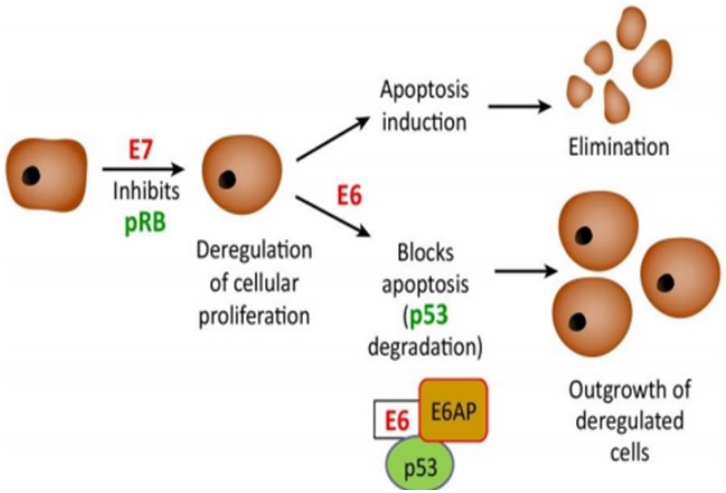


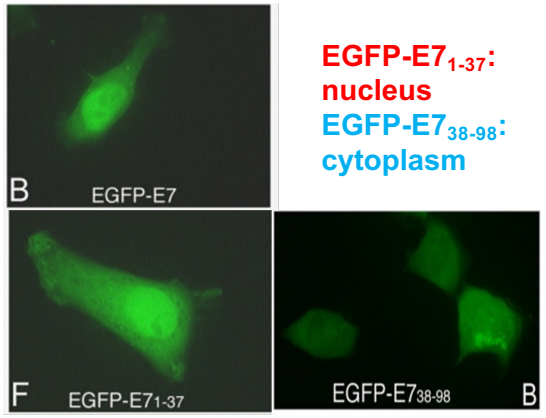
# Intracellular DC-Targeting Peptide & Optimal Antigen

D X F

## HPV 16 E7ΔNLS

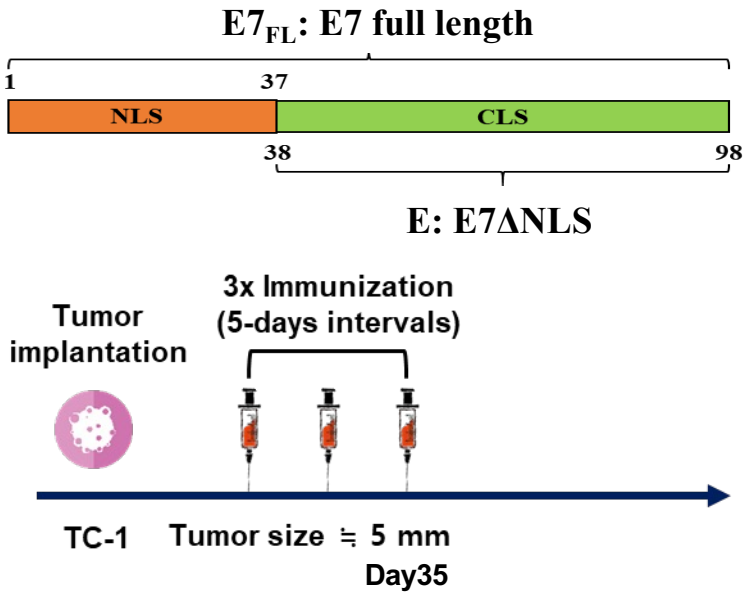


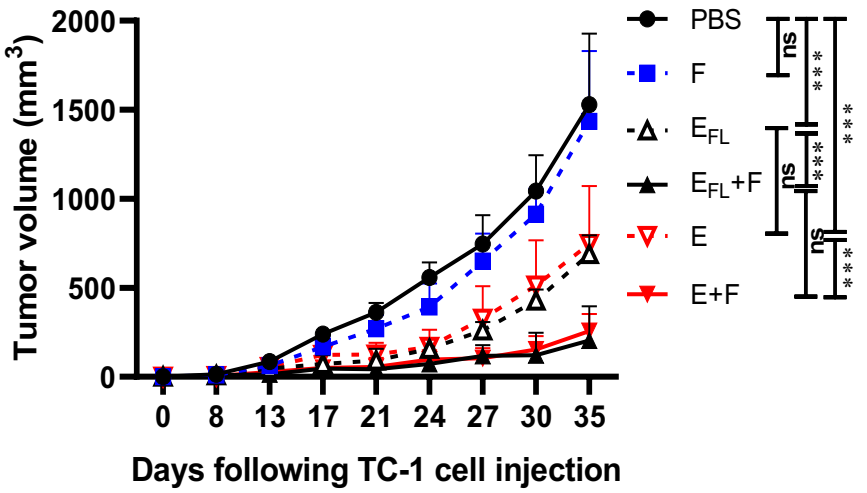



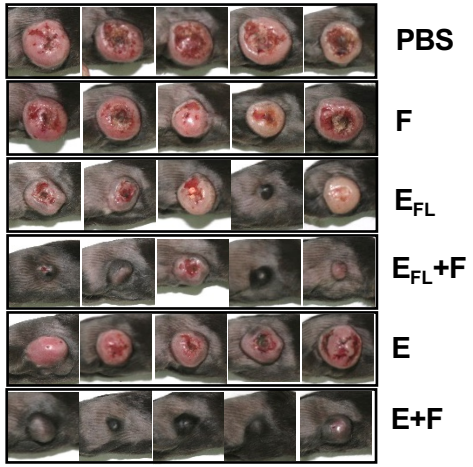




EGFP-E7$_{1-37}$: nucleus
EGFP-E7$_{38-98}$: cytoplasm

*Virology, 2009 Jan 5;383(1):60-8. 8*



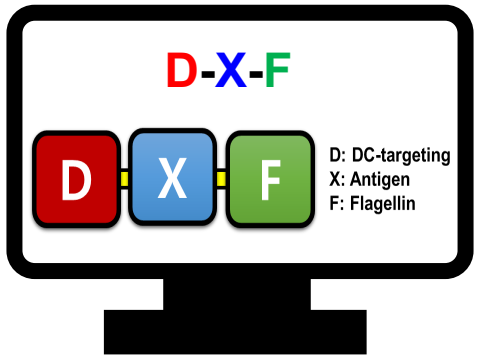



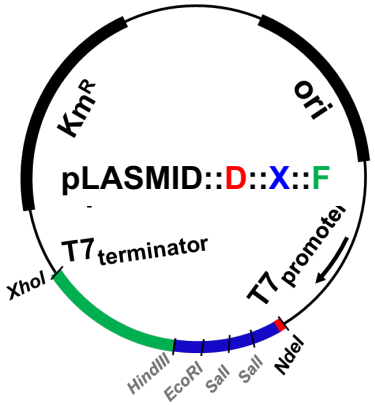
# Development of All-in-One vaccine

## Production and Validation

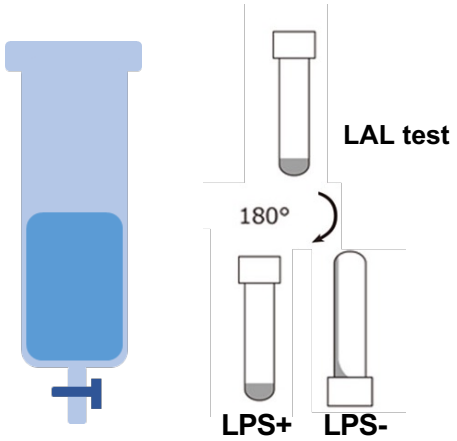
### Sequence design



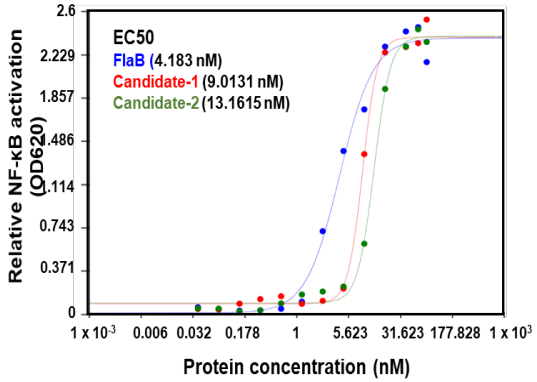

### Plasmid construction



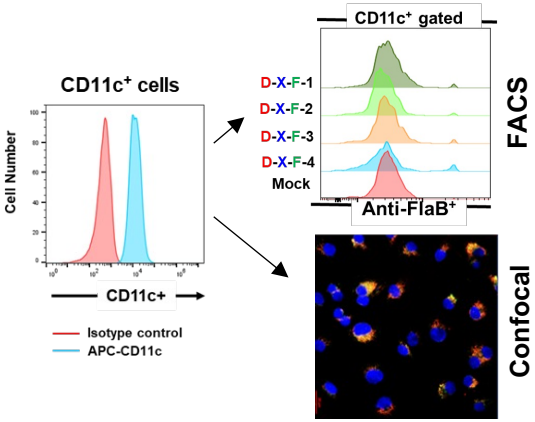

### Purification




### TLR5-stimulating activity

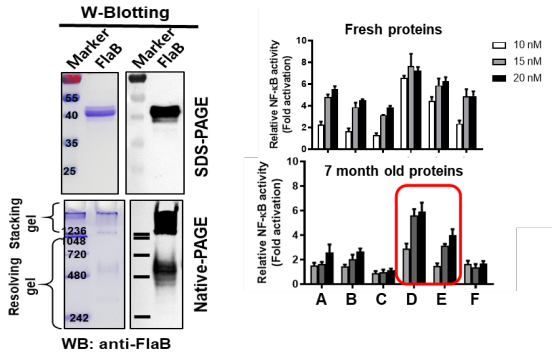



### DC-internalization




### Stability

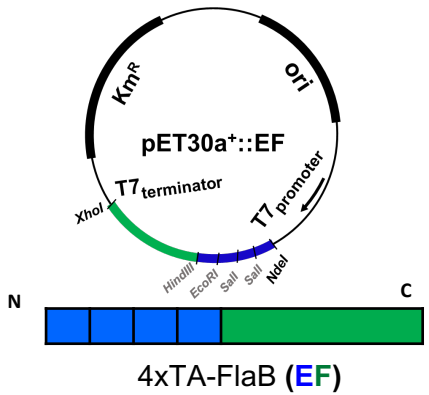
Lon term storage



# All-in-One vaccine: *In vitro* DC internalization

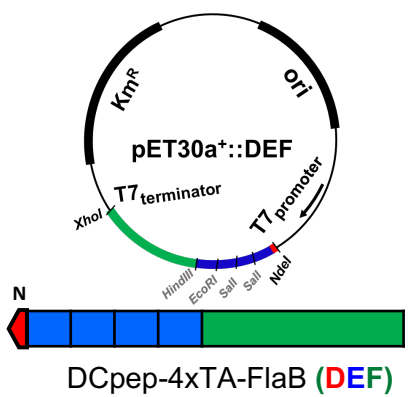
D X F

**EF**

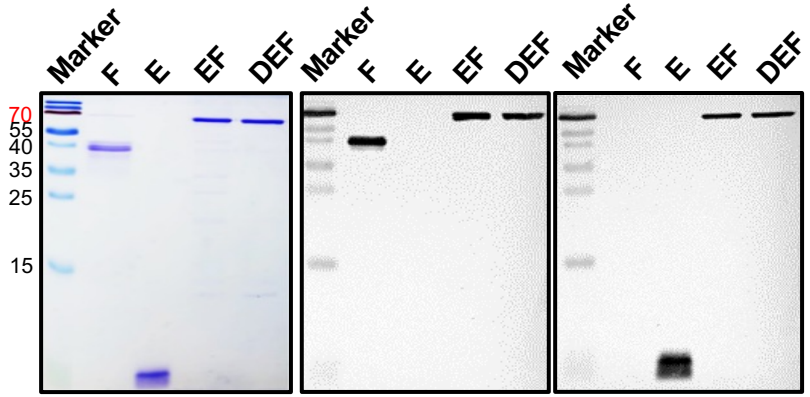



4xTA-FlaB (**EF**)

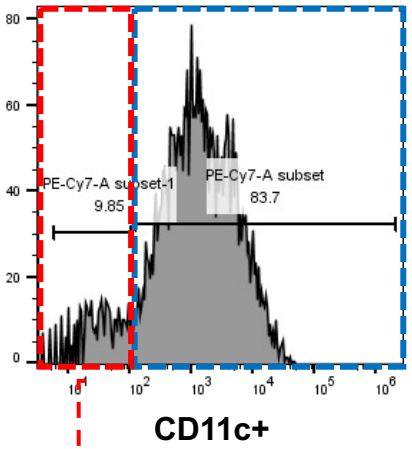
**DEF**

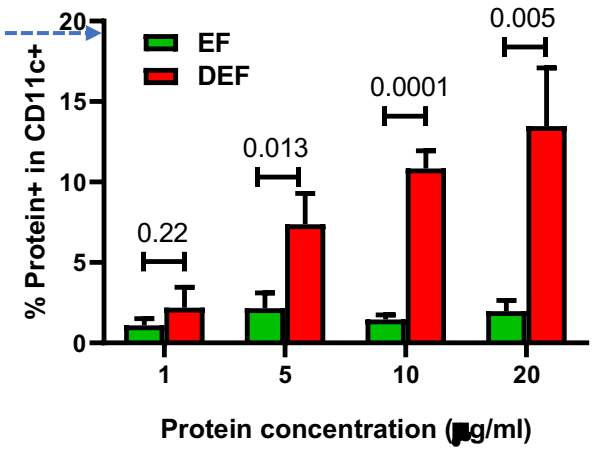


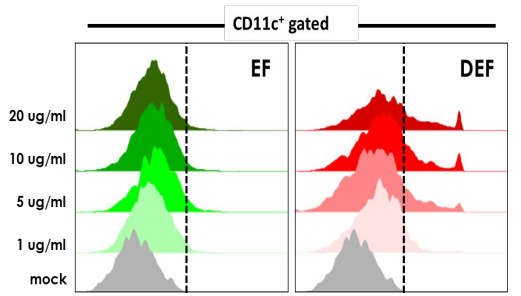

DCpep-4xTA-FlaB (**DEF**)

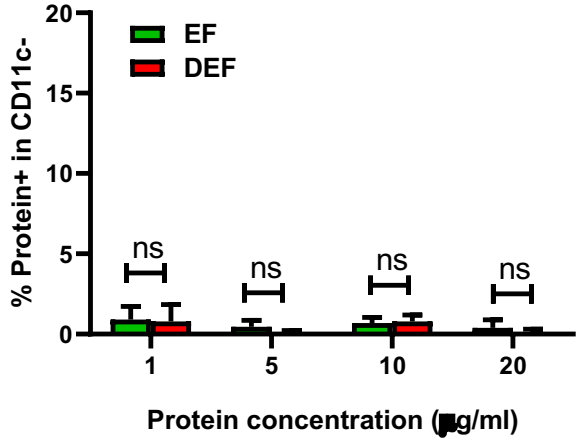


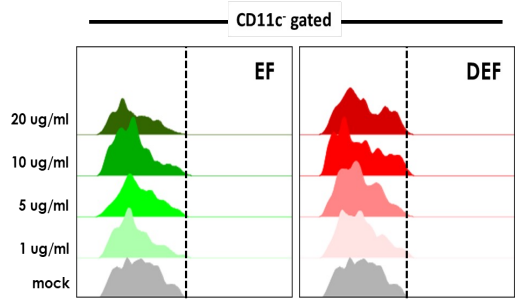

**BMDCs**



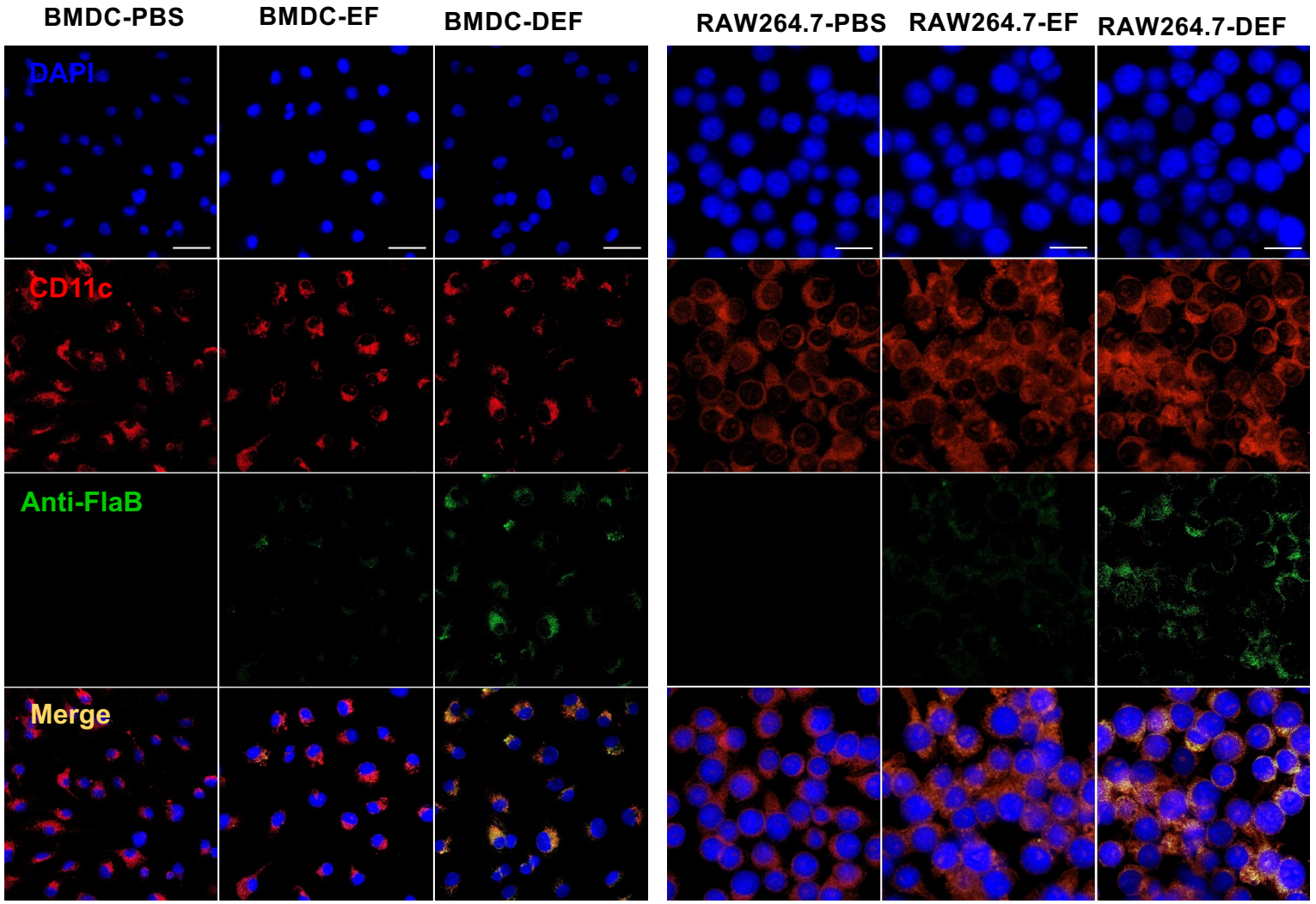



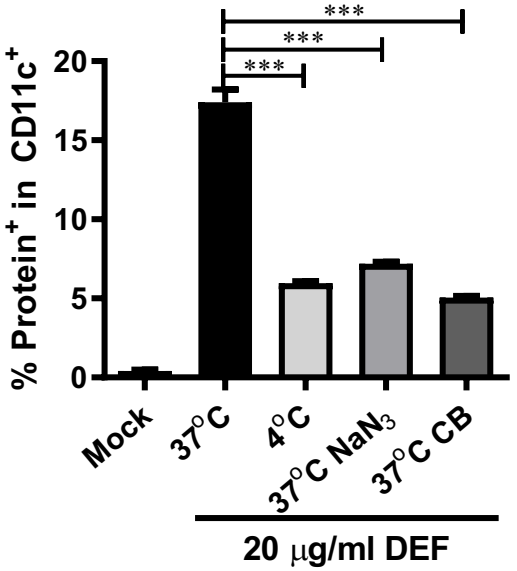



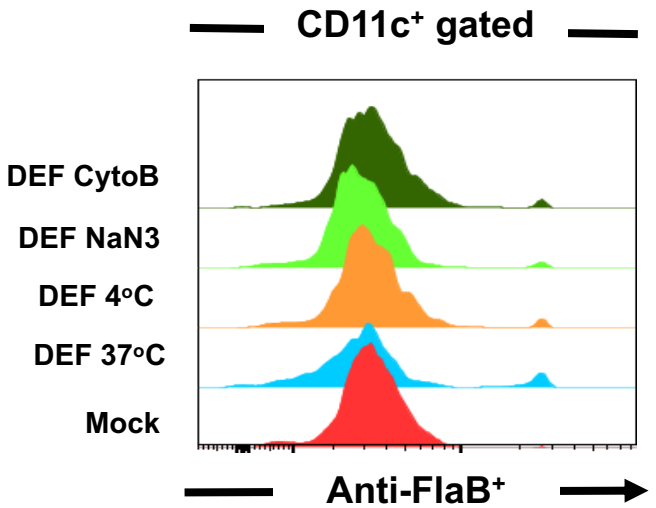




***In vitro***

# All-in-One vaccine: *In vitro* DC internalization



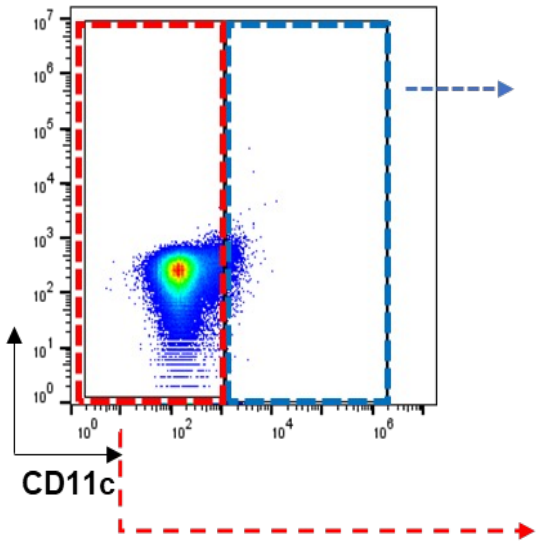



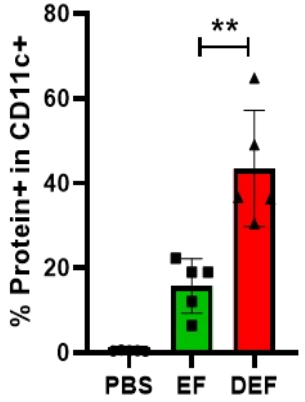

*In vitro*

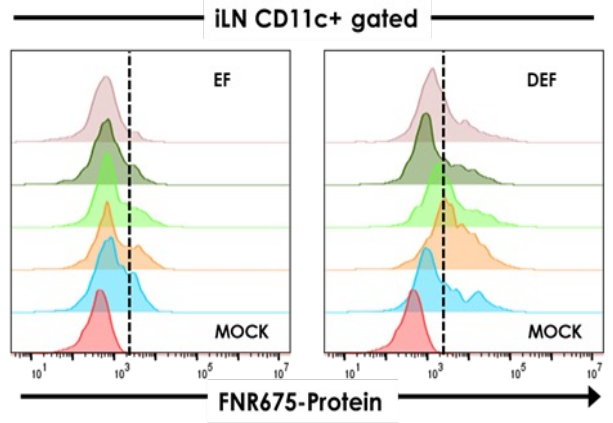
# All-in-One vaccine: *In vivo* DC internalization

D X F

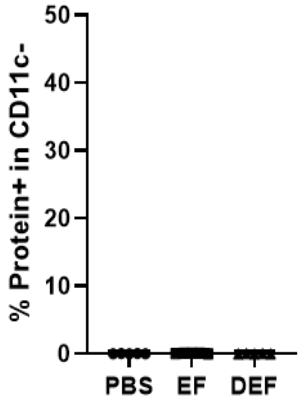
## Groin Administration -> iLNs

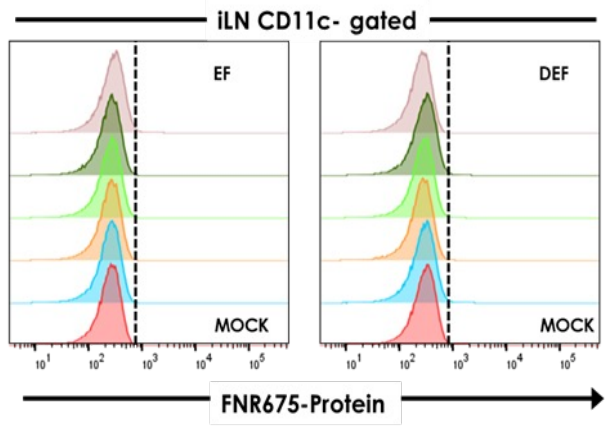


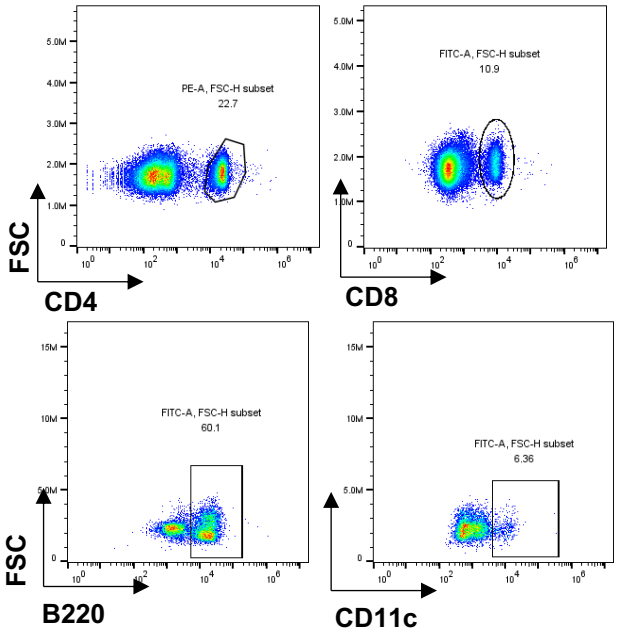



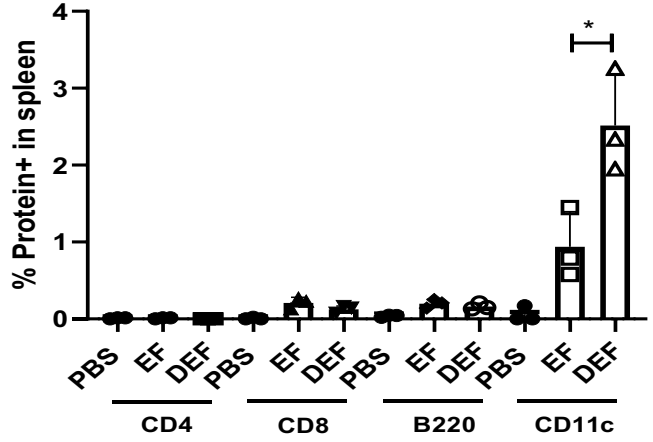

Supplementary Figure 4 (R1 revised version)



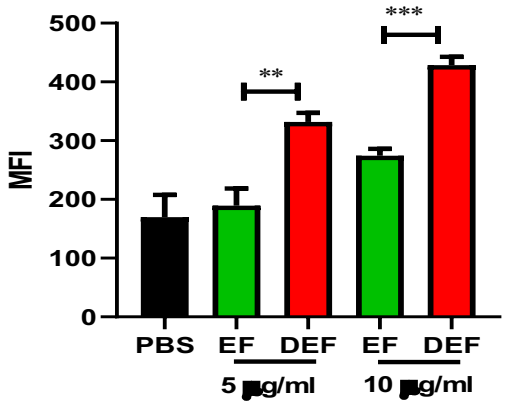

## Spleen

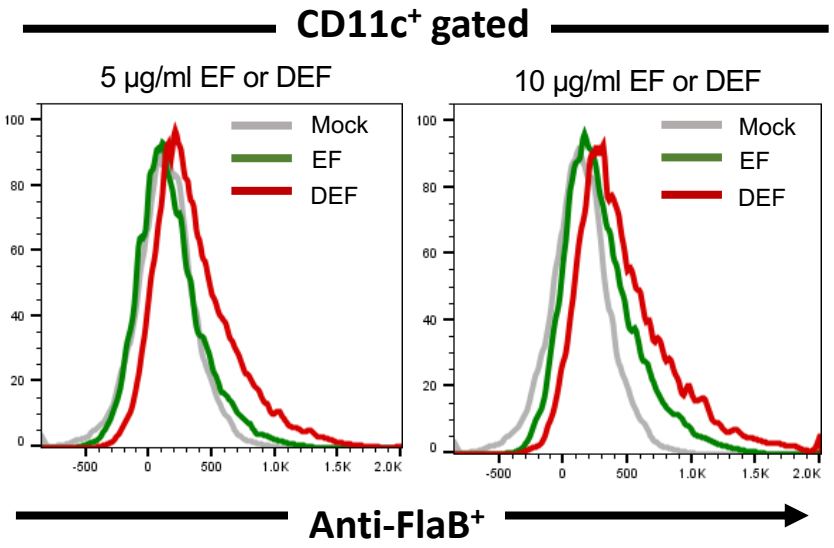


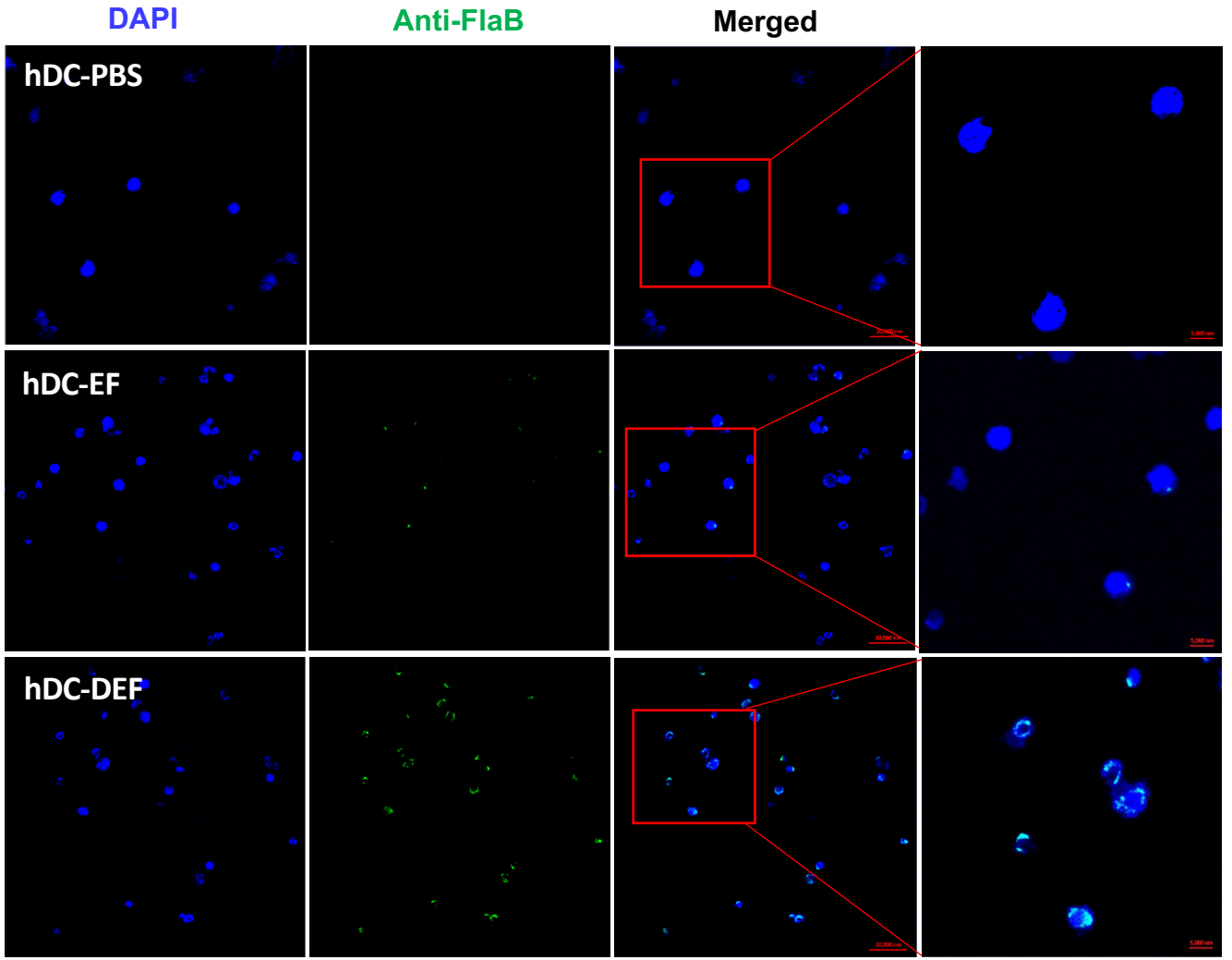




## *In vivo*

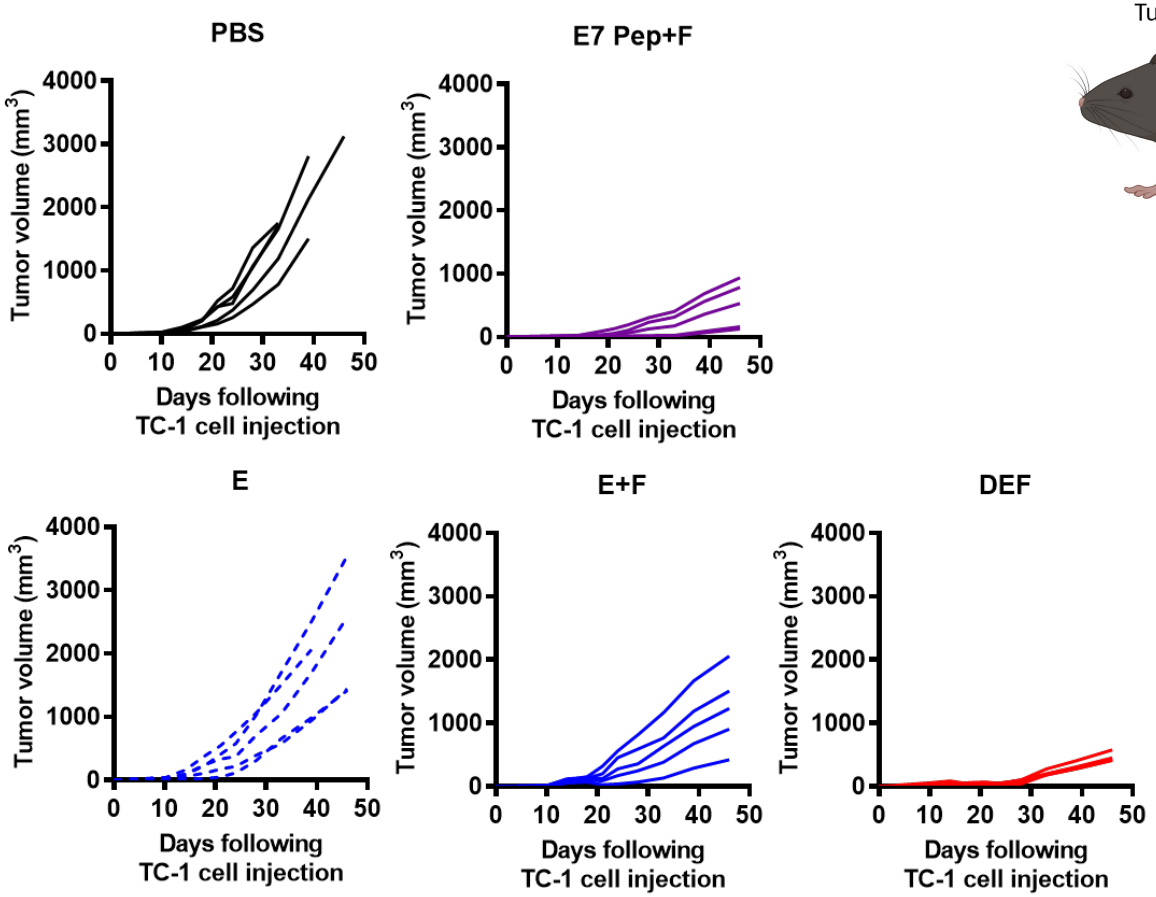
# All-in-One vaccine: human DC

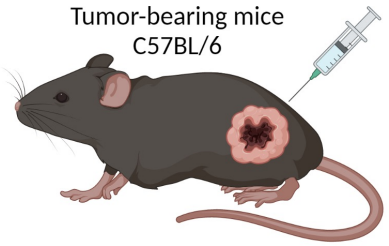


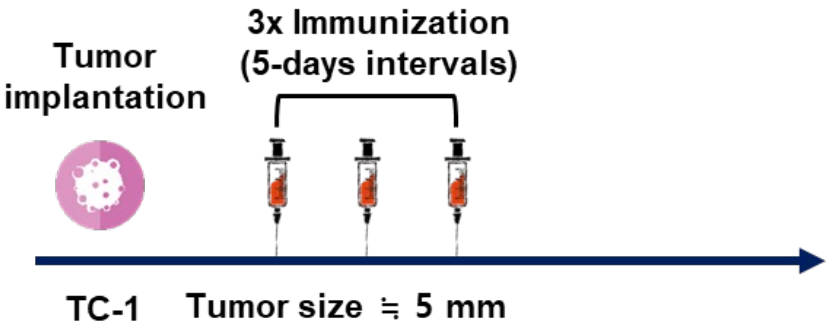



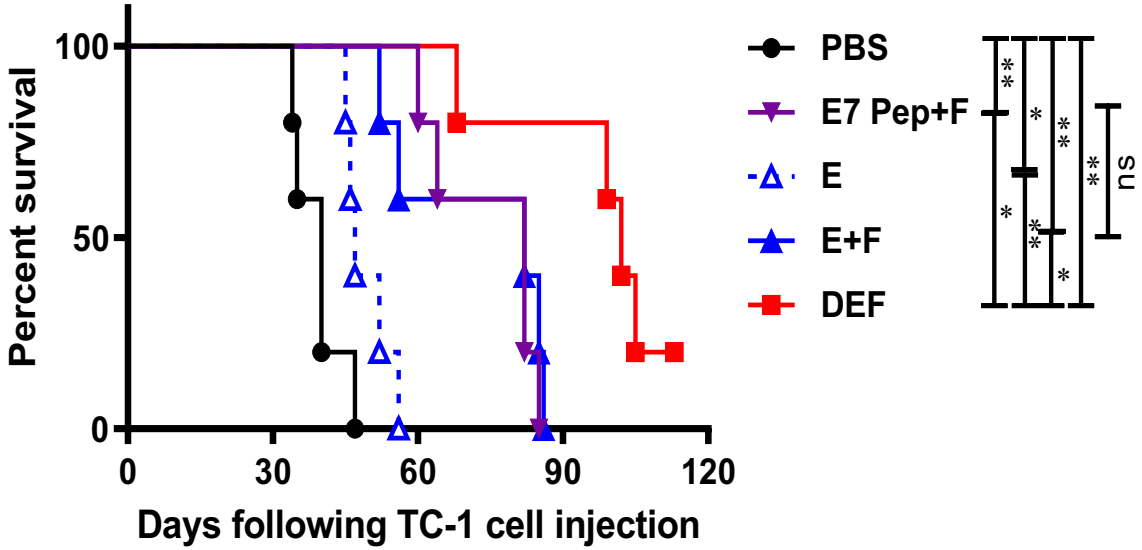



# All-in-One TCV: Efficacy



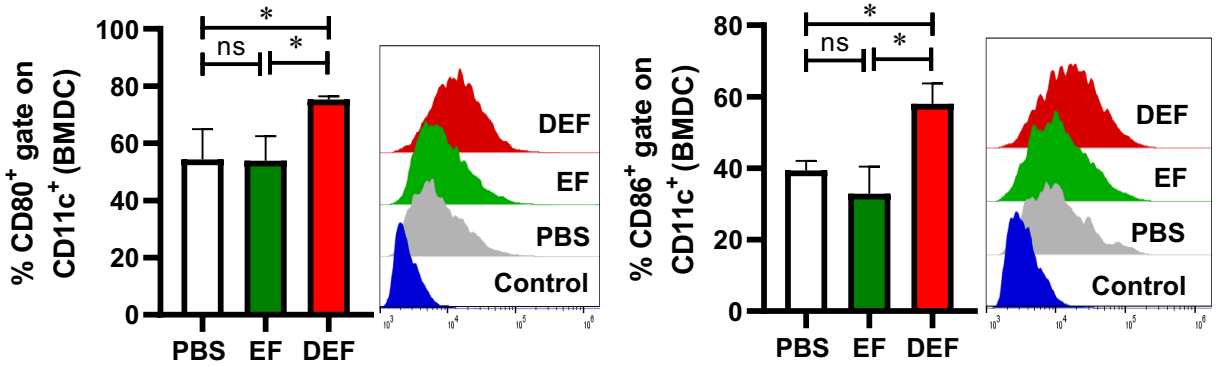

## Inhibition of Tumor Growth

## Long-Term Survival

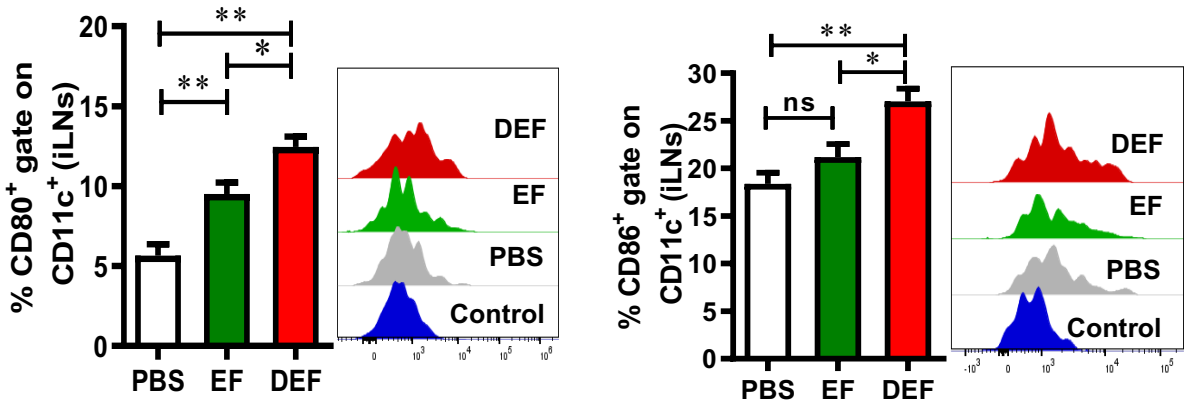
# All-in-One TCV: Mechanism of Action

D-X-F

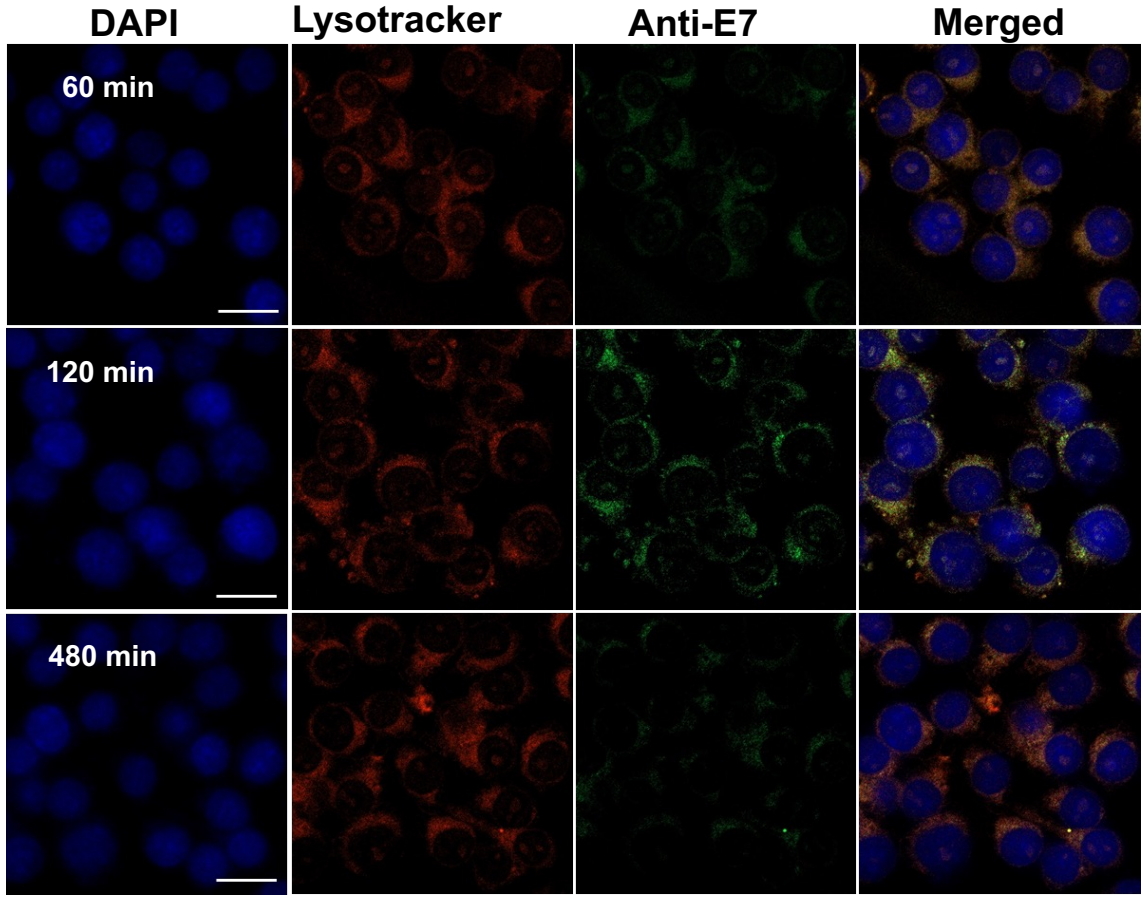
## *In vitro* DC Activation




## *In vivo* DC Activation




## Antigen Processing in CD11c+ Cells

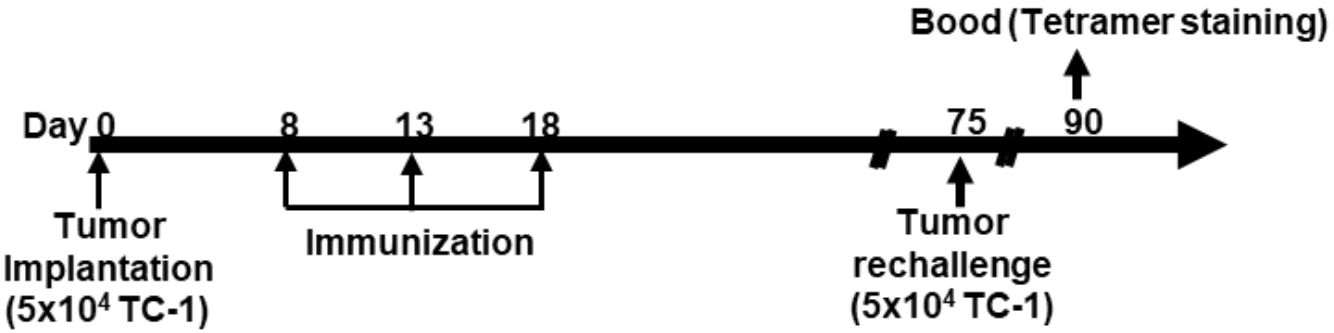


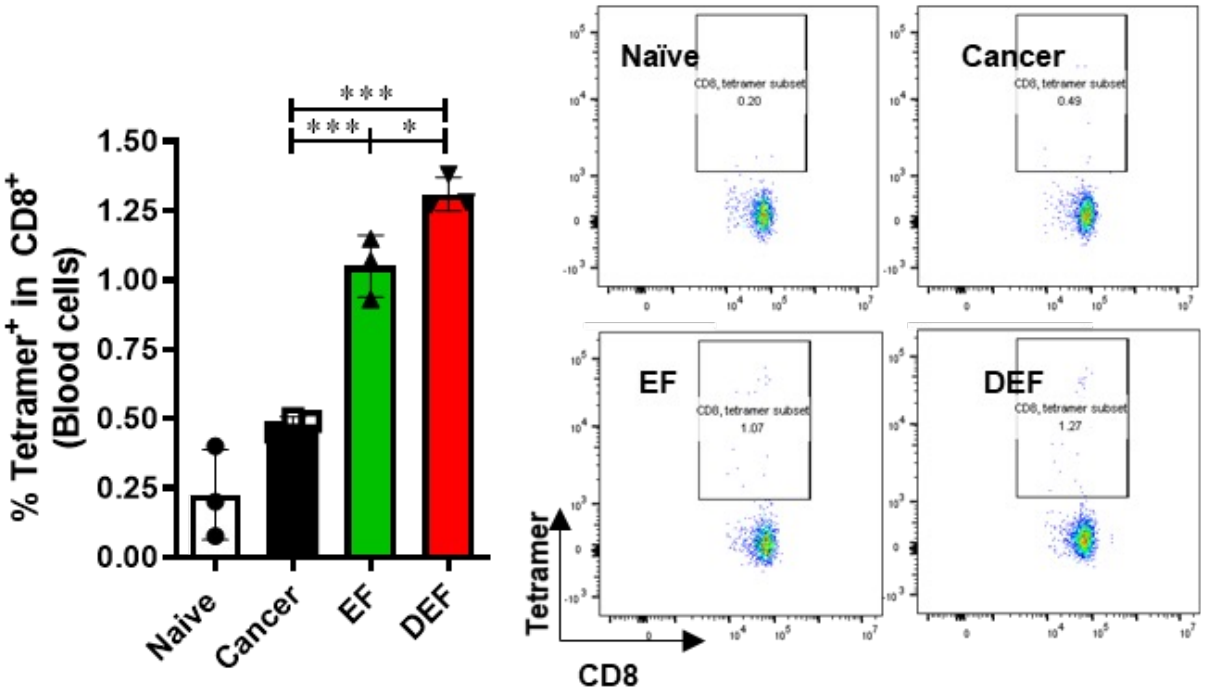

**Cytosolic DEFs should have been effectively processed by proteasomes for cross-presentation**

# All-in-One TCV: Mechanism of Action

D X F

## Antigen-specific immune responses

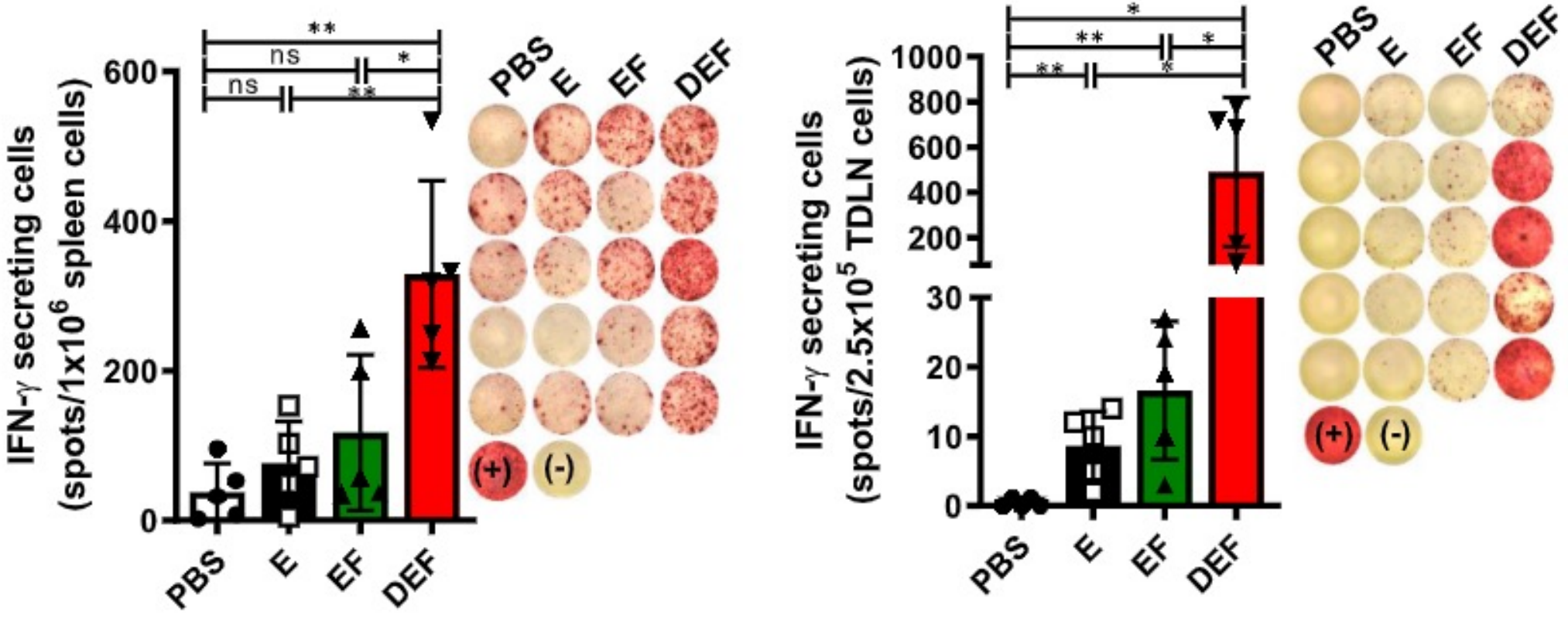


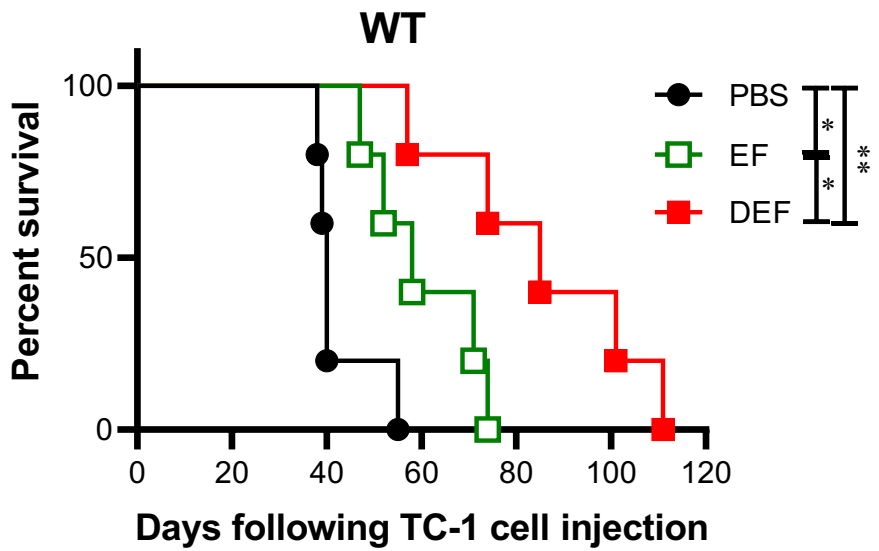
# All-in-One TCV: Mechanism of Action

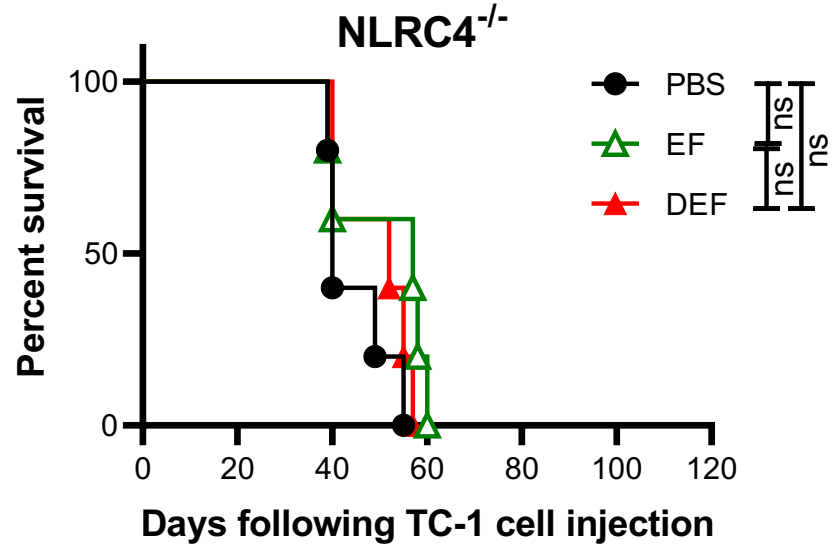
D · X · F

## Antigen-specific immune responses (tumor-bearing mice)



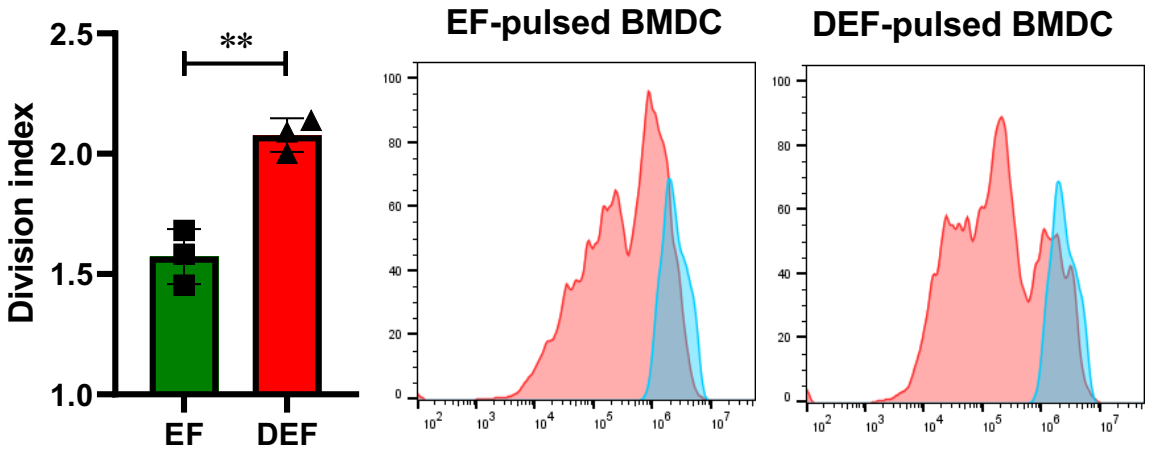
# All-in-One TCV: Mechanism of Action (TLR5 vs NLRC4)



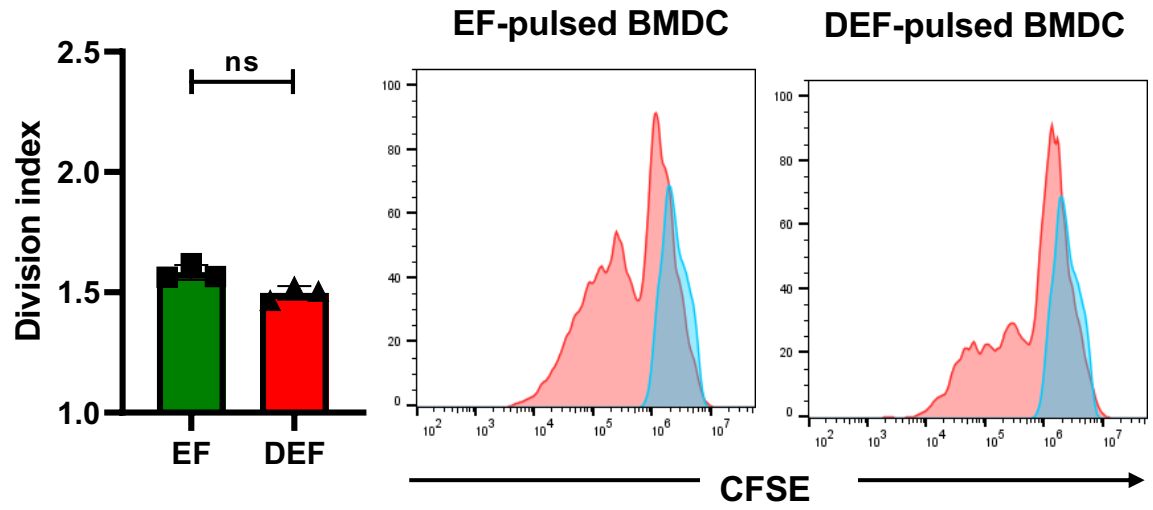




BMDC : CFSE-CD8⁺ (1:5)

WT BMDC




NLRC4-/- BMDC

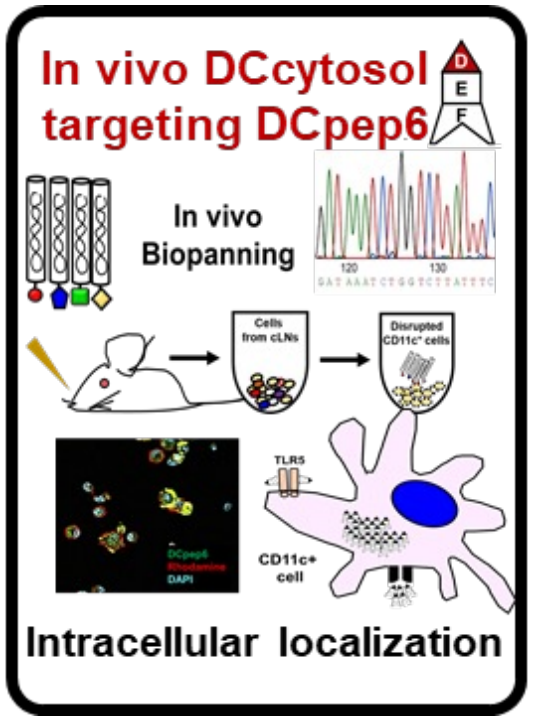



CD8⁺ cell only

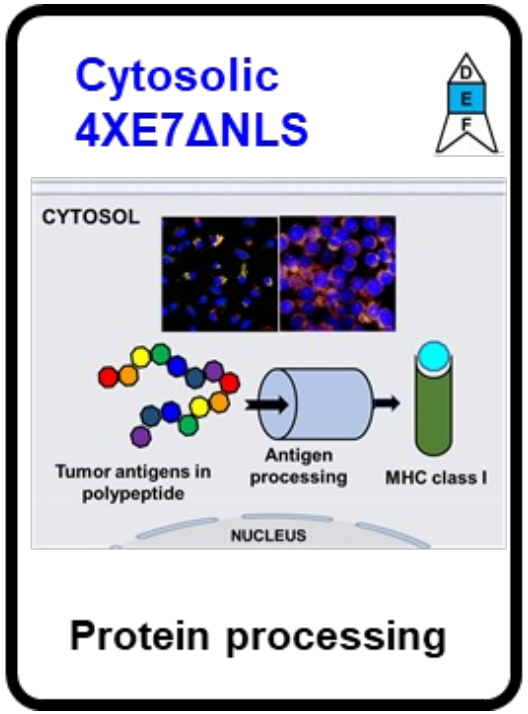
*Puth & Verma et. al. Biomaterials; 2022*

# All-in-One TCV Platform: Summary

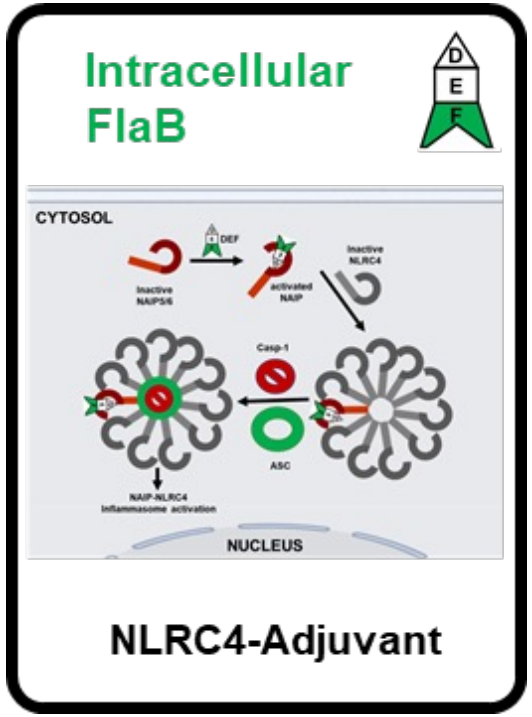

**D: DC-targeting/Internalization**
**X: Antigen**
**F: Flagellin Adjuvant**




**Intracellular localization**




**Protein processing**




**NLRC4-Adjuvant**

- ✓ **Broad Application**
  - TCV
  - Infectious Ds
  - Non-infectious Ds
- ✓ **Vaccine Upgrade**
  - Unmet-need vaccines
- ✓ **Ready to collaborate with other platforms**
  - mRNA Vaccine?

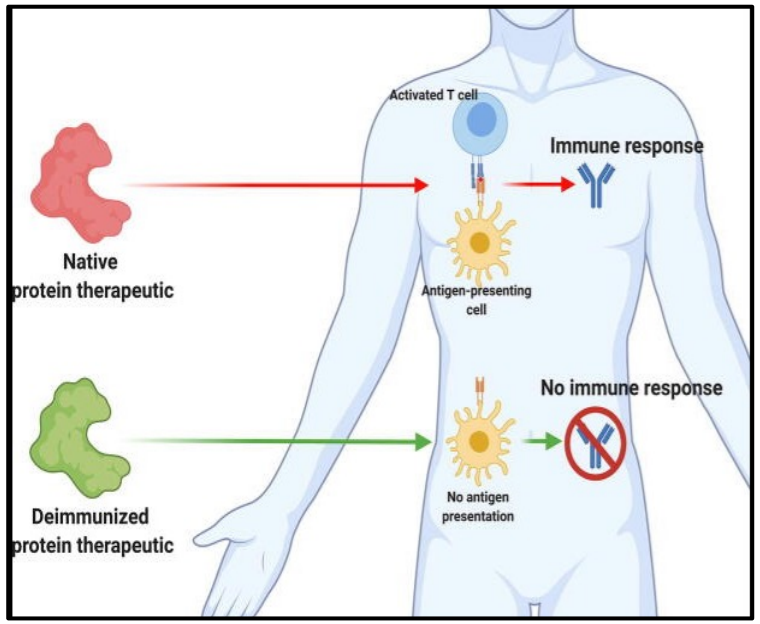
**Platform Technology / Ready-to-Use / Versatile Application / Addressing Unmet Needs**

# Flagellin adjuvanted vaccine:

# Deimmunization of FlaB, one more step toward clinical application

# Challenge of biotherapeutics

## POTENTIAL IMMUNOGENICITY




Comput Struct Biotechnol J. 2021; 19: 315–329.

### Anti-drug-antibodies

- ✓ Repeated drug administration
- ✓ Alteration of pharmacokinetics
- ✓ Reduced efficacy
- ✓ Allergic reactions (ex, anaphylaxis)

### Effective deimmunization methods for protein drugs is of utmost importance

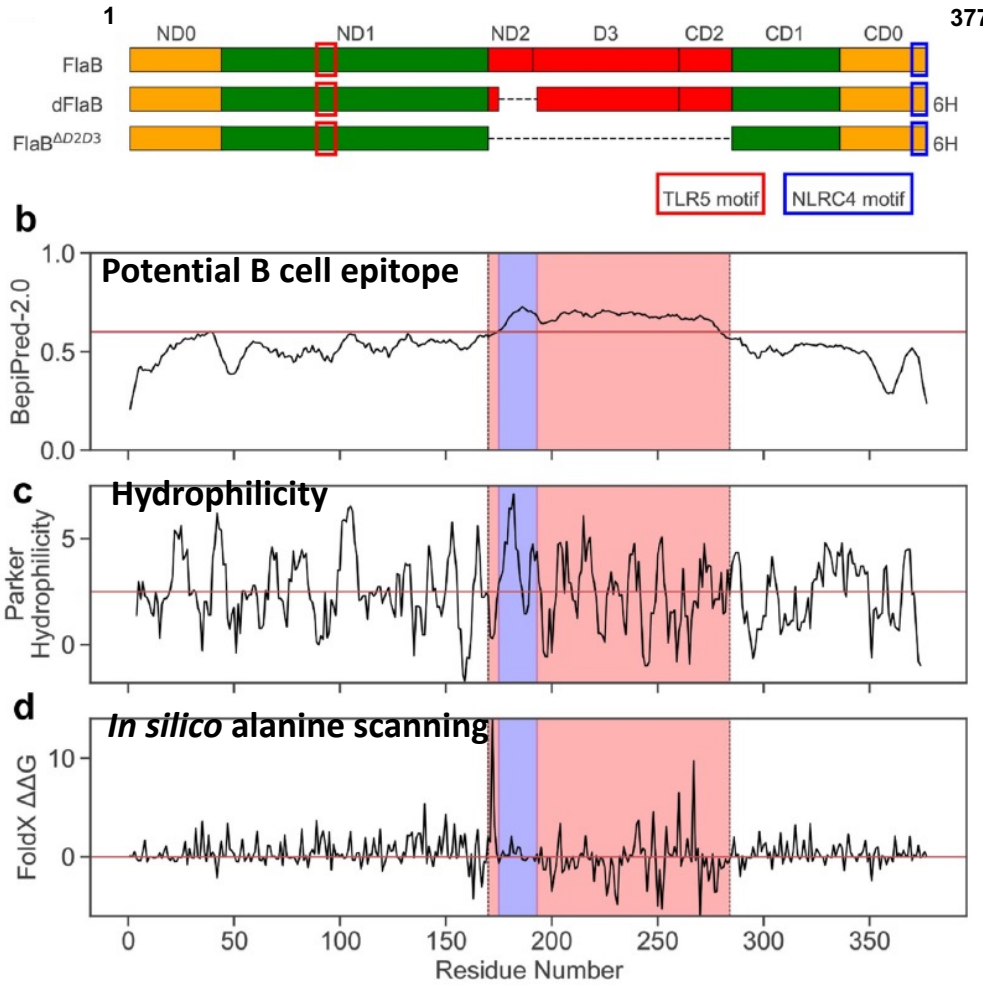
### Nonspecific shielding approaches

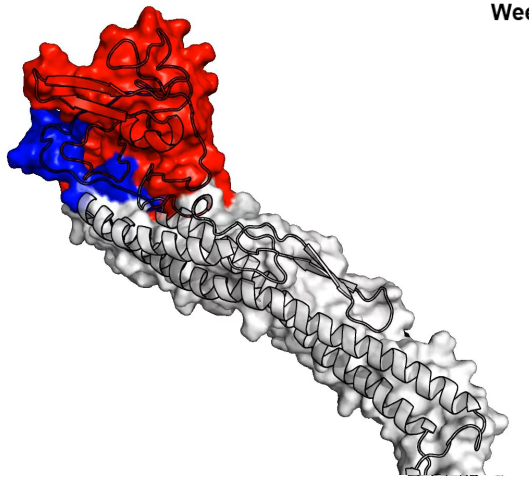
- PEGylation
- Fusion to polypeptides (e.g., XTEN or PAS)
- Reductive methylation
- Glycosylation
- Polysialylation

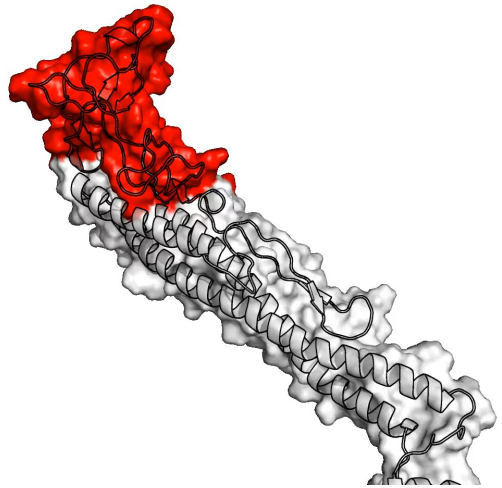
### Deletion of immunogenic epitopes

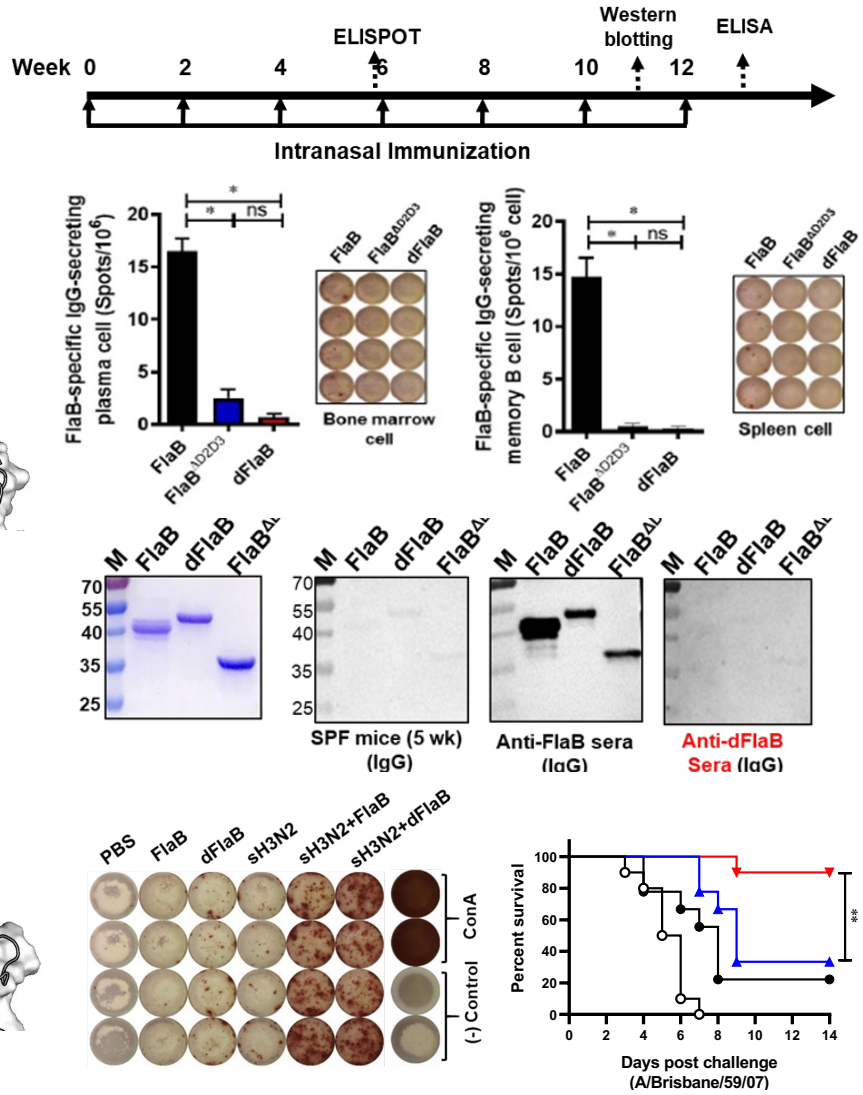
- Experimental approach
- Computational approach

# Deimmunized flagellin: Prediction of Immunogenic region








# Deimmunized flagellin as a mucosal vaccine adjuvant

*Khim Koemchhoy et. al. npj Vaccines (2021)*

# Thank you for your attention!

CVRDC

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Hard-tissue Biointerface Research Center

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