Cancer vaccine discovery capability

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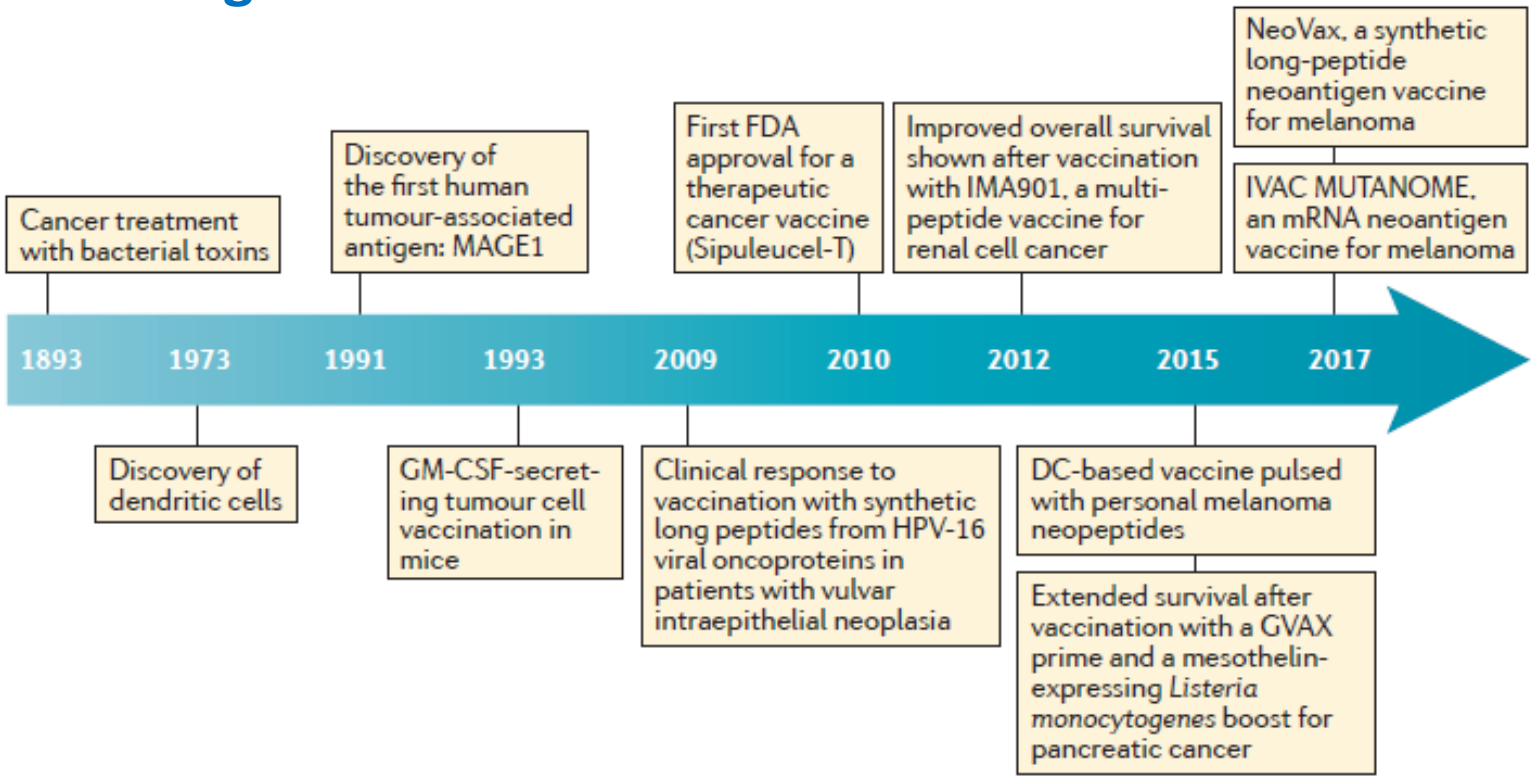
Outline

- **Background**
- **Case studies of cancer vaccine discovery**
  - Tumor antigen prediction and *in vivo* immunogenicity validation
  - Determine immunogenicity of peptides using Elispot *in vitro* assay
  - *In vivo* validation of predicted peptides using B16F10 tumor model
  - *In vivo* validation of predicted peptides using CT26 tumor model
  - *In vivo* validation of peptide-loaded DC vaccine using B16F10 tumor model

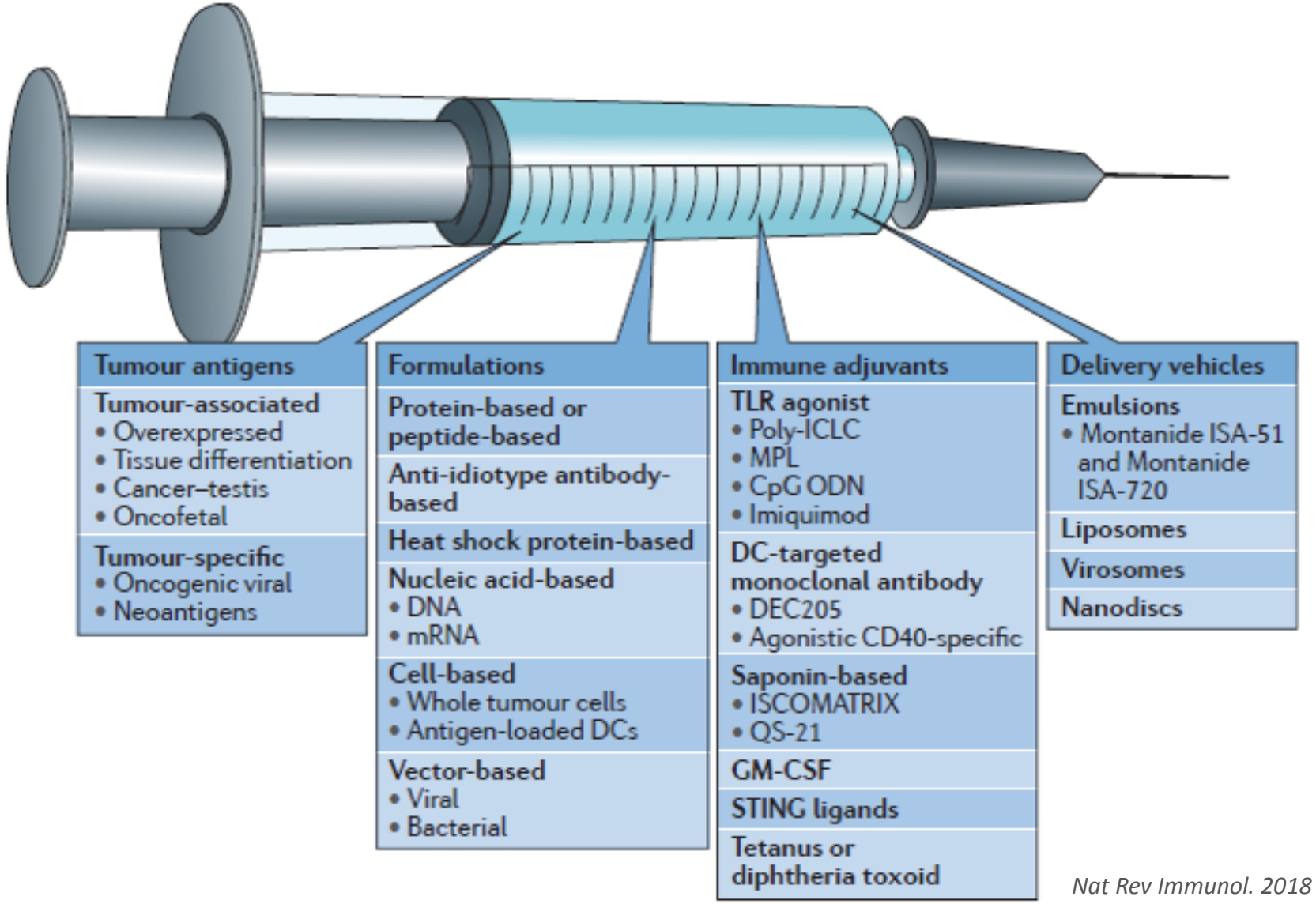
History of tumor antigens and cancer vaccines



Trends Immunol. 2017 Aug;38(8):577-593

- Cancer vaccines have long been envisioned as a key tool of effective cancer immunotherapy.
- The clinical benefit of therapeutic cancer vaccines has been established. Clinical benefit in cancer patients was mostly noted as prolonged survival.
- In 2010, the autologous DC-based prostate cancer vaccine Sipuleucel-T (Provenge; Dendreon) became the first human therapeutic cancer vaccine to be approved by the US Food and Drug Administration (FDA).

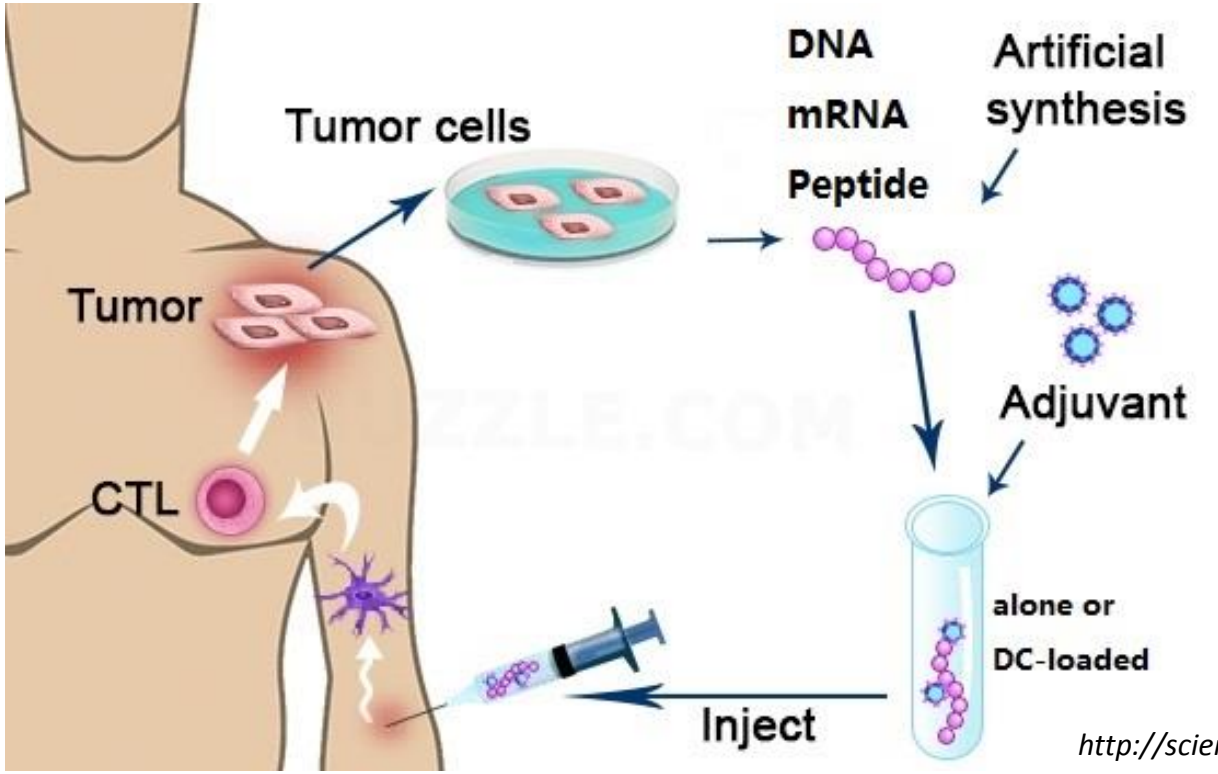
Four key components of cancer vaccines



Nat Rev Immunol. 2018 Mar;18(3):168-182

- There are four key components of cancer vaccines: tumor antigens, formulations, immune adjuvants and delivery vehicles.

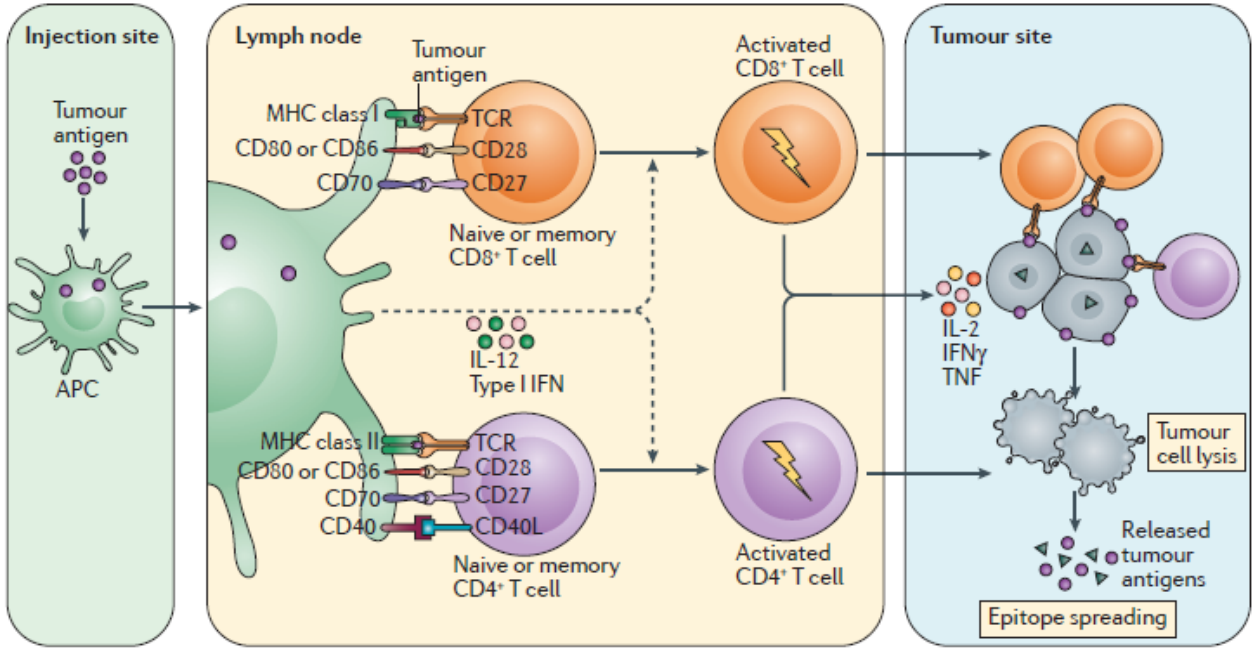
Cancer vaccine therapy



http://sciencedrivennutrition.com/vaccines-and-autism

- Tumor-specific antigens (TSA), a single peptide/mRNA or a cocktail of peptides/mRNAs, were purified from cancer cells of the patient himself or synthesized artificially.
- The peptide/mRNA vaccine is formulated with adjuvant or loaded with DCs, and then is injected into the patient.
- The APCs of the patient's immune system engulf these peptides or translate mRNAs in to peptides , and present them on the surface in order to educate the other immune cells.
- The educated immune cells, when encounter the same antigen on a cancerous cell, bring about the destruction of that cell.

Mechanisms of an effective cancer vaccine



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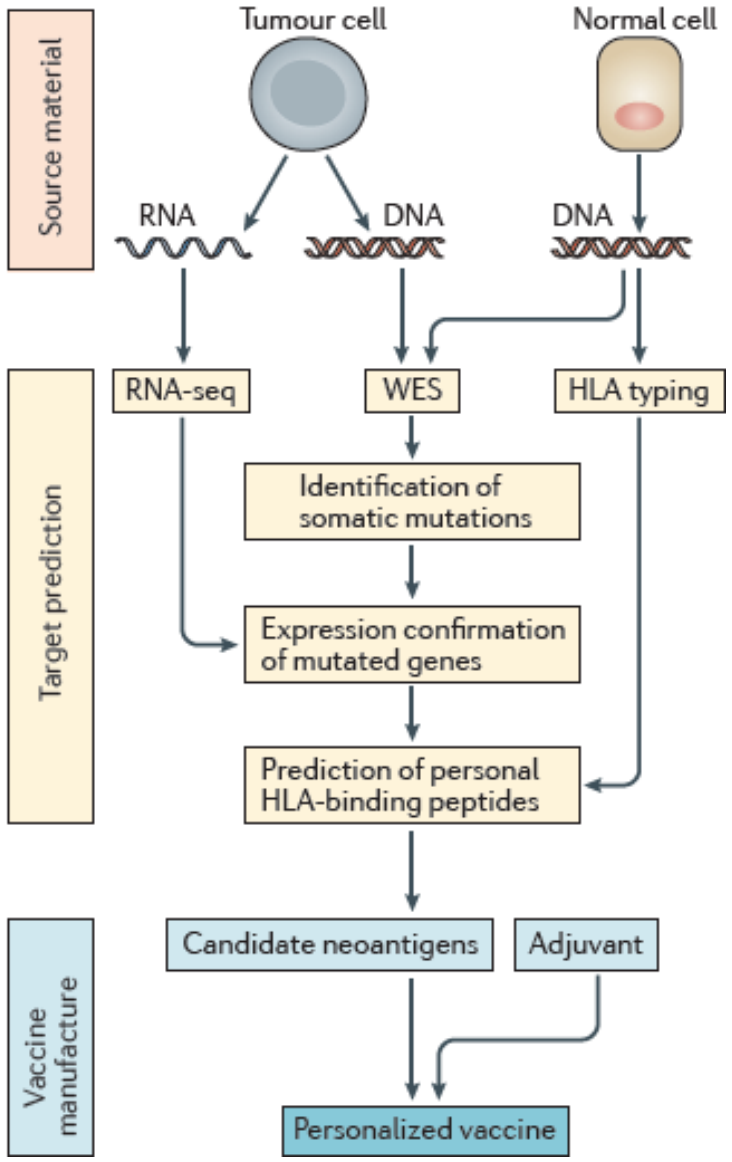
- The antigen-loaded DCs traffic through the lymphatics from the injection site to the draining lymph nodes, where mature DCs present the tumor-derived peptides on MHC class I molecules and MHC class II molecules to promote the generation and expansion of activated tumor-specific CD8+ and CD4+ T cell populations, respectively.

- tumor-specific CD4+ and CD8+ T cells traffic to the tumor site, and upon encountering their cognate antigens, they can kill tumor cells through cytotoxicity and the production of effector cytokines, such as IFNγ and tumor necrosis factor (TNF).

- In turn, the lysed tumor cells release tumor antigens that can again be captured, processed and presented by APCs to induce polyclonal T cell responses, thereby increasing the antigenic breadth of the antitumor immune response and leading to the process of epitope spreading.

The typical workflow for cancer vaccine discovery

- DNA and RNA are extracted from single-cell suspensions of tumor cells and matched normal tissue cells.
- Somatic mutations of tumor cells are discovered by whole-exome sequencing (WES). RNA sequencing (RNA-seq) narrows the focus to mutations of expressed genes. Clinical HLA typing is carried out on DNA from normal tissue.
- The potential antigenicity of neo-epitopes identified by WES and RNA-seq is assessed by predicting the affinity of the neo-epitopes for binding to the HLA type of that individual (using NetMHCpan), thereby generating candidate vaccine epitopes.
- Validated epitopes are selected for incorporation into the personalized cancer vaccine, which is administered to patients in combination with an immune adjuvant.



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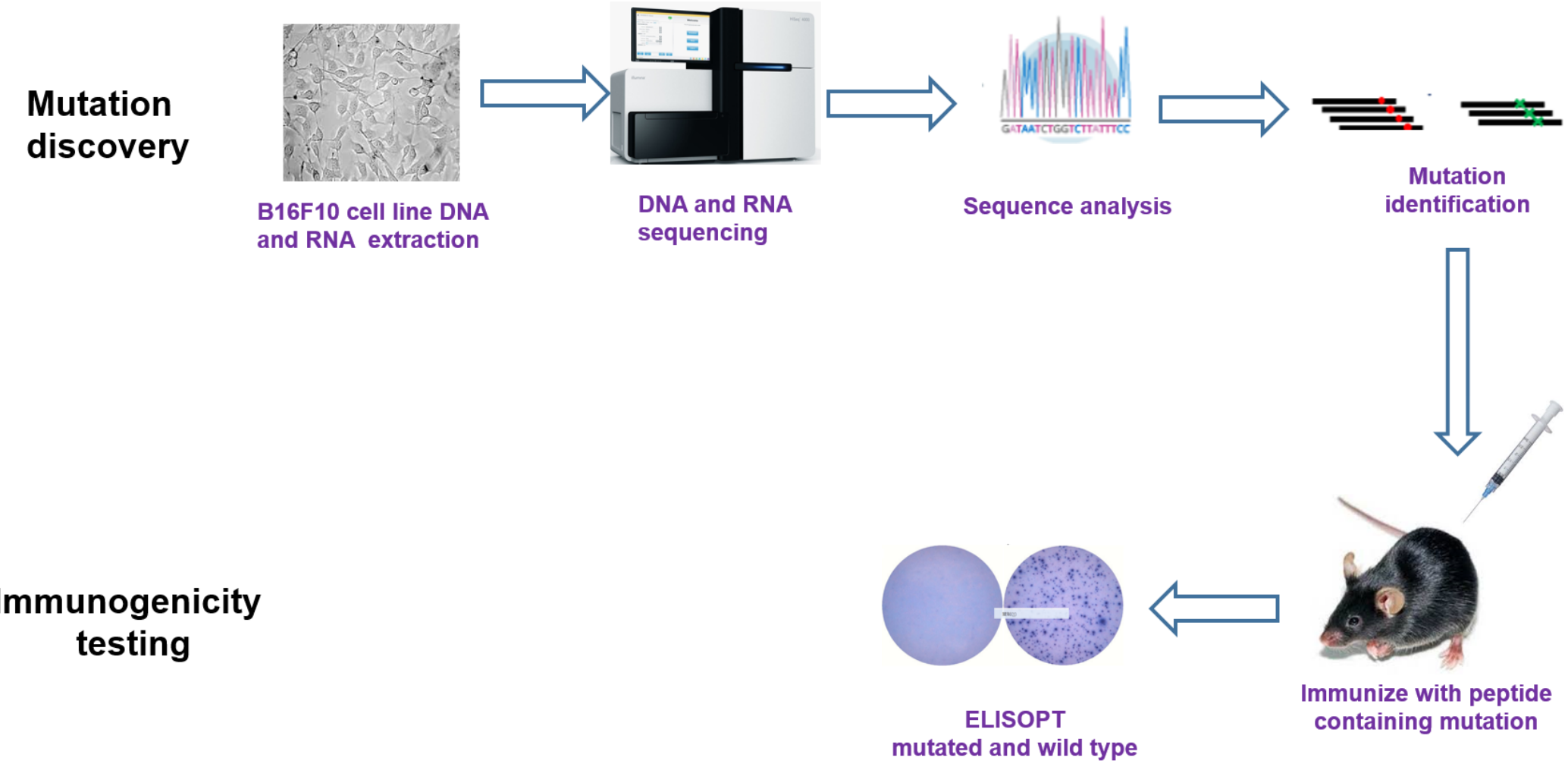
A case study of cancer vaccine discovery

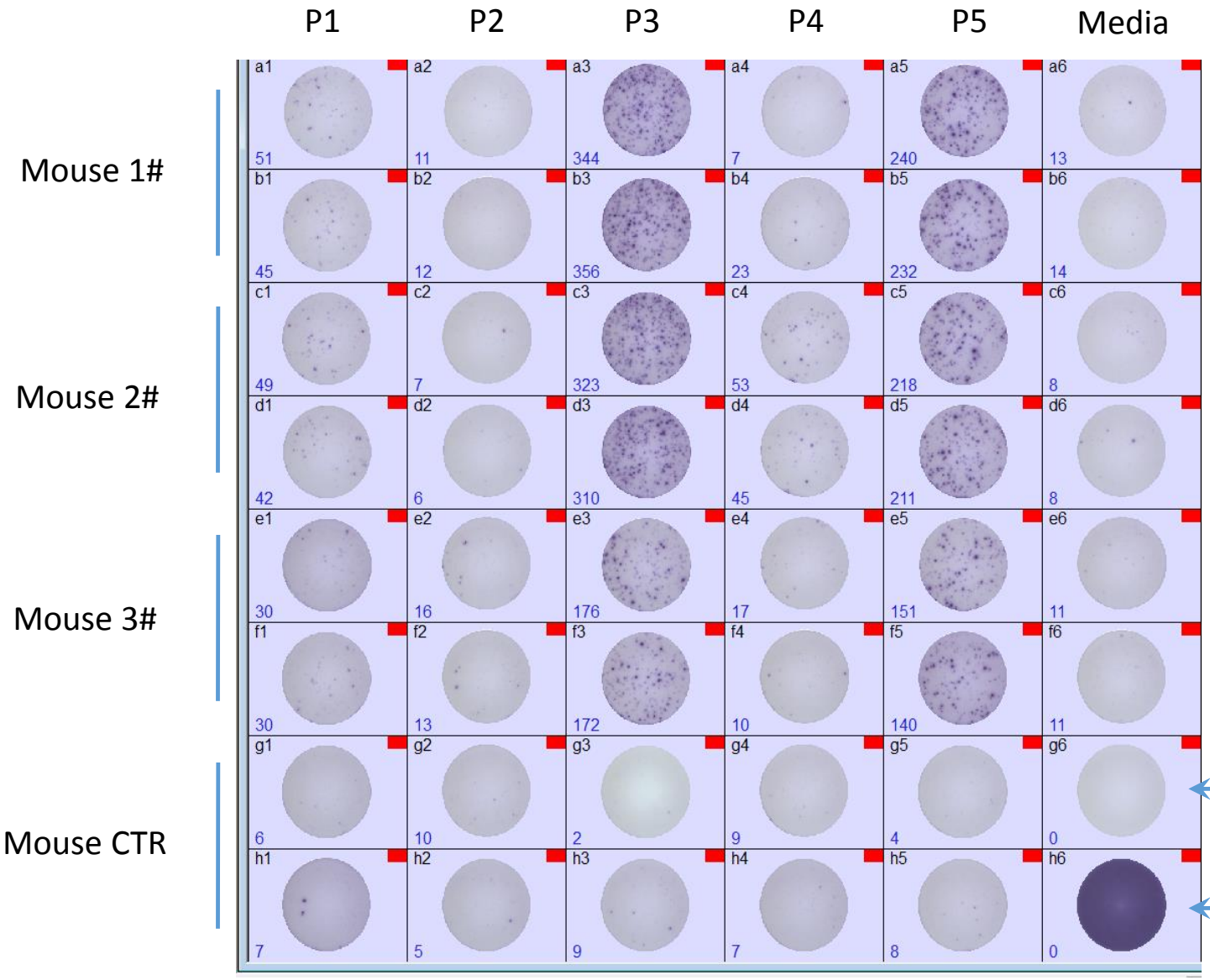
Tumor antigen prediction and *in vivo* immunogenicity validation: B16F10 tumor



- Potentially immunogenic somatic point mutations in B16F10 mouse melanoma were identified by NGS.
- The *in vivo* immunogenicity was tested by peptide vaccination of mice measuring elicited T-cell responses by ELISPOT assay.

A case study of cancer vaccine discovery

Determine immunogenicity of peptides using Elispot in vitro assay



P3 and P5: strong immunogeneicity
P1 and P4: weak immunogeneicity
P2: no immunogeneicity

Note:
Mouse CTR: mouse immunized by non-relevant peptides.

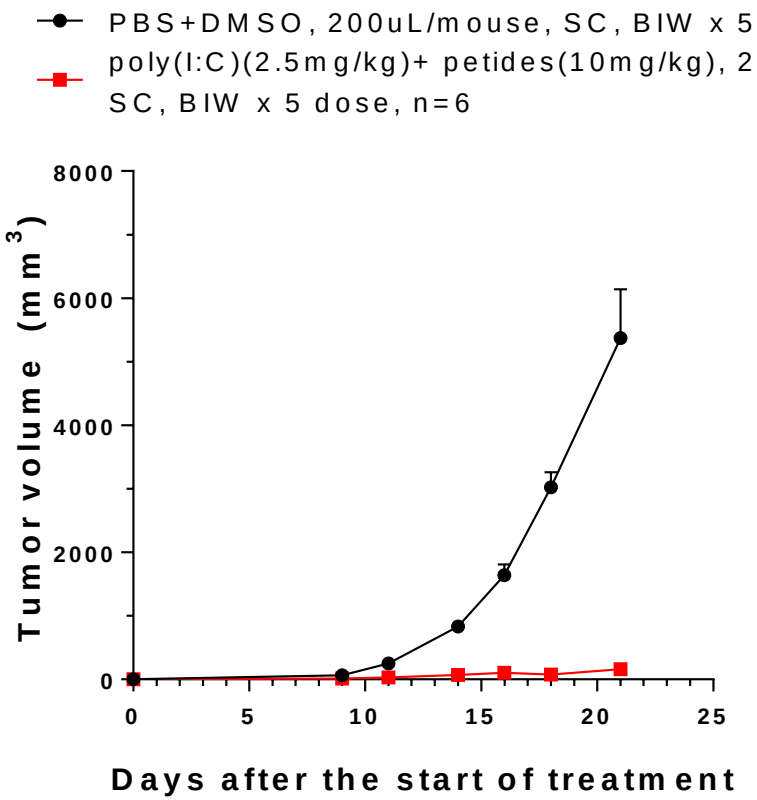
Media: no peptide added to cultured cells.

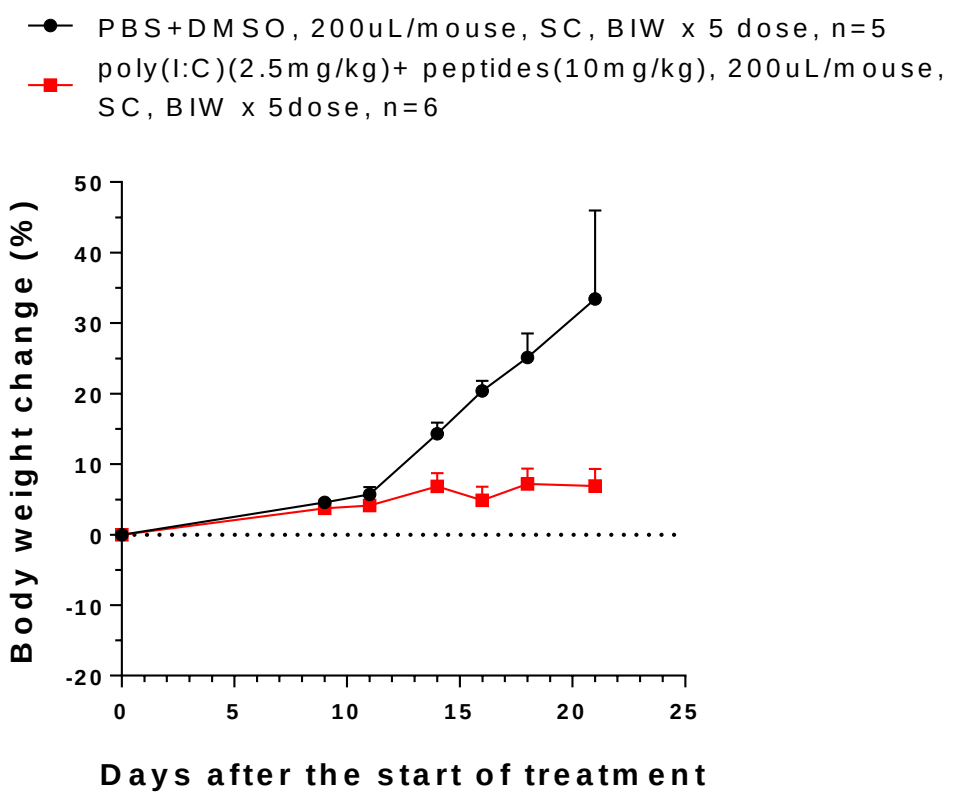
Media
No cells

rIFNγ

A case study of cancer vaccine discovery

In vivo validation of peptide vaccine: B16F10 tumor model

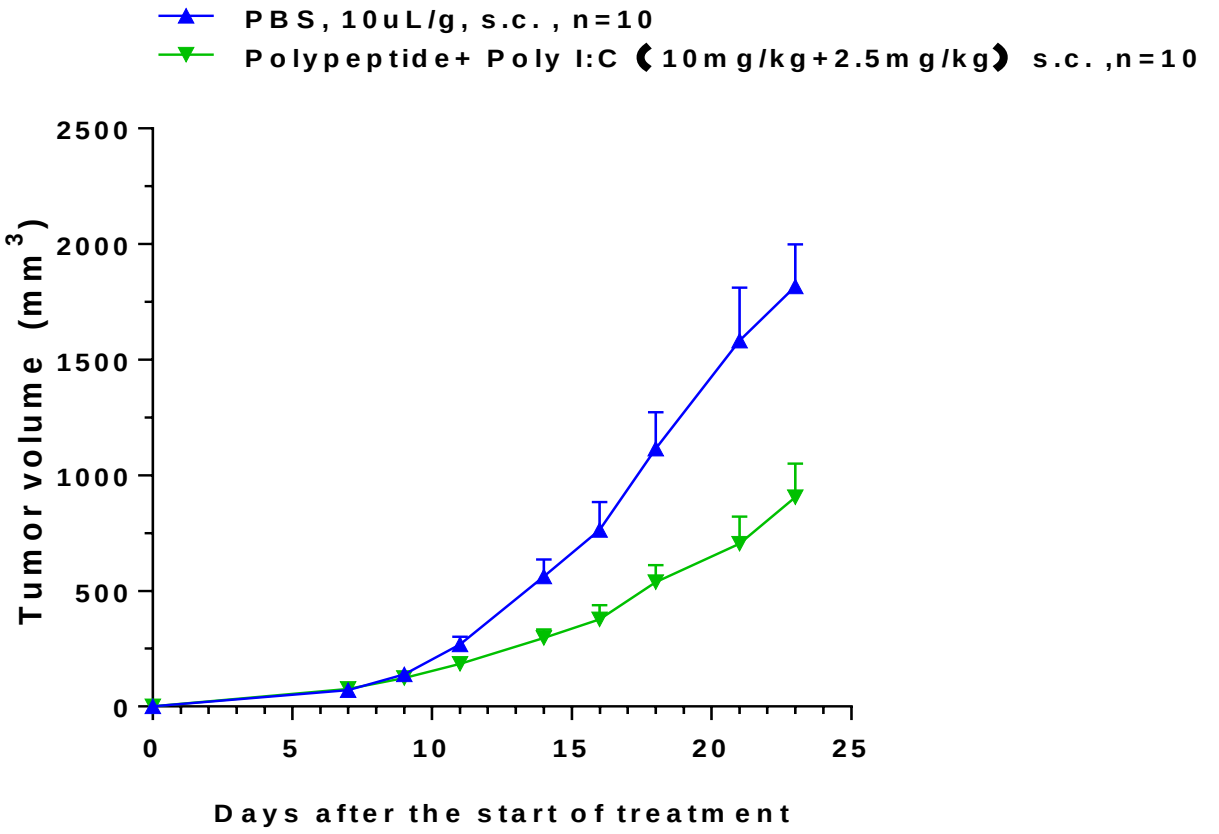




- Elispot validated peptides were pooled and S.C administrated into mouse bearing B16F10 tumor.
- The *in vivo* tumor growth inhibition effect was evaluated under peptide treatment.

A case study of cancer vaccine discovery

In vivo validation of peptide vaccine: CT26 tumor model



- 4 MHC I-restricted peptides and 4 MHC II-restricted peptides were pooled and S.C administrated into mouse bearing CT26 tumor.
- The *in vivo* tumor growth inhibition effect was evaluated under peptide treatment.

A case study of cancer vaccine discovery

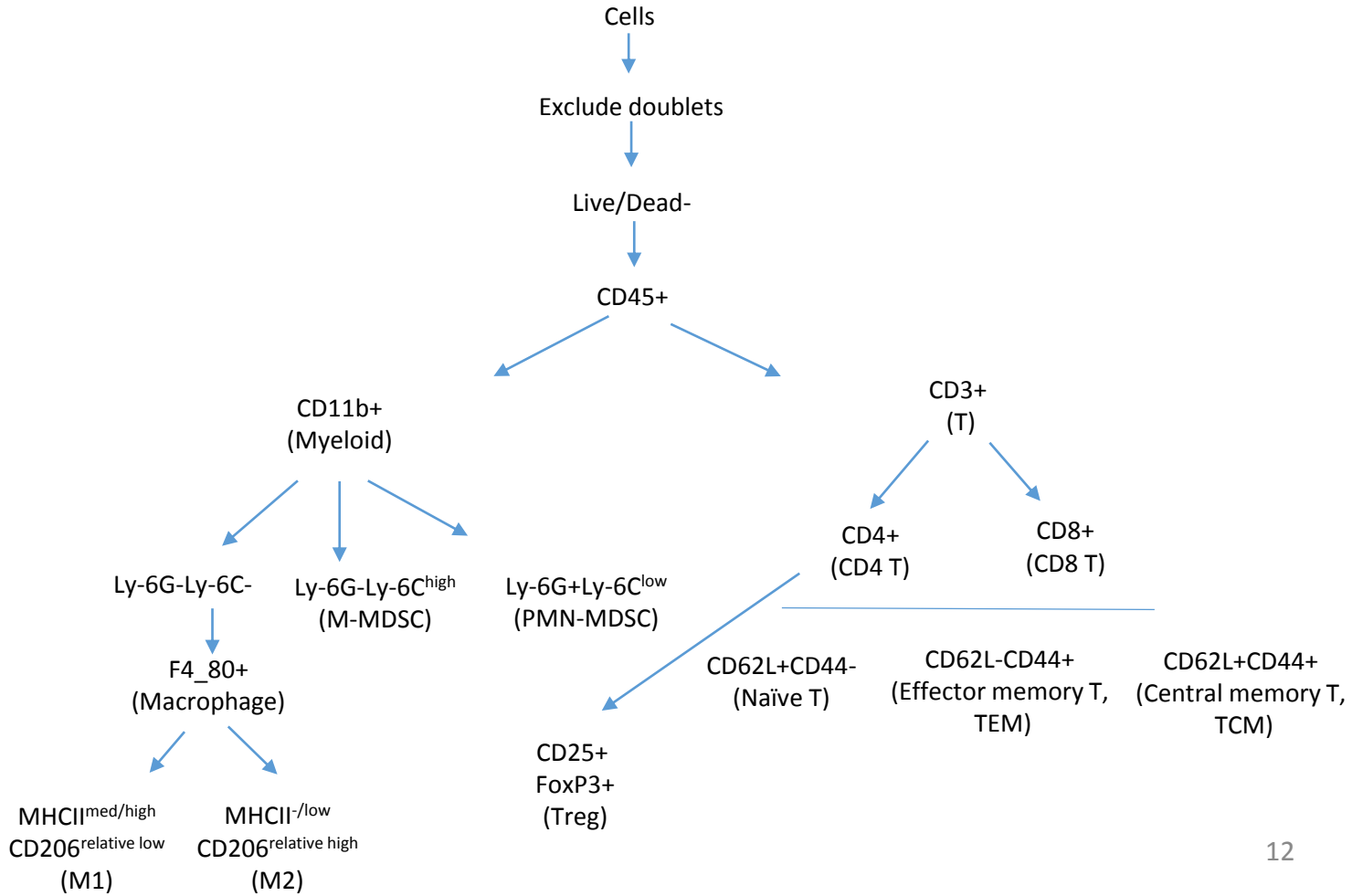
In vivo validation of peptide vaccine: CT26 tumor model

This panel was designed to analyze:

- The percentage of T, CD4 T, CD8 T, Treg, central memory CD4/CD8 T, effector memory CD4/CD8 T and naïve CD4/CD8 T populations in CD45+ cells in tumor and spleen.
- The percentages of Myeloid, M-MDSC, PMN-MDSC, Macrophage, M1/M2 Macrophage in CD45+ cells in tumor and spleen

Panel
CD45
CD4
CD8
Live/Dead
MHCII
CD206
CD25
CD62L
CD44
F4_80
Ly-6C
FoxP3+
CD11b+
Ly-6G
CD3

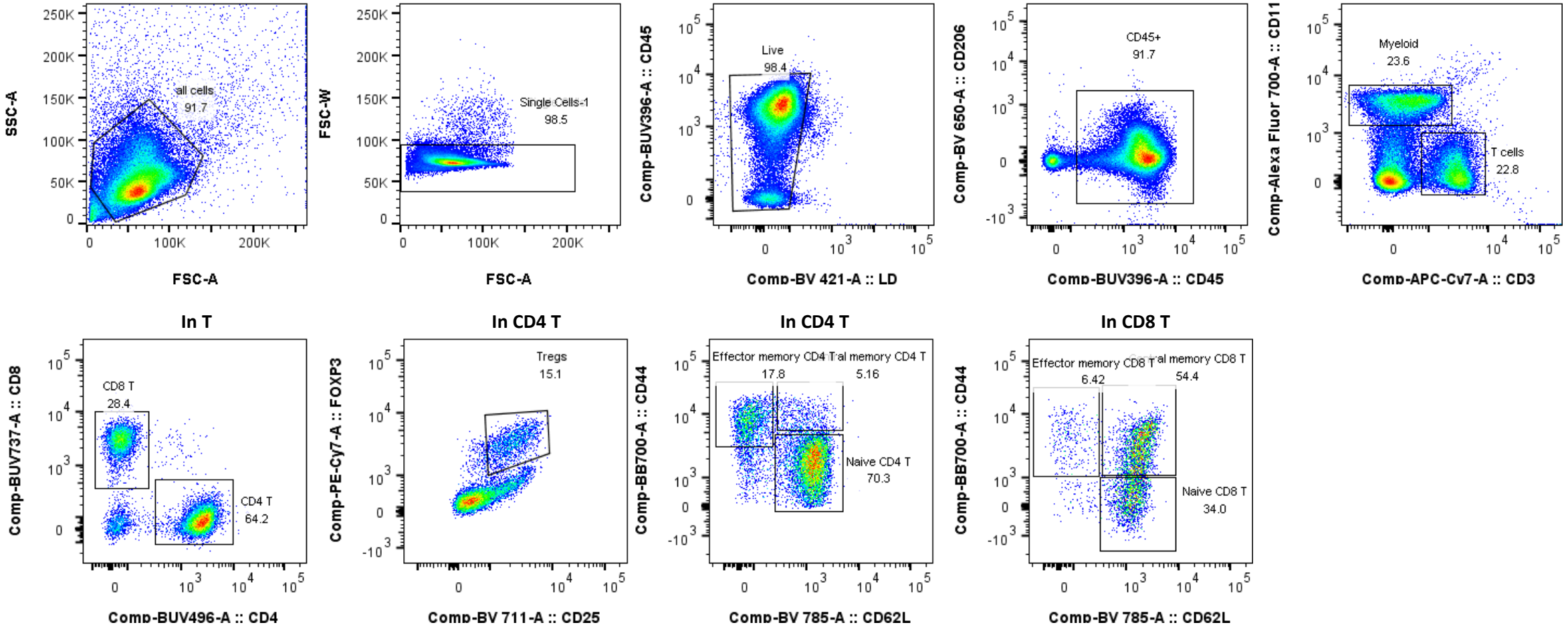
Gating strategy for immune cell subpopulaiton analysis



A case study of cancer vaccine discovery

In vivo validation of peptide vaccine: CT26 tumor model

Gating strategy for spleen



A case study of cancer vaccine discovery

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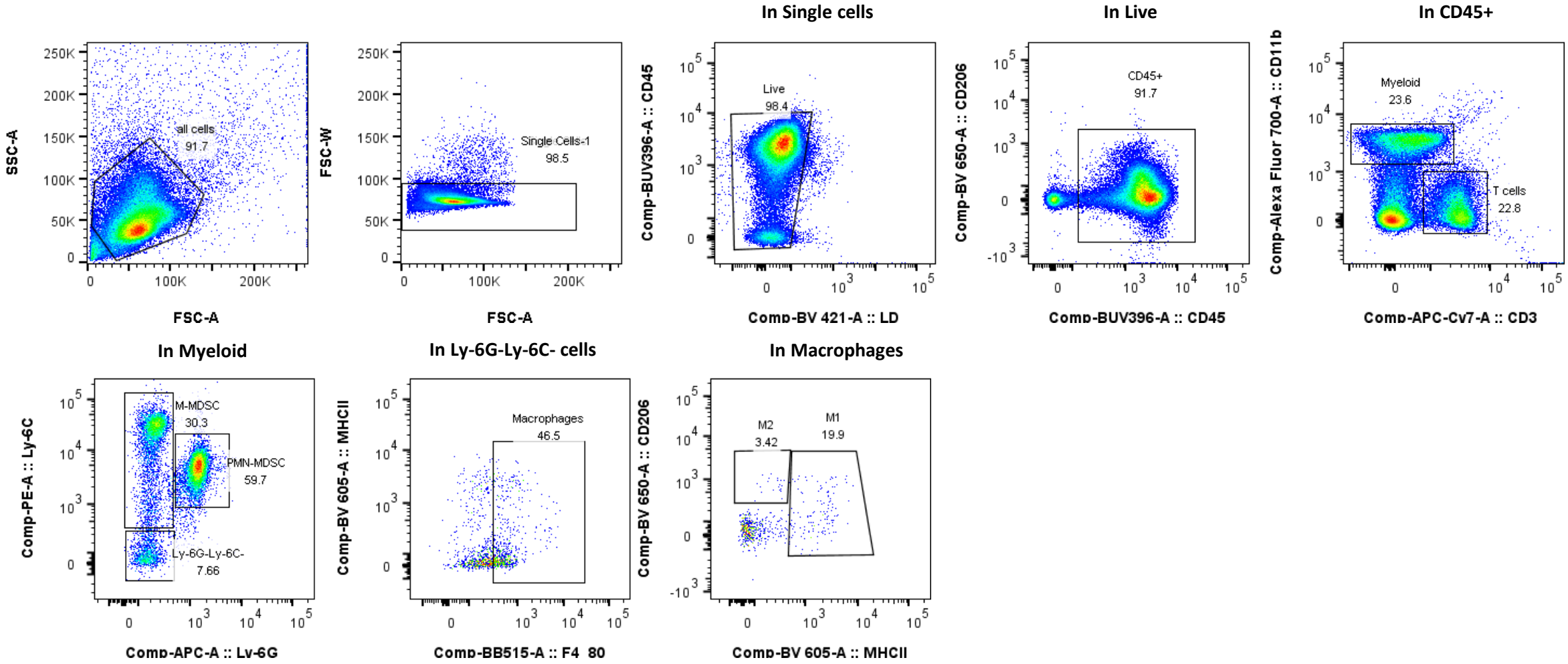
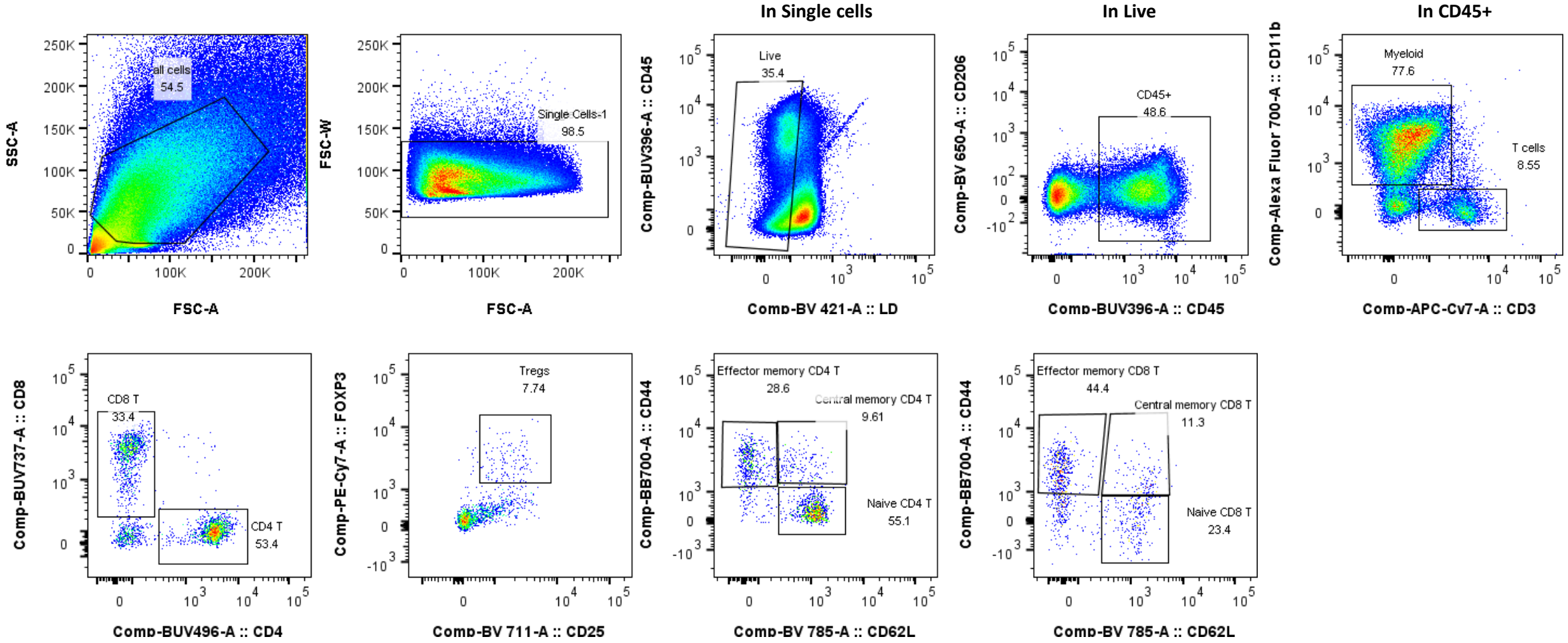


A case study of cancer vaccine discovery

In vivo validation of peptide vaccine: CT26 tumor model

Gating strategy for tumor



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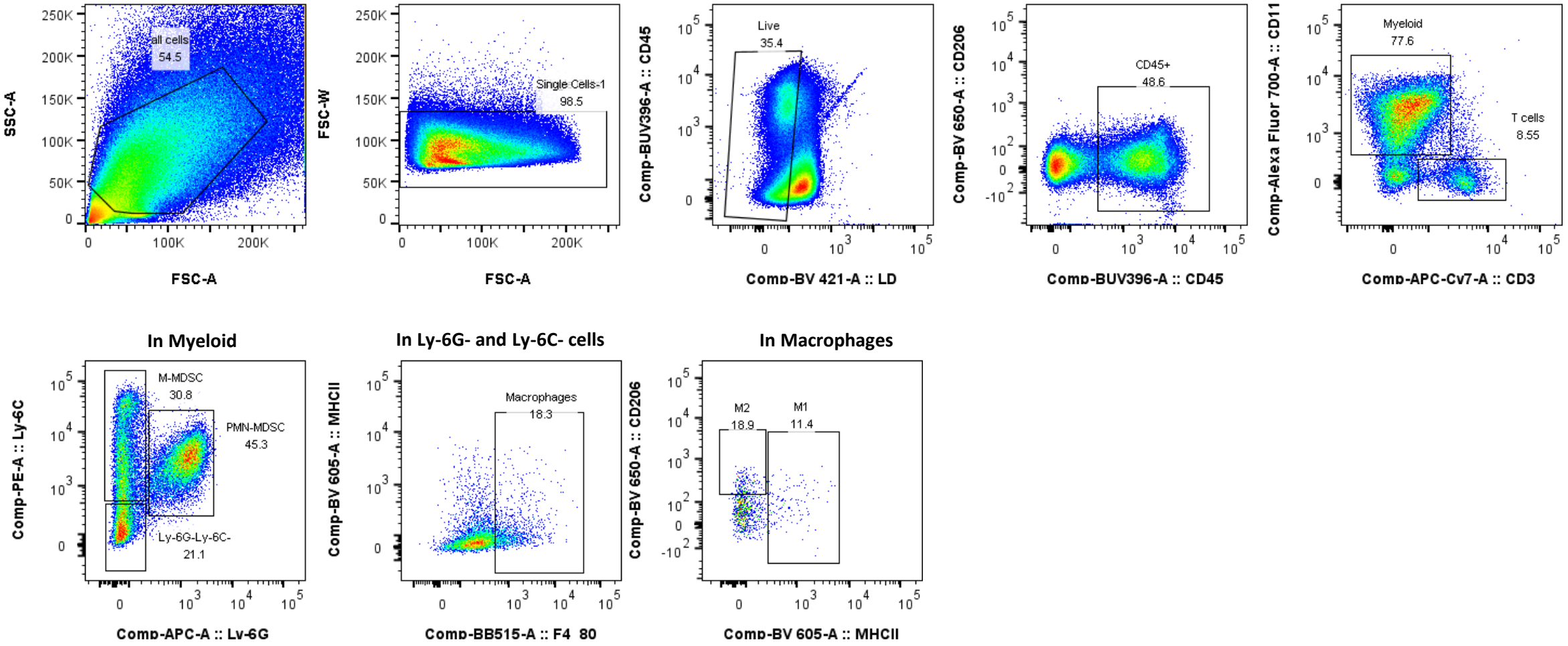
In vivo validation of peptide vaccine: CT26 tumor model

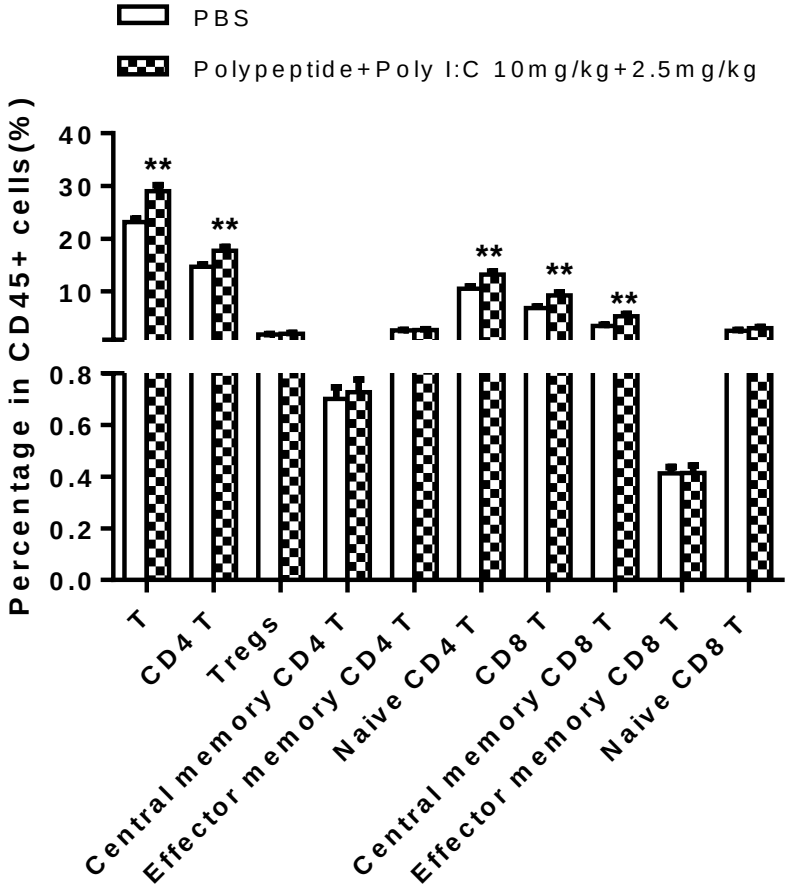
Gating strategy for tumor

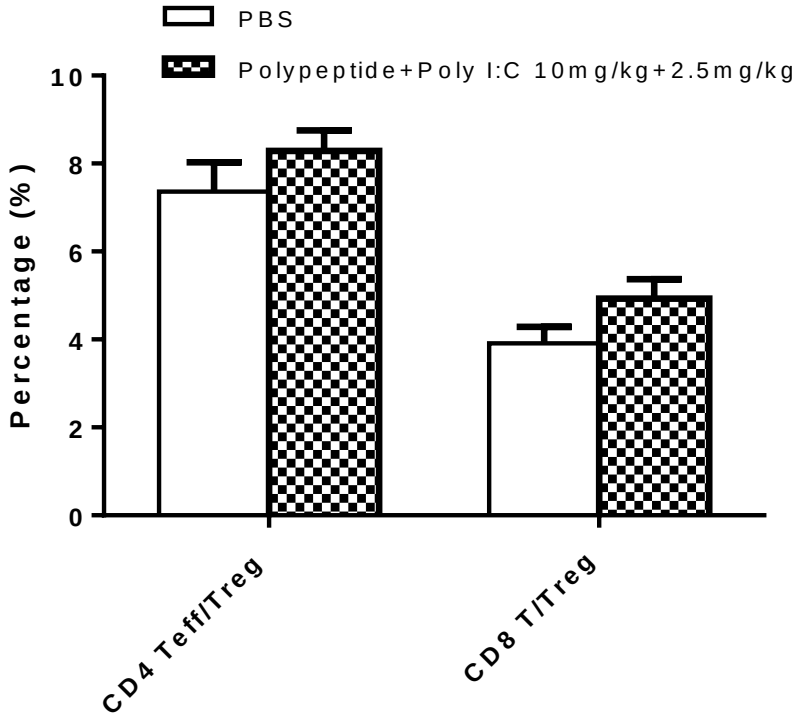


A case study of cancer vaccine discovery

In vivo validation of peptide vaccine: CT26 tumor model

T populations in spleen



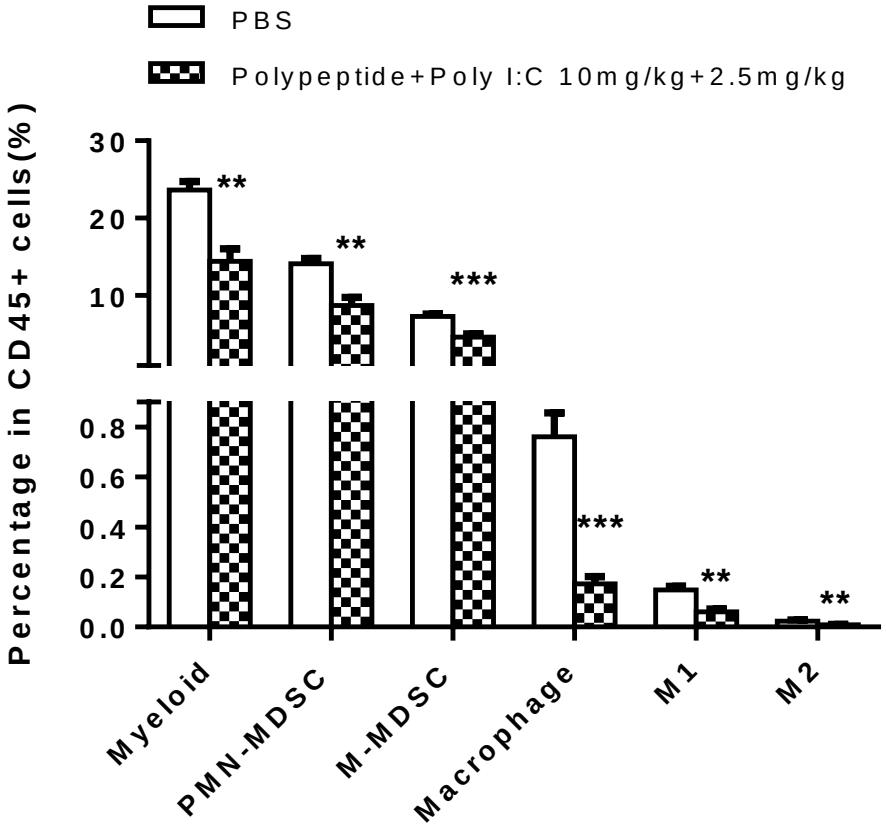


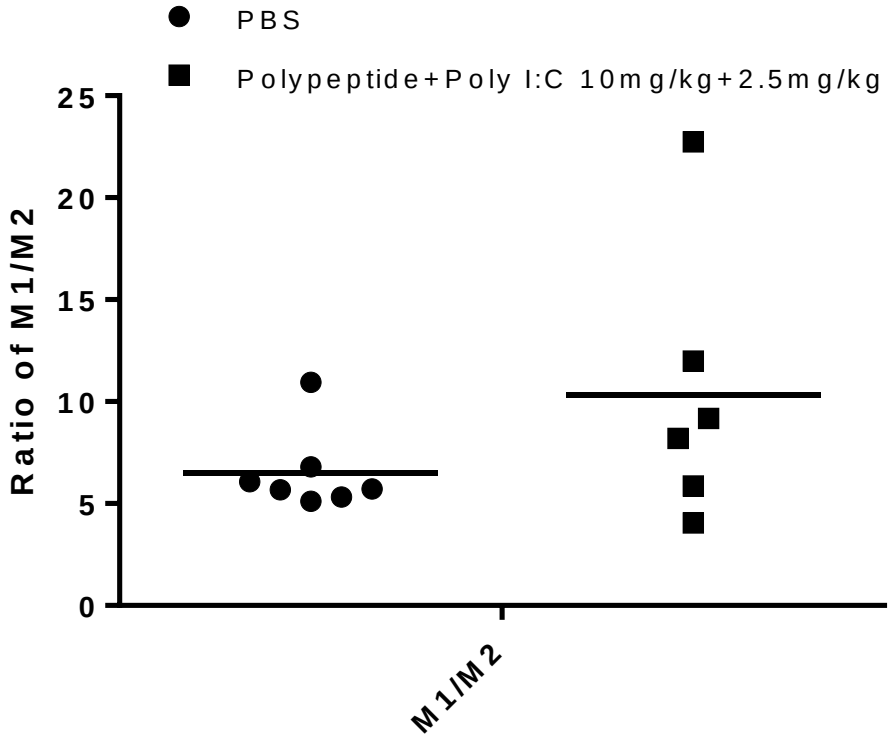
Independent-Samples T test was used for statistical analysis. * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Error bars represented Standard Error of Mean (SEM).

A case study of cancer vaccine discovery

In vivo validation of peptide vaccine: CT26 tumor model

Myeloid populations in spleen

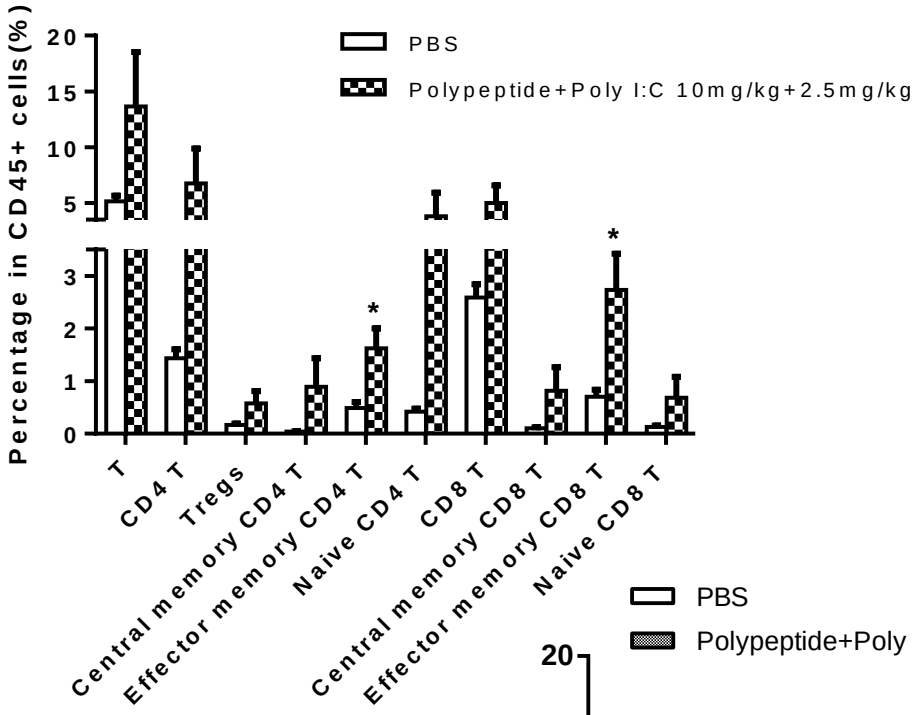


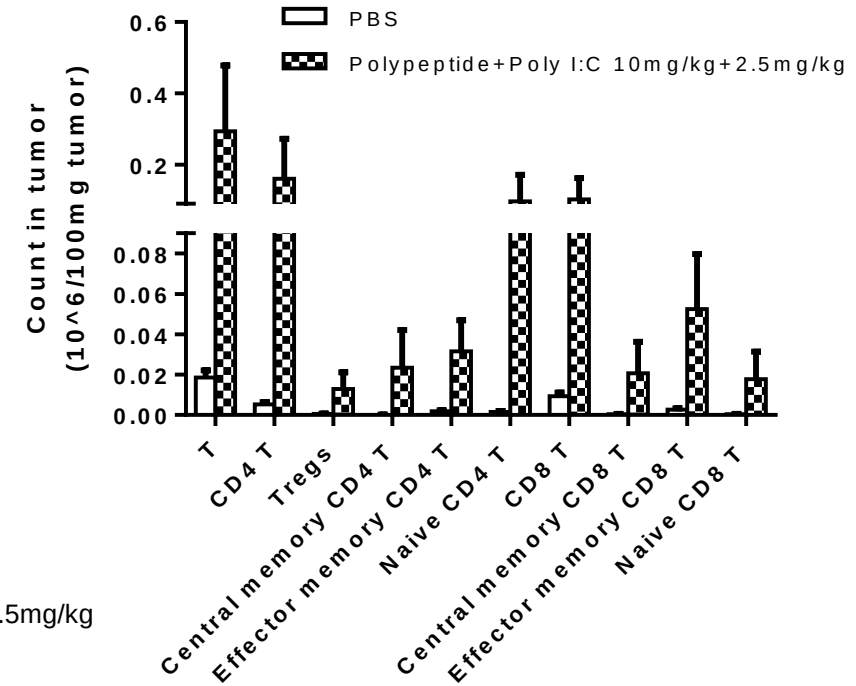


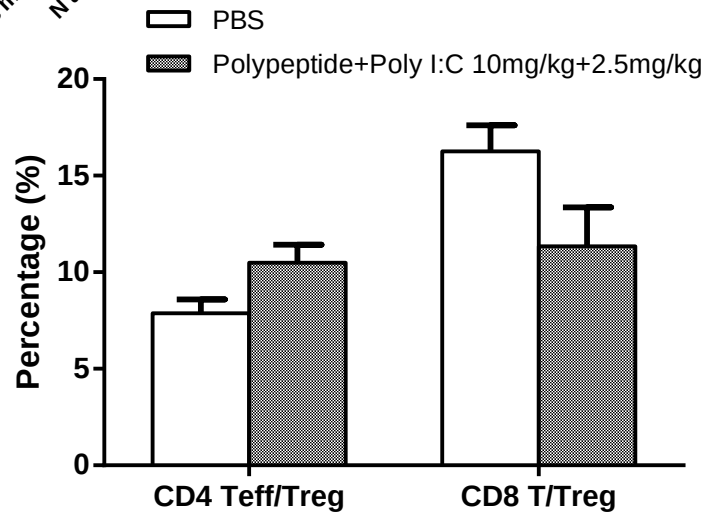
A case study of cancer vaccine discovery

In vivo validation of peptide vaccine: CT26 tumor model

T populations in Tumor







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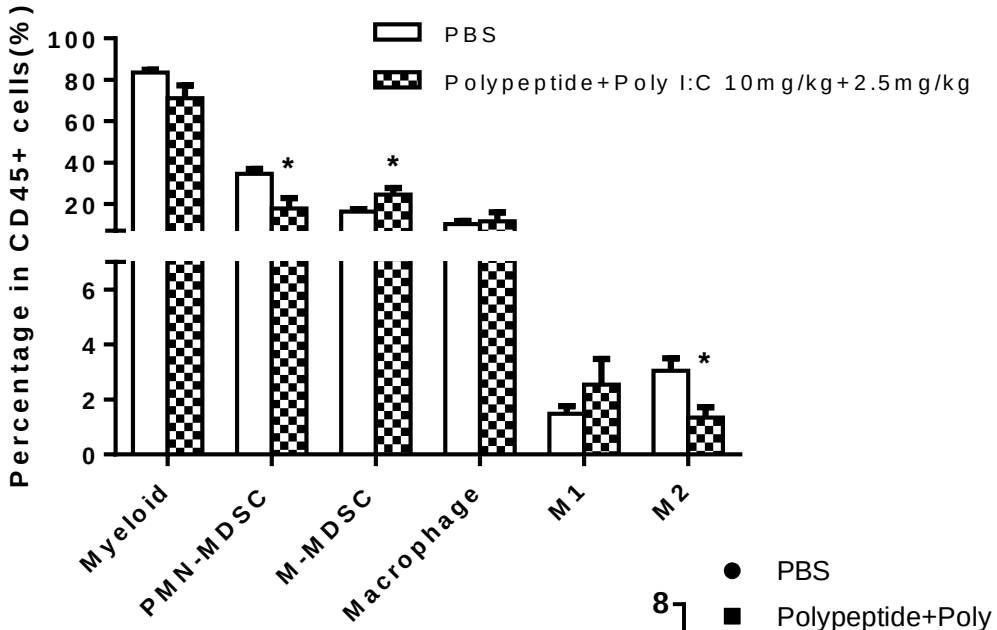
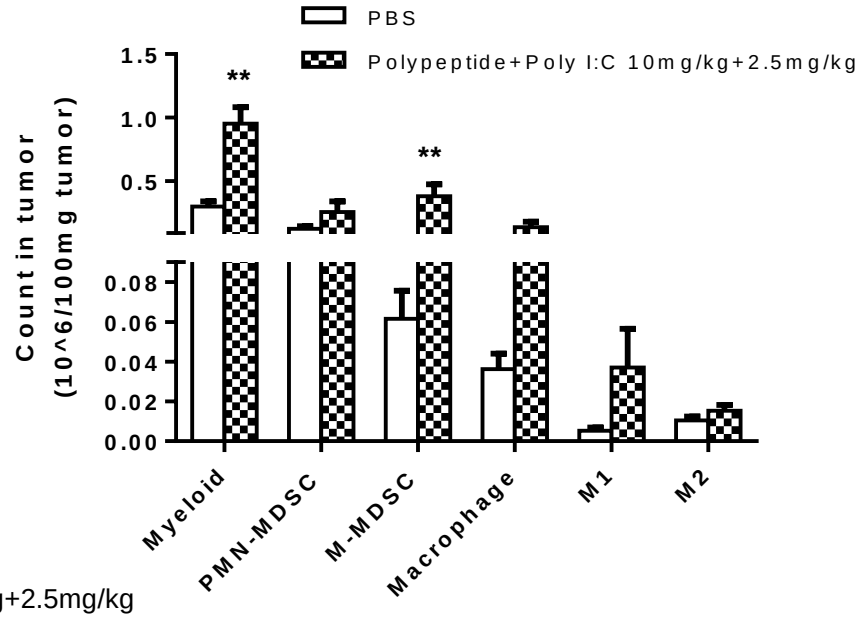
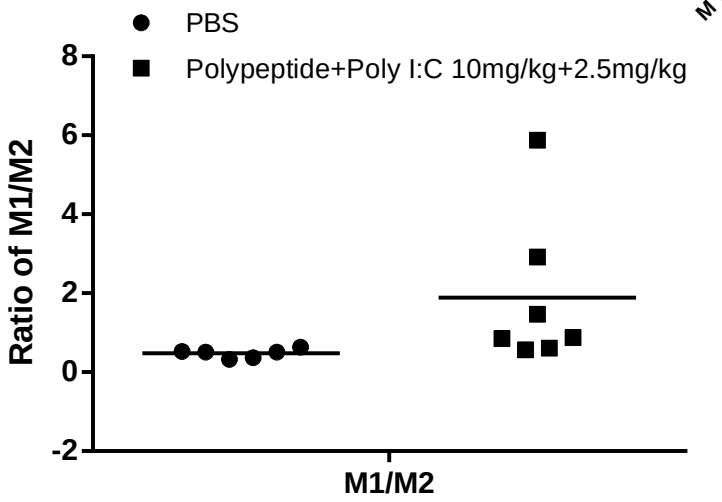
Myeloid populations in Tumor

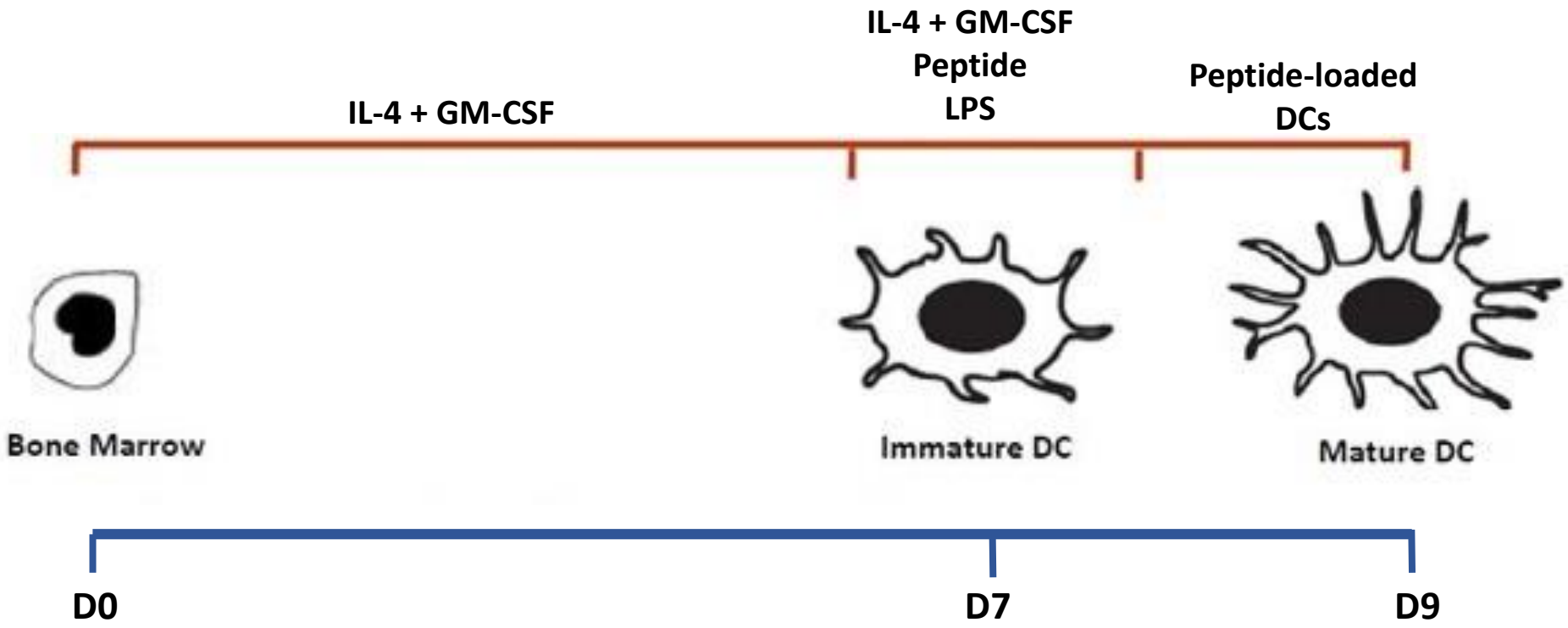






Generation of Peptide-Loaded DC-Vaccine for Cancer Therapy

Peptide-loaded DC vaccine: Experimental procedure

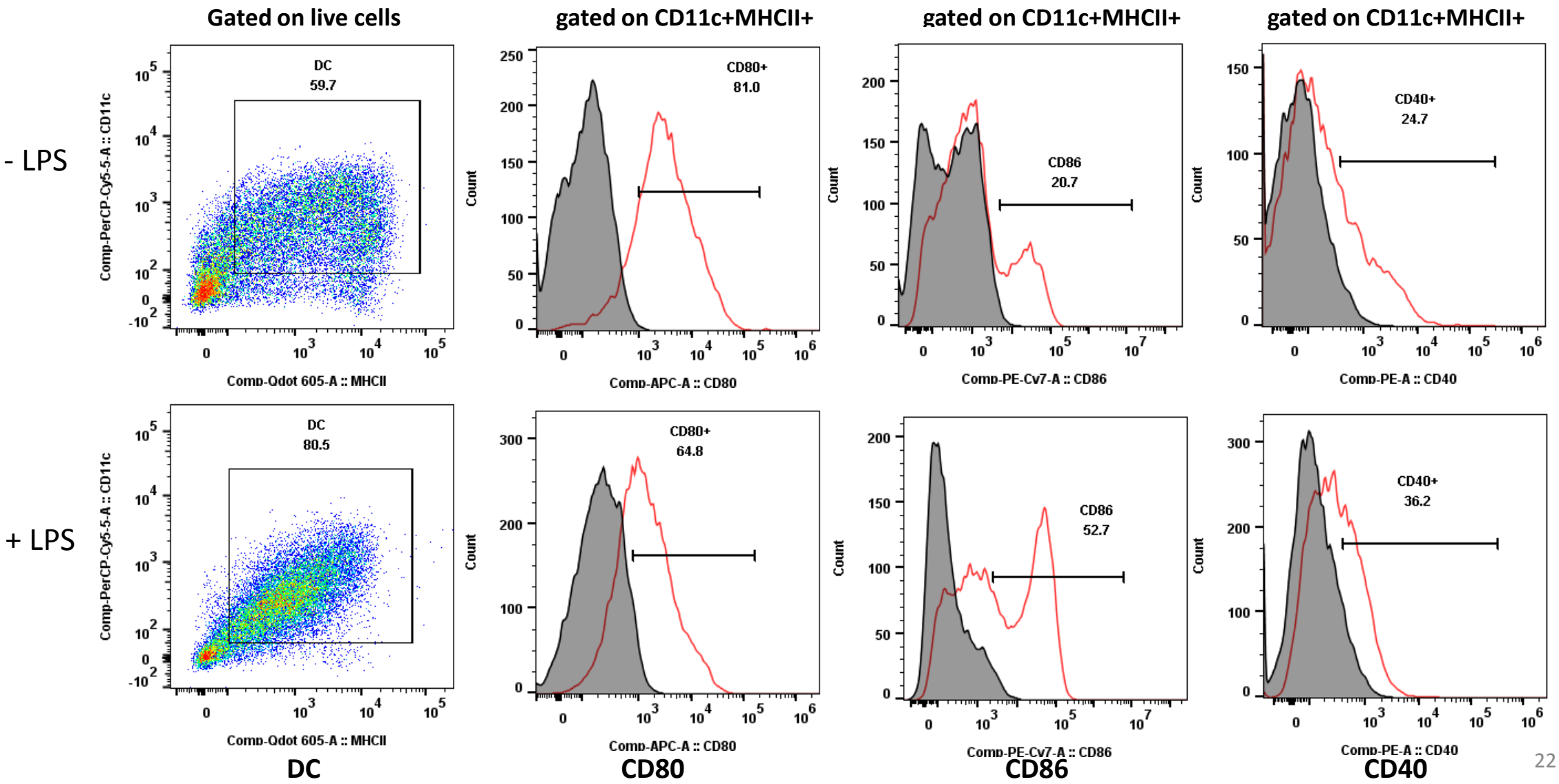


- Murine bone marrow cells were cultured in RPM-1640 complete medium with mIL-4 and mGM-CSF for 7 days. Immature DCs were generated at D7. Immature DCs were cultured in RPM-1640 complete medium with mIL-4, mGM-CSF, LPS and peptide for 2 days to generate peptide-loaded mature DC that were used as a therapeutic agent for cancer therapy subsequently.

Generation of Peptide-Loaded DC-Vaccine for Cancer Therapy

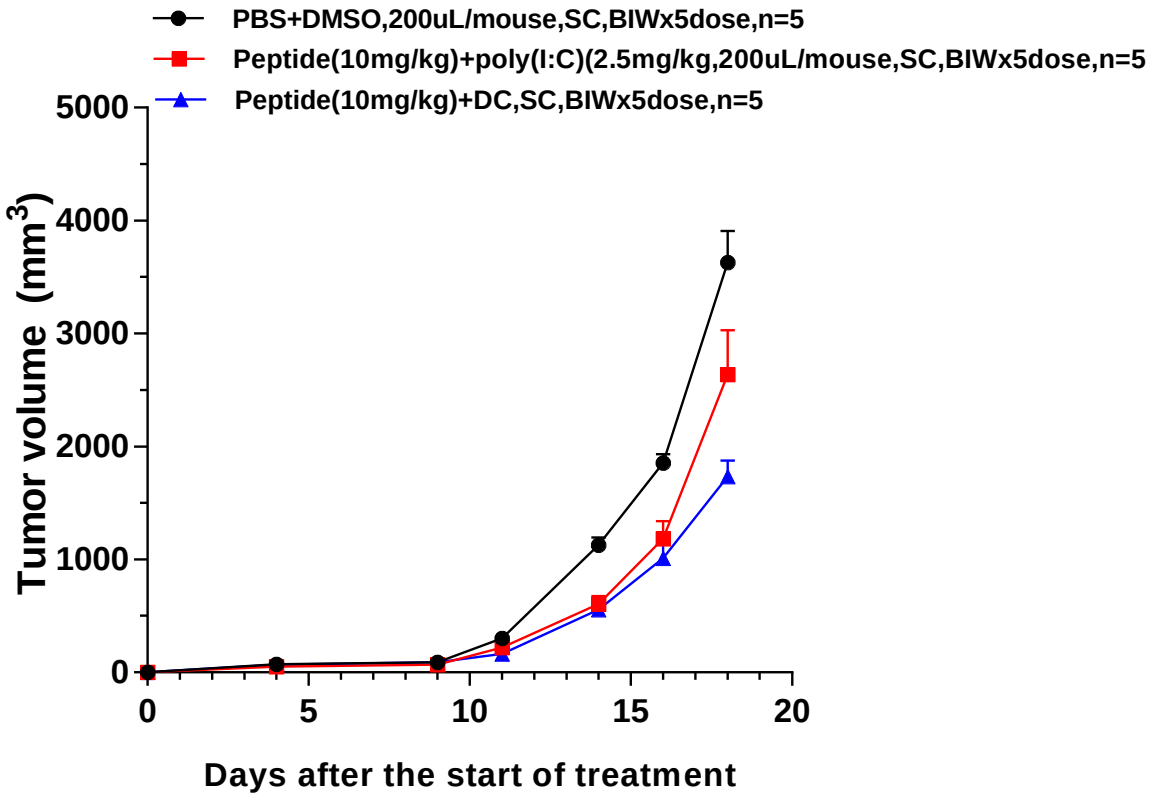
Peptide-loaded DC vaccine: B16F10 tumor model

in vitro induction and maturation of murine bone marrow derived DCs

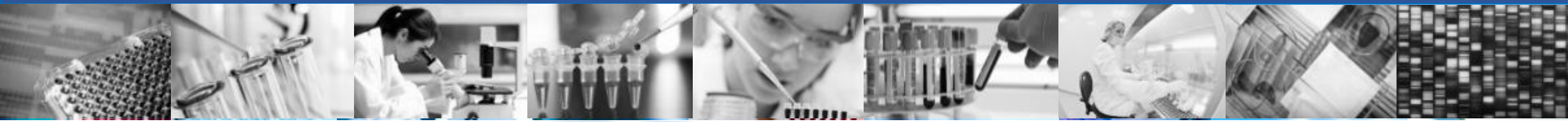


A case study of cancer vaccine discovery

Peptide-loaded DC vaccine: B16F10 tumor model



- Mature bone marrow derived DCs were loaded with peptide.
- The *in vivo* tumor growth inhibition effect was evaluated under peptide-loaded DCs treatment or peptide treatment alone.



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