



# *Plant-Based VLPs as a Cutting-Edge Vectorization Platform for Cancer Immunotherapy*

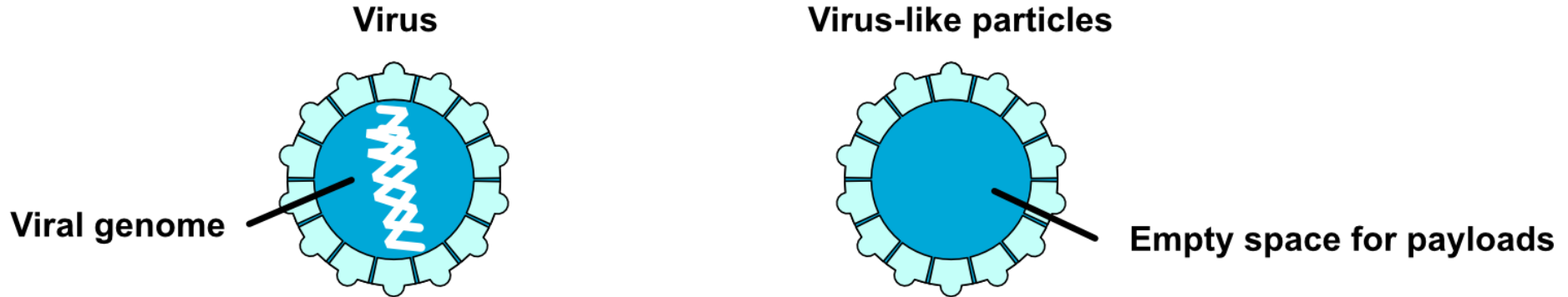
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Vianney Poignavent CSO



# Viruses and their virus-like particles

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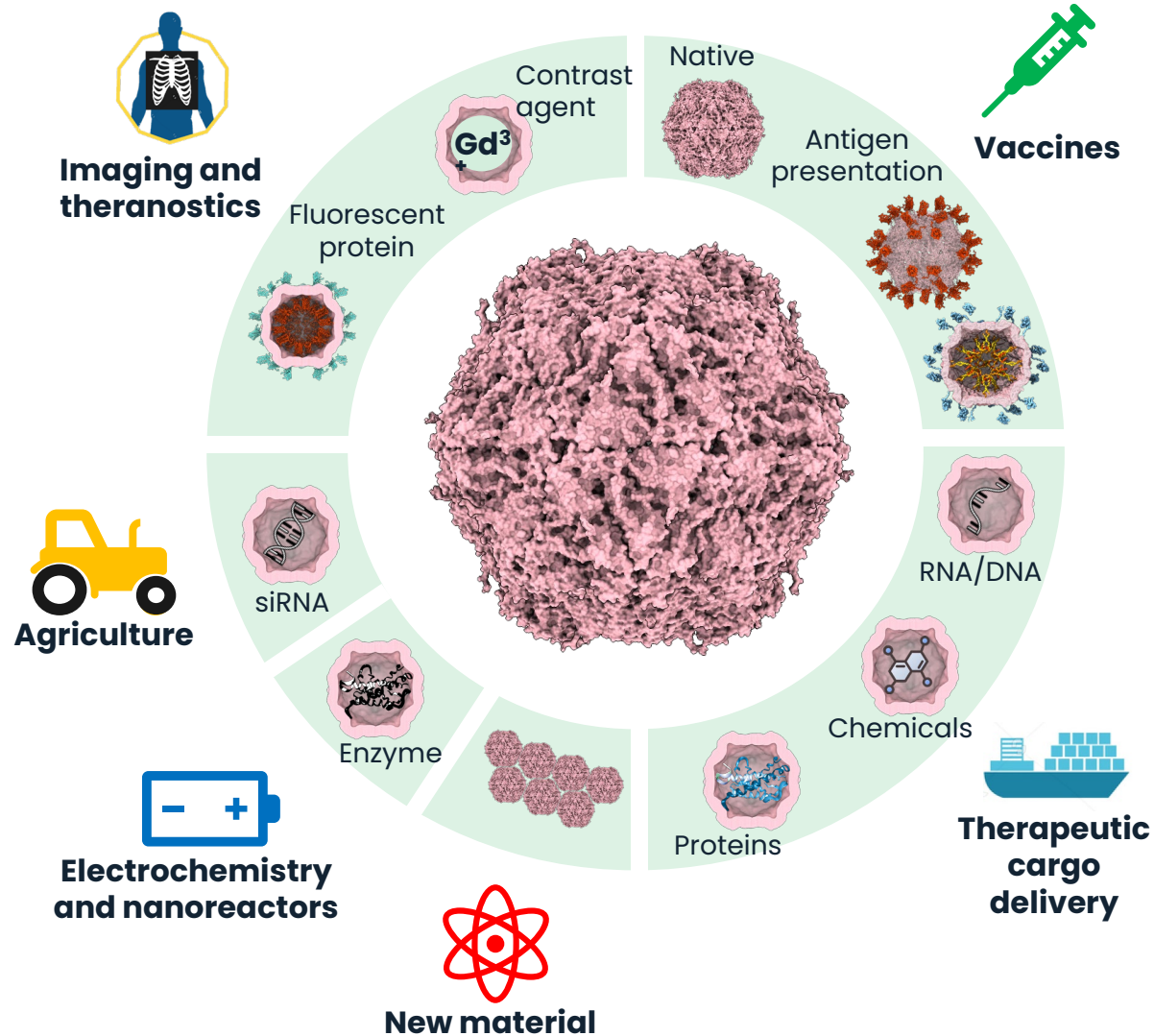


**Viruses and VLP are natural nanocarrier**

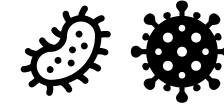
**VLP results from viral structural protein auto-assembling, devoided of genome and non-infectious.**

- Repeated and ordered protein structure suitable to raise immune responses
- Surface modifications for repetitive antigens or targeting agents presentation
- Nanocage for payloads

# VLP : the swiss knife vectorization system



## Therapeutic Prophylactic



Infectious disease



Cancer



Autoimmune disease and allergy



Central nervous system

Most developed applications are **VLP-based vaccines for infectious disease**

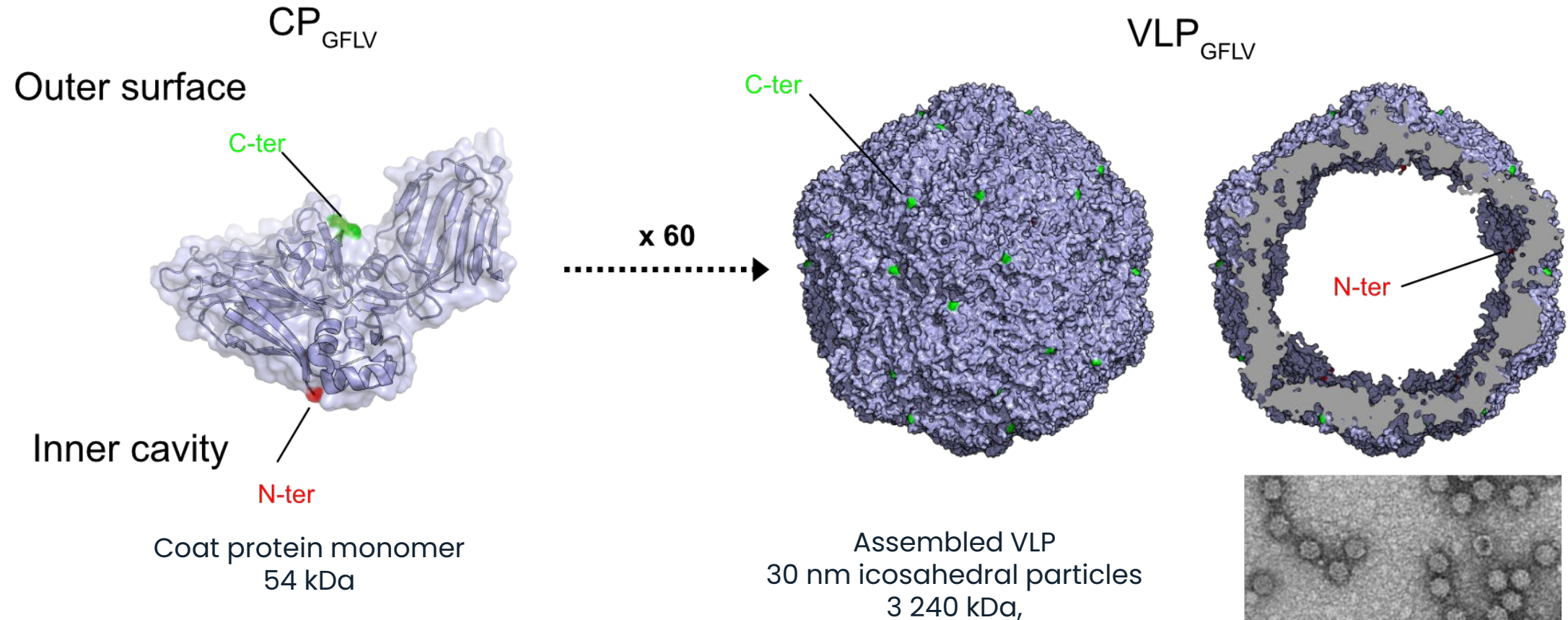
1<sup>st</sup> licenced VLP vaccine in 1986 against Hepatitis B

VLP engineering methods : genetic fusion, chemical coupling, ligand-based display

# Genetic engineering of Grapevine fanleaf virus VLP

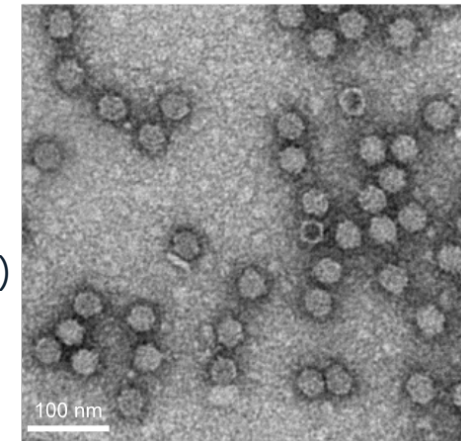


N- and C- terminus are exposed at the inner and outer surface of the VLP, respectively



Crystal structure 2,7 Å (schellenberger et al., 2011)

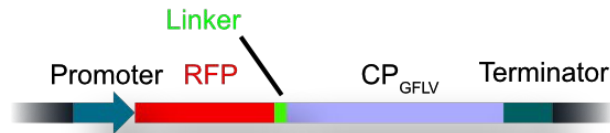
VLP<sub>GFLV</sub> are naturally self-assembling in plant expression system



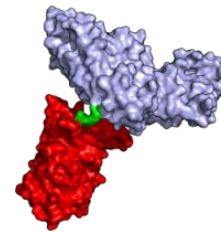
# Genetic engineering of VLP<sub>GFLV</sub>



Genetic construct

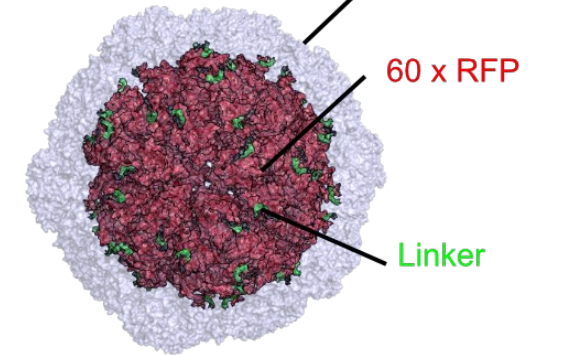


RFP-CP<sub>GFLV</sub>

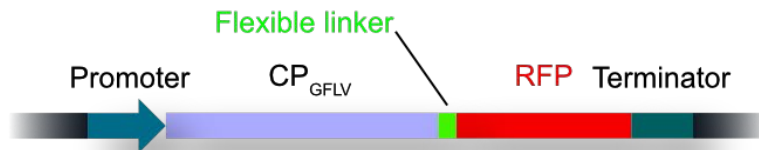


X 60

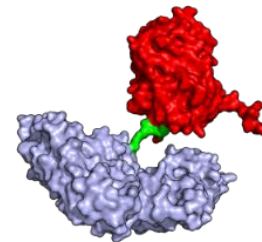
RFP-VLP<sub>GFLV</sub>



Genetic construct

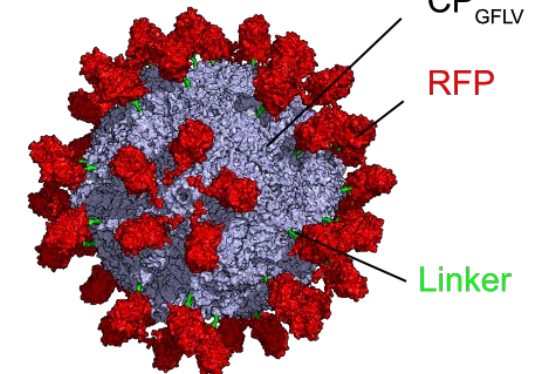


CP<sub>GFLV</sub>-RFP



X 60

RFP-VLP<sub>GFLV</sub>



In silico models (raptorX)

**N-ter and C-ter genetic fusion lead to encaging and display of foreign protein into VLP respectively.**

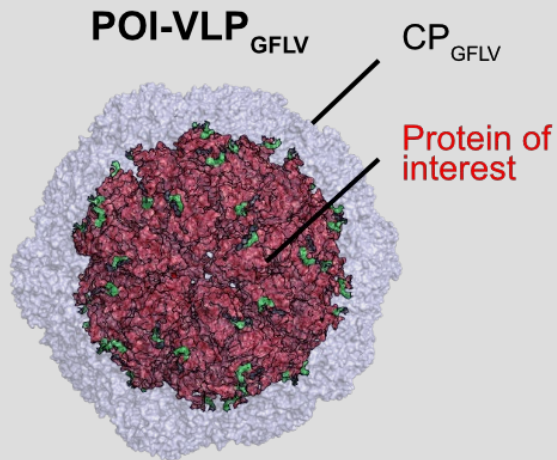


# Highly modular VLP platform for drug vectorization



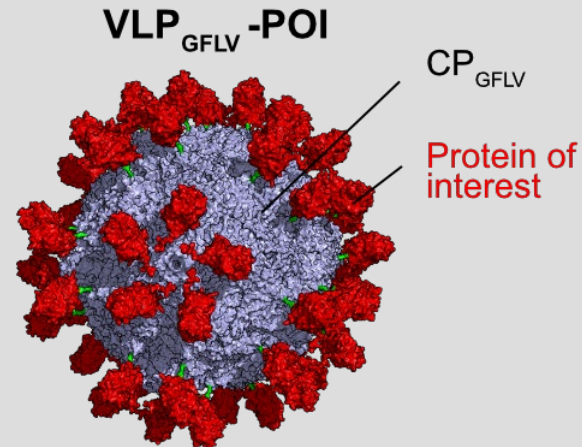
Two simple and robust methods to engineer our VLP system

## Genetic fusion



### Payload

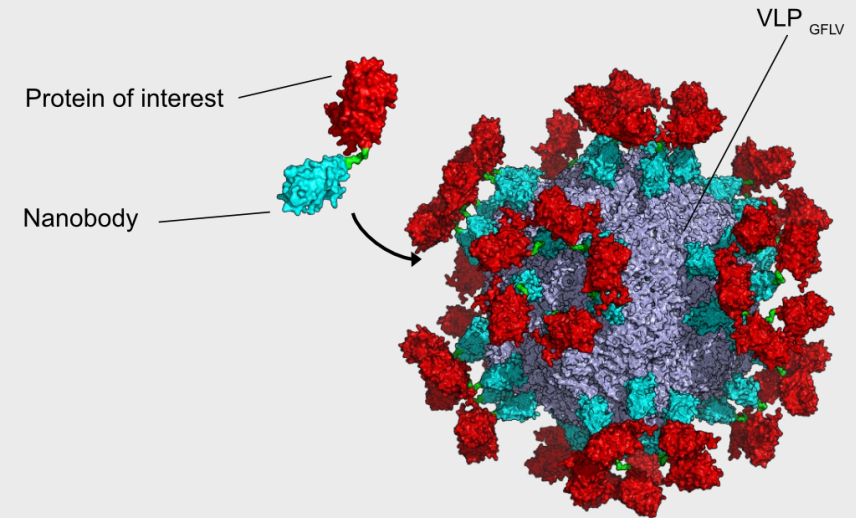
Fluorescent protein, antibody fragment, enzyme, **string of neopeptides** (~110 AA)



### Surface display

**Antibody fragment**, protein A domain, cytokine, viral and fluorescent protein

## Ligand-based display



### Surface display

Antibody, bispecific antibody fragment, enzymes, small molecules

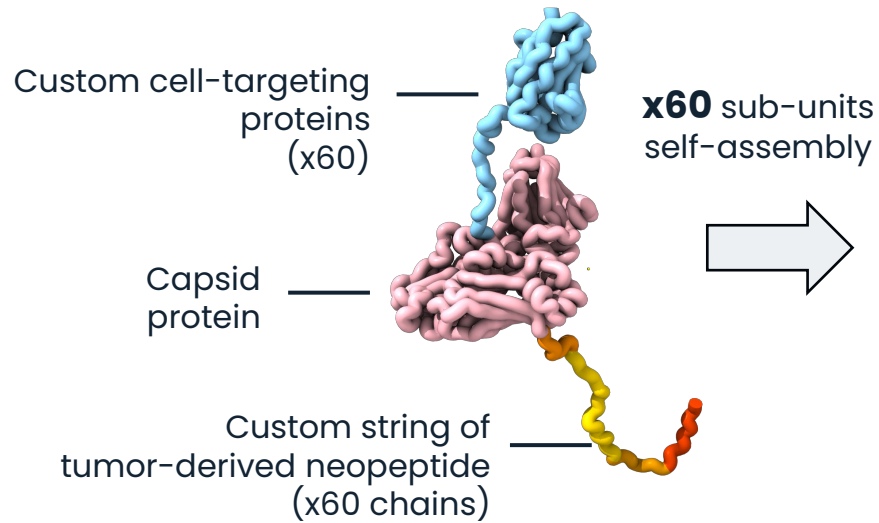


# *1<sup>st</sup> application – A immunization system for therapeutic vaccination*

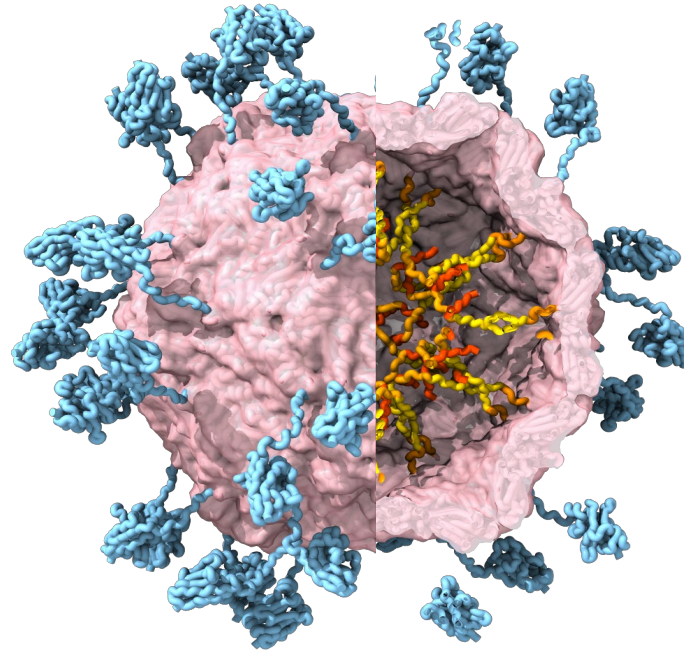
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**VLP sub-unit**



**Functional assembled VLP**

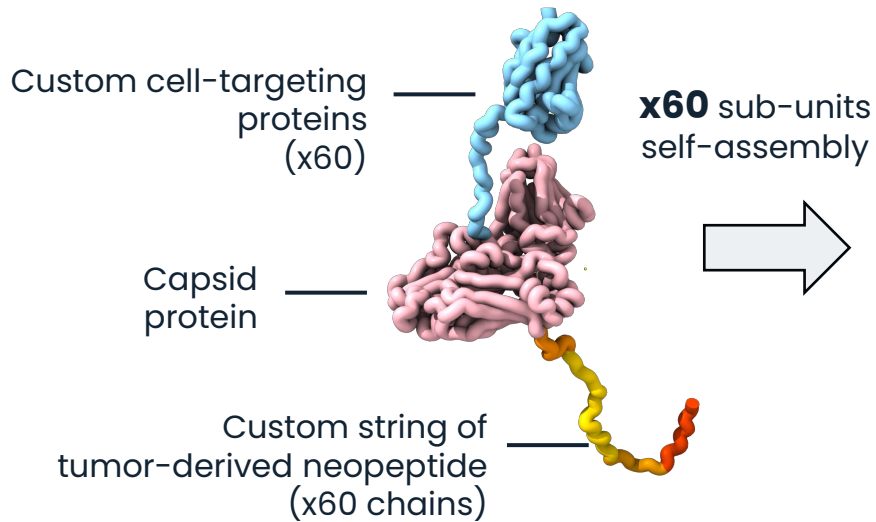


# 1<sup>st</sup> application – A immunization system for therapeutic vaccination

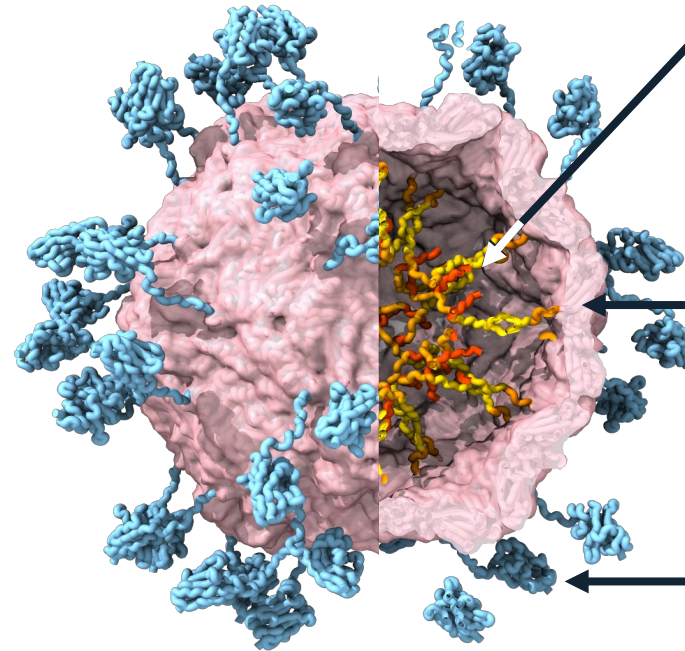


1<sup>st</sup> R&D collaboration on MSS metastatic colon cancer

## VLP sub-unit



## Functional assembled VLP



### 1. Custom payload

**60 x neoantigen strings** for a high-performance immune response  
**>100 AA**



GUSTAVE  
ROUSSY  
CANCER CAMPUS  
GRANDS PARIS



CGFL  
CANCER RESEARCH  
FUNDATION

### 2. Stable nanocarrier

**Neoantigens loading and protection,**  
custom inner/outer surface engineering



### 3. Custom cell targeting

Cell-targeting proteins allows **cell penetration and antigens release**



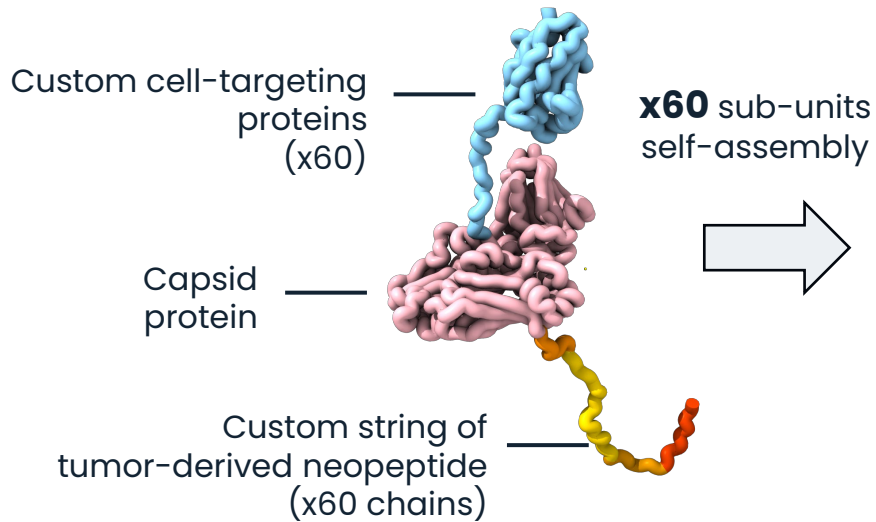


# 1<sup>st</sup> application – A immunization system for therapeutic vaccination

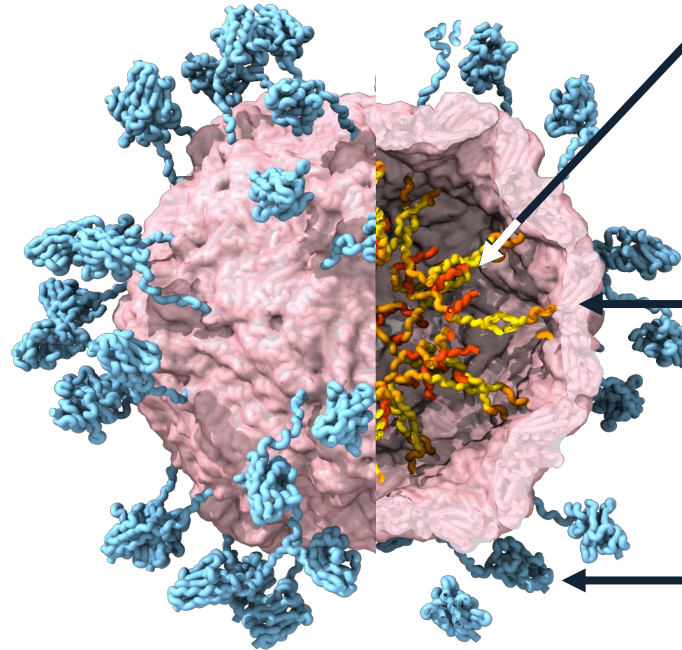


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## VLP sub-unit



## Functional assembled VLP



### 1. Custom payload

**60 x neoantigen strings** for a high-performance immune response  
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GUSTAVE  
ROUSSY  
CANCER CAMPUS  
GRANDS PAYS



CGFL  
CENTRE GUSTAVE ROUSSY  
LABORATOIRE D'ONCOLOGIE  
FONCTIONNELLE

### 2. Stable nanocarrier

**Neoantigens loading and protection,**  
custom inner/outer surface engineering



### 3. Custom cell targeting

Cell-targeting proteins allows **cell penetration and antigens release**



### 4. Plant-based expression

**Human pathogens-free,** fast,  
economic and scalable bioproduction



A single step for sub-unit  
expression and VLP assembly in  
the plant's leaves in **one week**



Nicotiana benthamiana

# Mode of Action – Precise DC-targeting with endosomal routing of peptide antigens



*In vitro preparation*



*In vivo action by sub-cutaneous injection*

1

Immunization system engineering

2

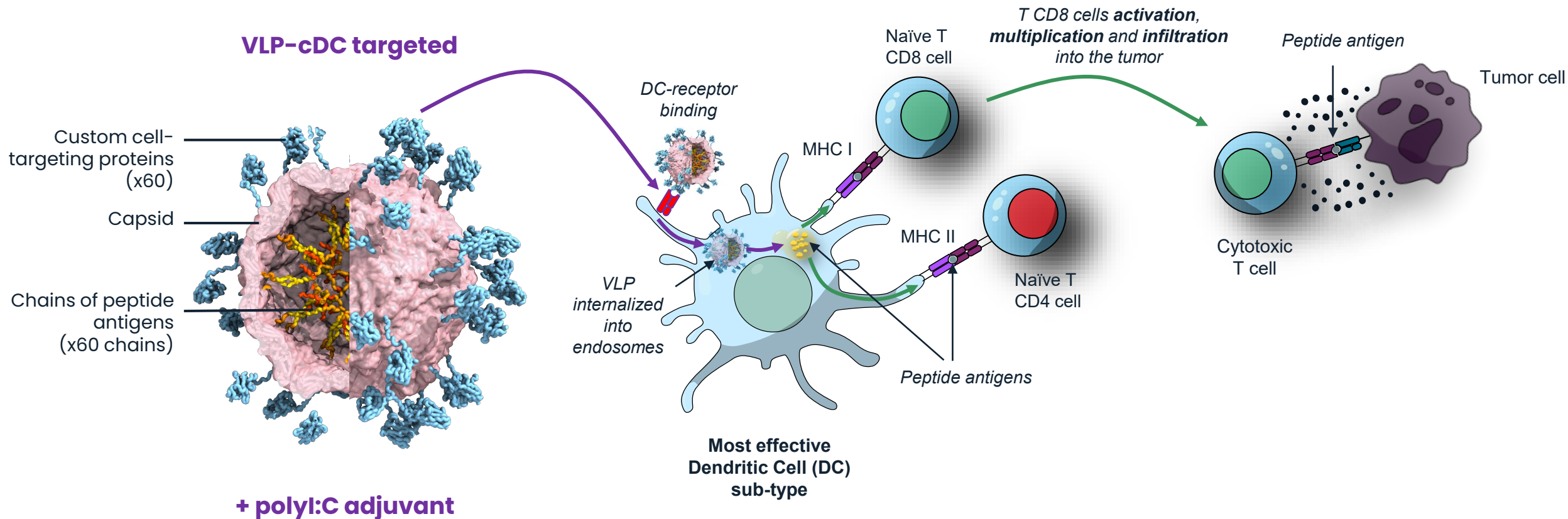
VLP delivery and antigens processing

3

T-cell activation into the lymph nodes

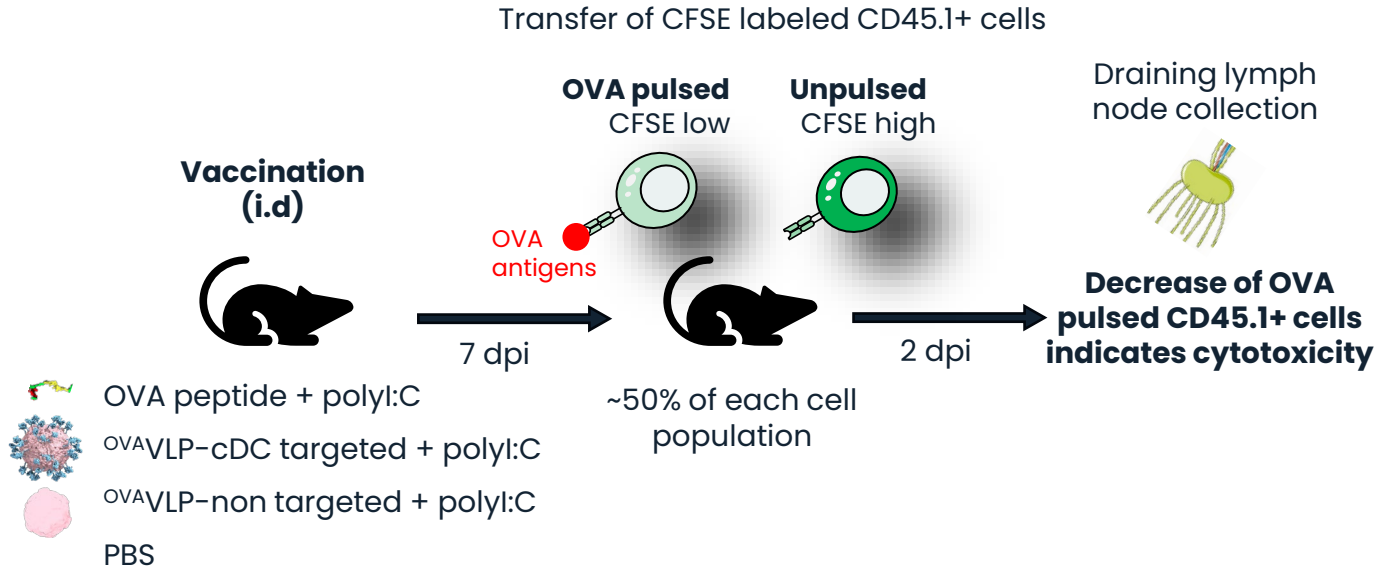
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Tumor-specific cytotoxicity

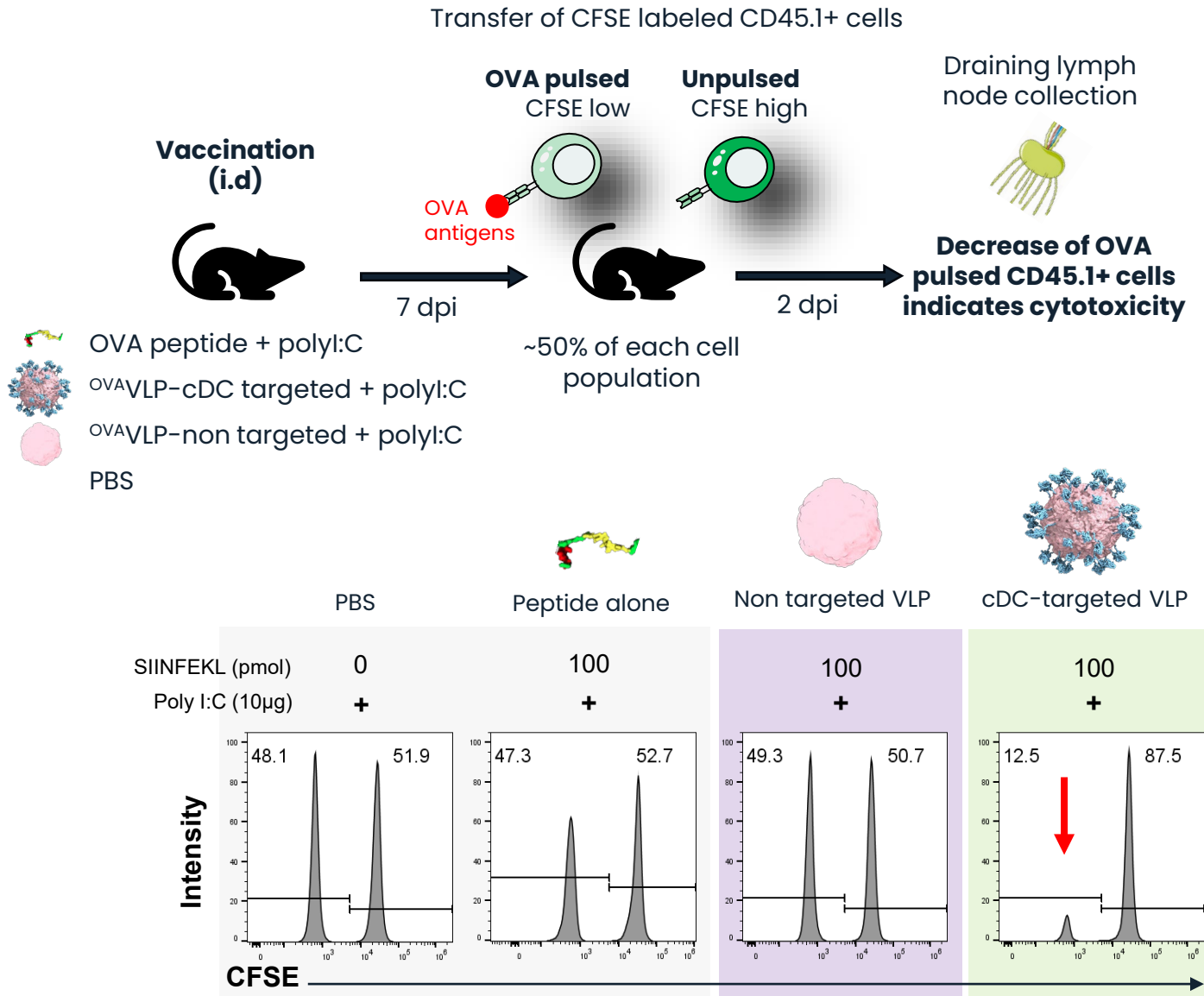




# VLP vaccination induces potent in vivo cytotoxic activity

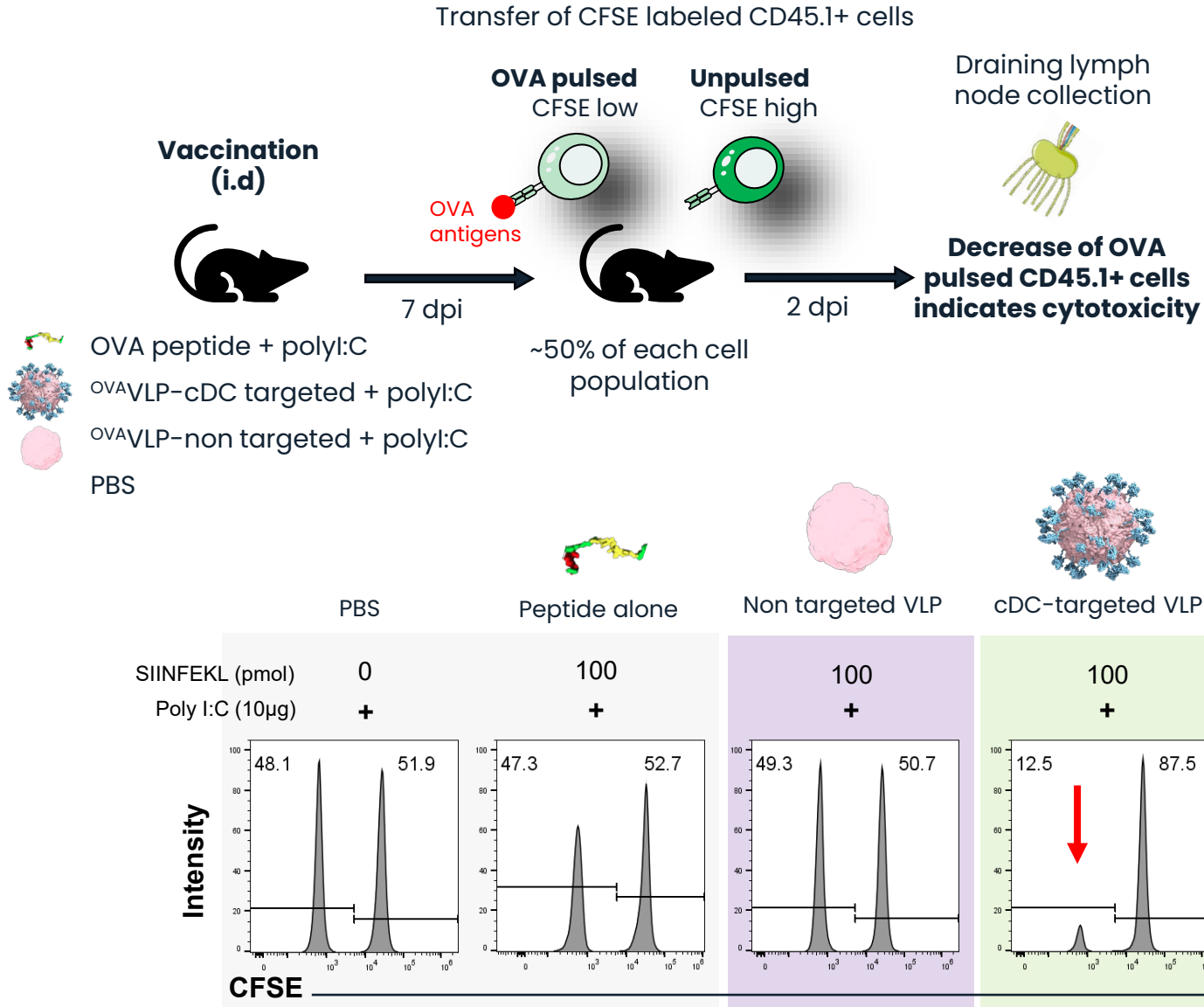


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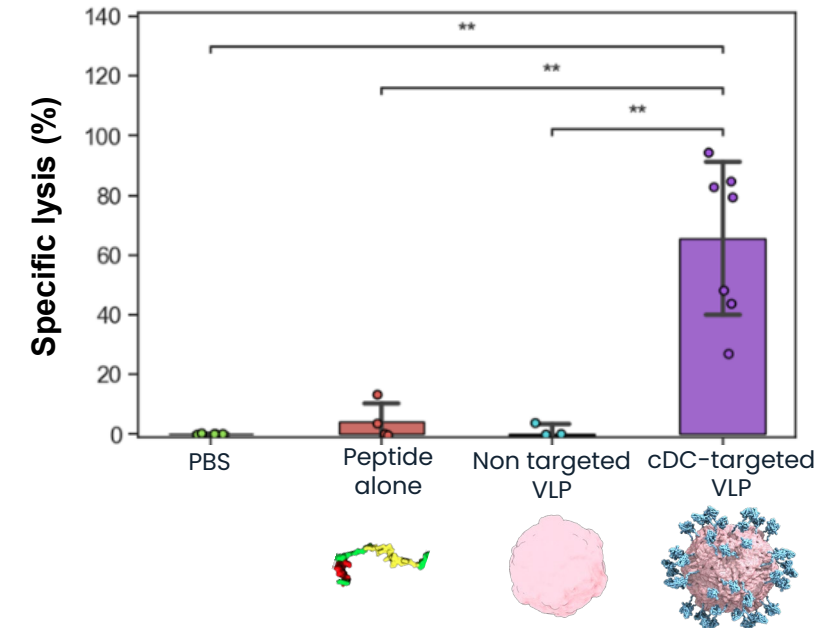




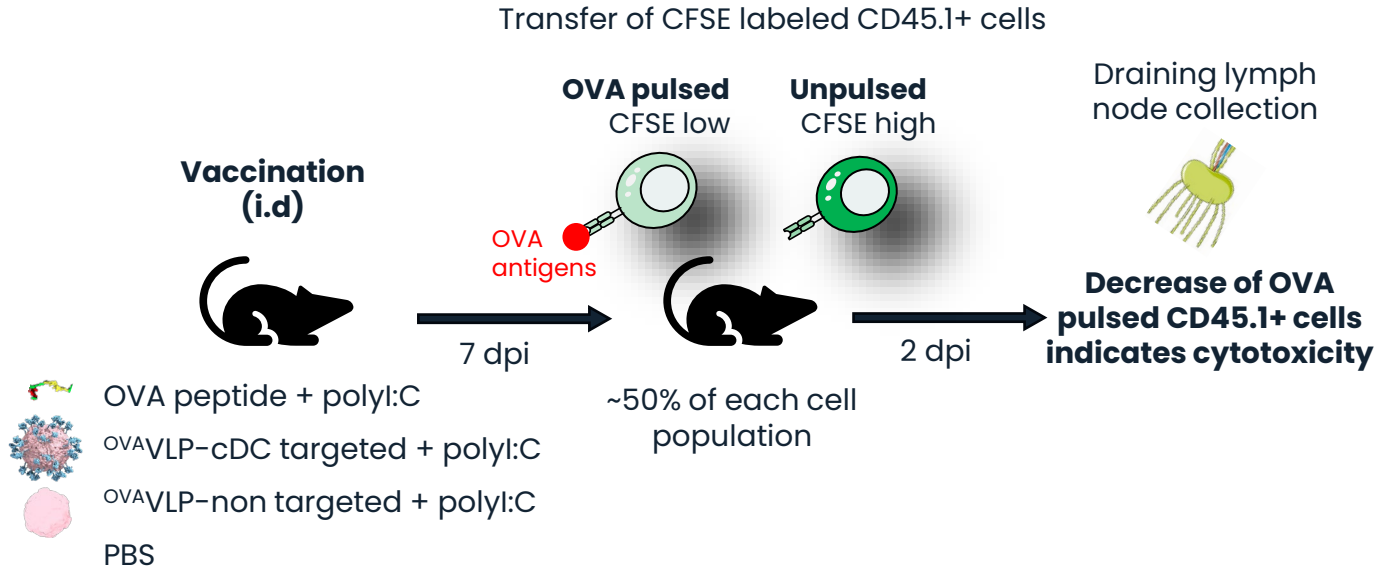
# VLP vaccination induces potent *in vivo* cytotoxic activity



VLP vaccination induces proliferation of T CD8+ and specific cytotoxicity

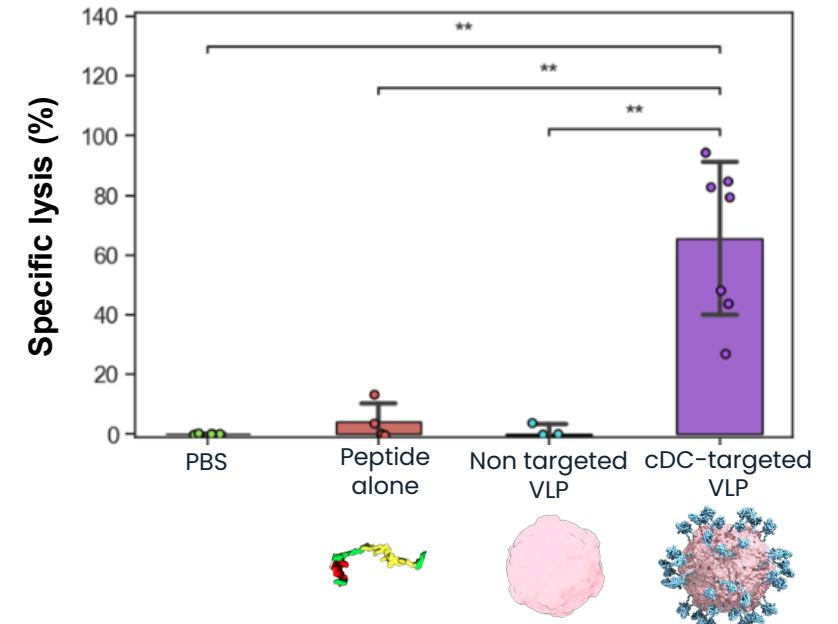
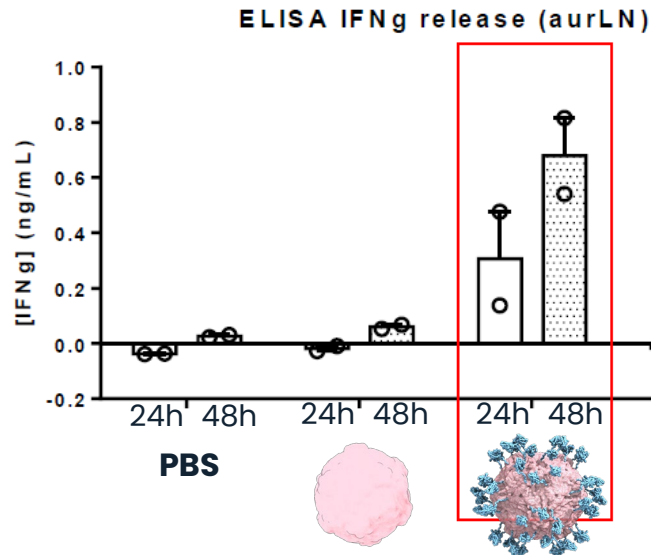


# VLP vaccination induces potent in vivo cytotoxic activity



VLP vaccination induces proliferation of T CD8+ and specific cytotoxicity

Activated T CD8+ produce IFN $\gamma$



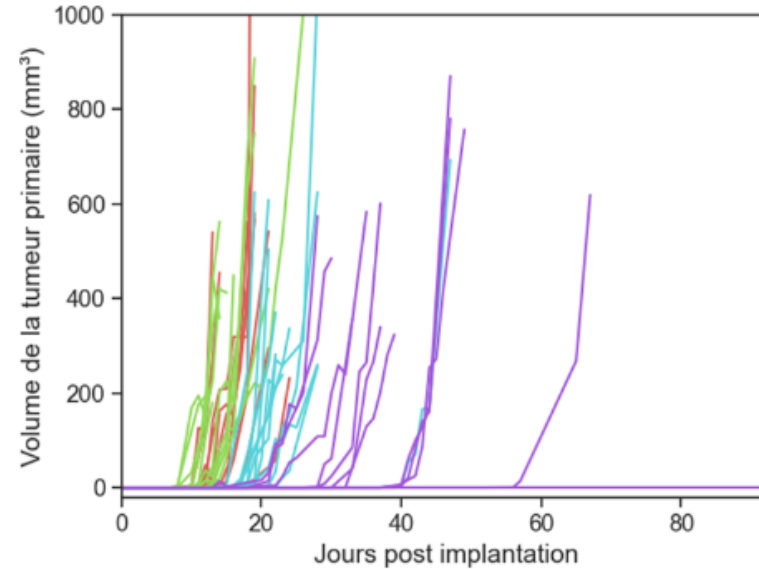
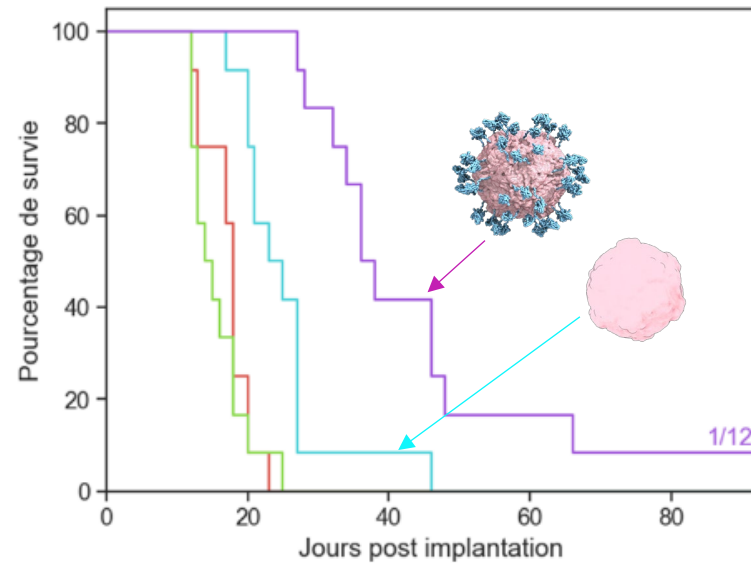


# VLP vaccination improves mouse survival



VLP vaccination  
B16-OVA injection

7 dpi



ns [ — Non traitée  
— Adjuvant seul ]  
\*\* [ — VLP non ciblée  
— VLP ciblée ]

\*\*\*

VLP targeting DC slows tumor growth and improves survival

VLP vaccination protects mice from melanoma challenge (only one injection, low dose)

# Humoral responses against the VLP

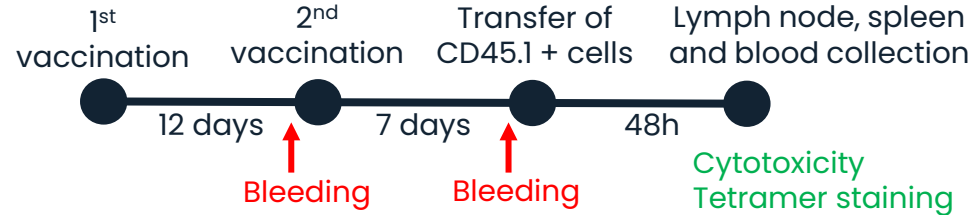


## 1<sup>st</sup> vaccination

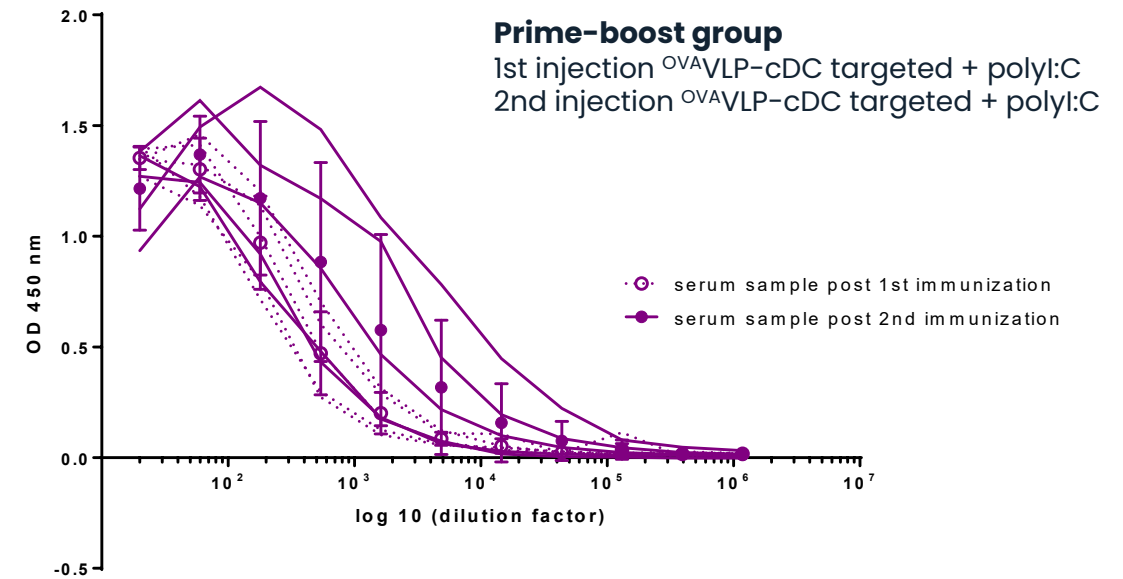
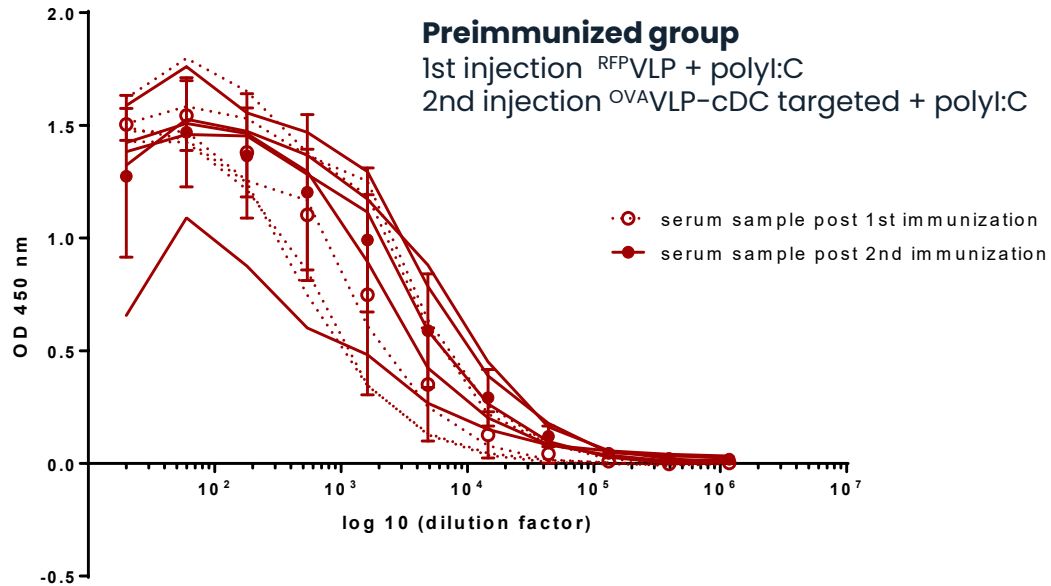
RFPVLP + polyI:C  
OVAVLP-cDC targeted + polyI:C  
PBS

## 2<sup>nd</sup> vaccination

OVAVLP-cDC targeted + polyI:C  
PBS



ELISA : @VLP IgG titration



VLP vaccination induce strong humoral responses with anti-VLP IgG production in mouse serum

Preimmunized and Prime boost regimen present a similar IgG titration profile

# Impact of anti-VLP humoral responses on TCD8+ cytotoxicity

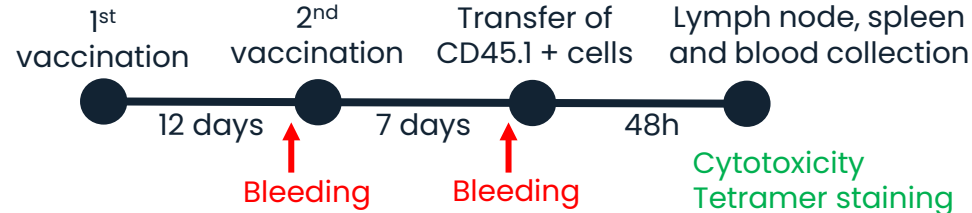


## 1<sup>st</sup> vaccination

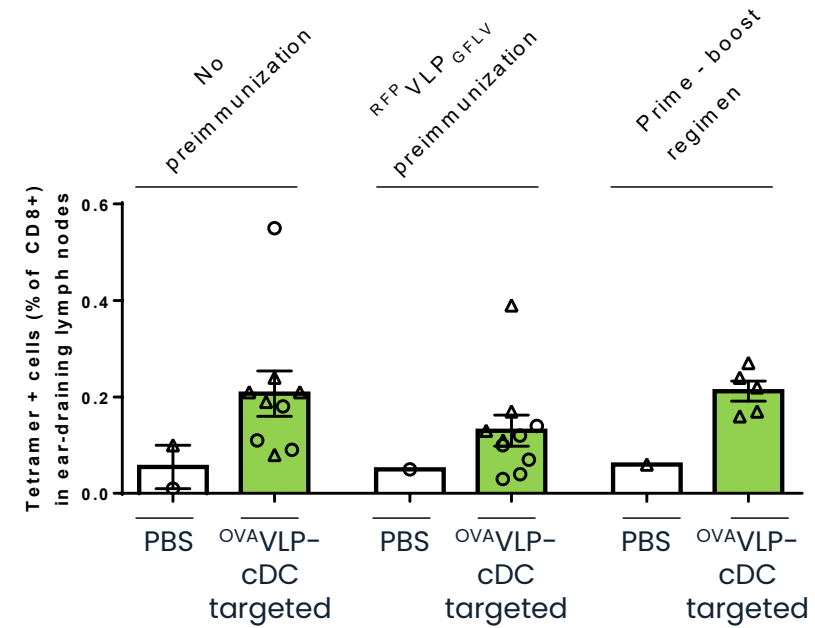
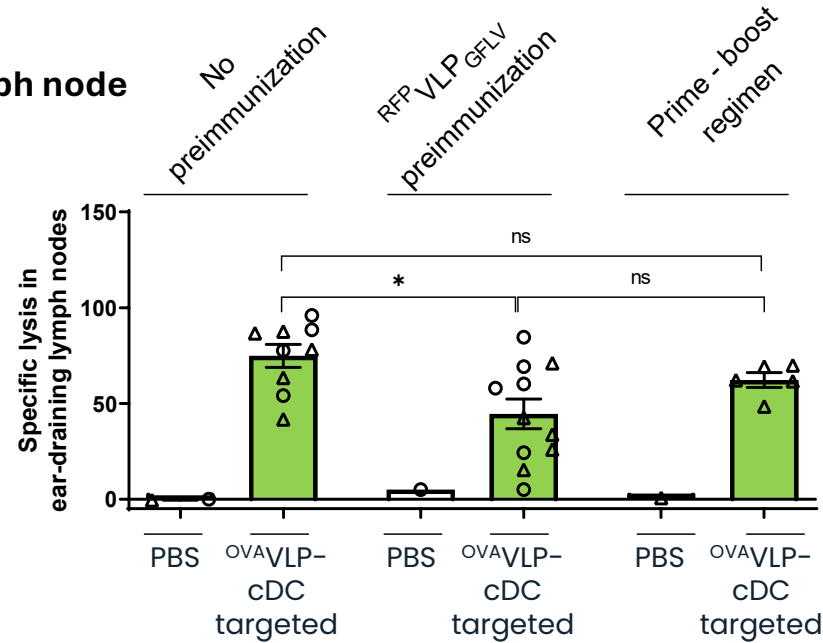
RFP VLP + polyI:C  
OVA VLP-cDC targeted + polyI:C  
PBS

## 2<sup>nd</sup> vaccination

OVA VLP-cDC targeted + polyI:C  
PBS



## Draining lymph node



Humoral response against VLP slightly decrease but do not abolish cytotoxic responses and tetramer positive cells (similar results in the spleen)

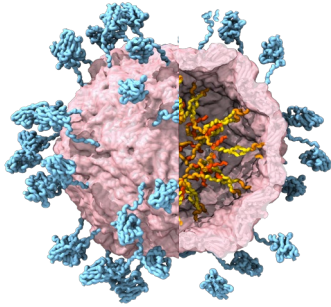
**Cytotoxic responses are still efficient despite IgG production in a prime-boost regimen**



# Design of VLP constructs for efficacy testing on colorectal model MC38



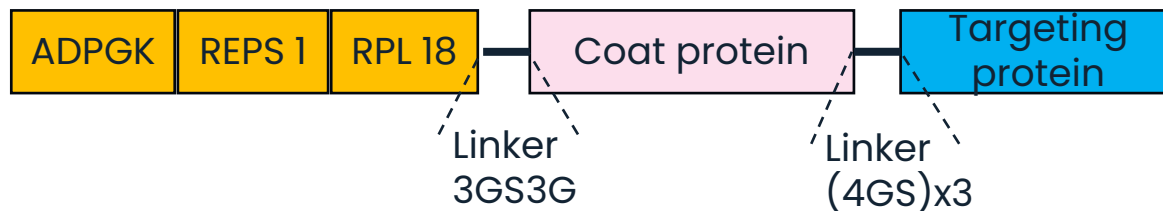
## VLP construct



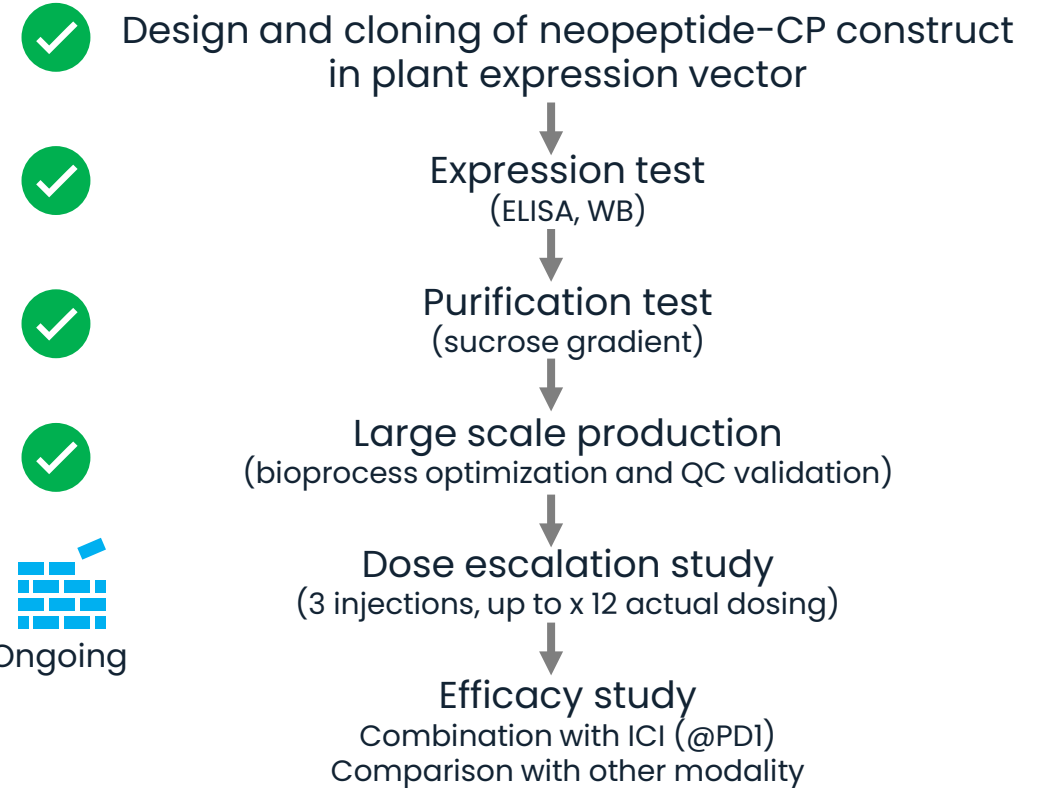
VLP containing a string of 3 long neopeptides

Gene name	Neopeptide sequence
• RPL 18 :	KAGGKILTFD <b>R</b> LALESPK
• ADPGK :	HLELASMTN <b>M</b> ELMSSIVHQ
• REPS 1 :	RVLELFRAAQL <b>A</b> NDVVLQIME

Design of the neopeptide-CP fusion protein



## Work Flow

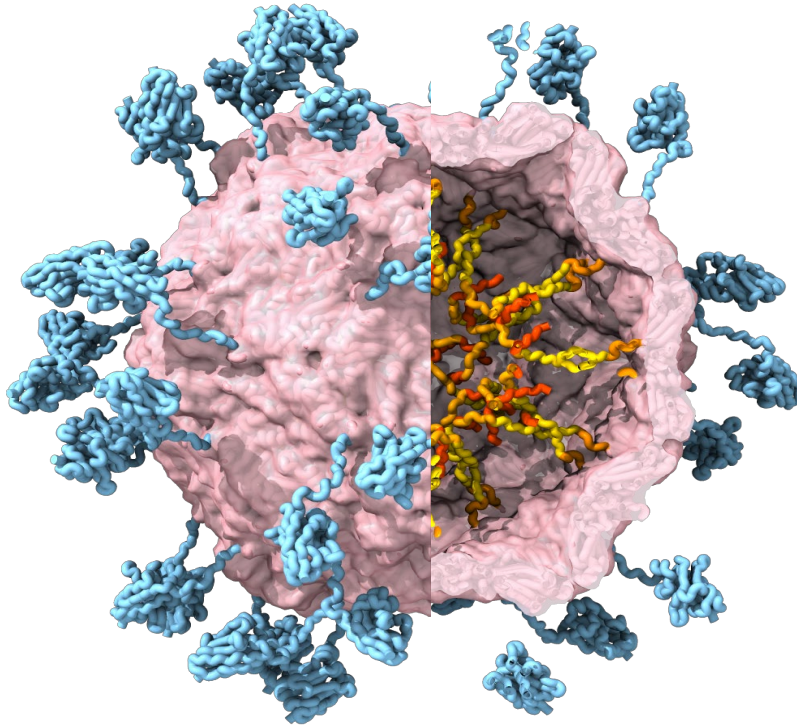


**Expected robust antitumoral responses with repeated injections**, best dose selection

Studies with CT26 and human organoids models are planned soon



## VLP nanocarrier



## VLP unique advantages

- Trigger **potent cytotoxic T cell** responses
- **Delivery capacity** to specific cells in vivo
- **Ease of customization**, tailor-made VLP
- **High density** of surface **display and encapsulation** capacity
- **Simple manufacturing**, one step production process
- Potential **good safety** profile

**Theoretically unlimited modification capacity and therapeutic applications**

We are **seeking clinicians** and **industrial partners** to develop our products  
(and seeking cash obviously, we are a start-up! 😊)

# Acknowledgement



## Academic laboratories



## Start-up incubator



## Technology transfer office



## Funding



## Training





# Thank you !

*For any question, please contact :*

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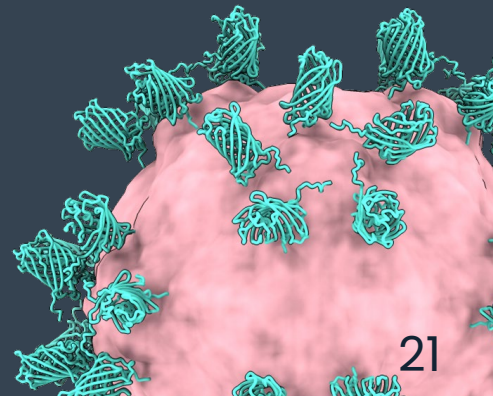
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# *Annexes*

