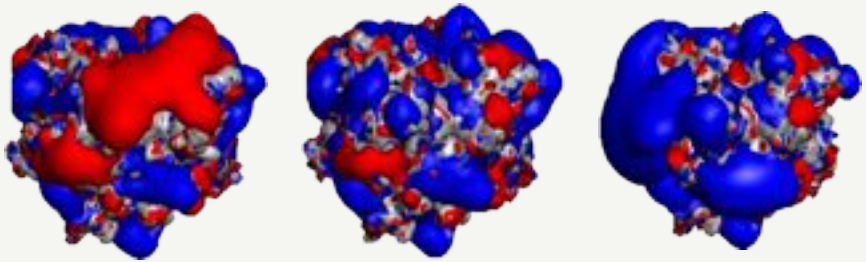
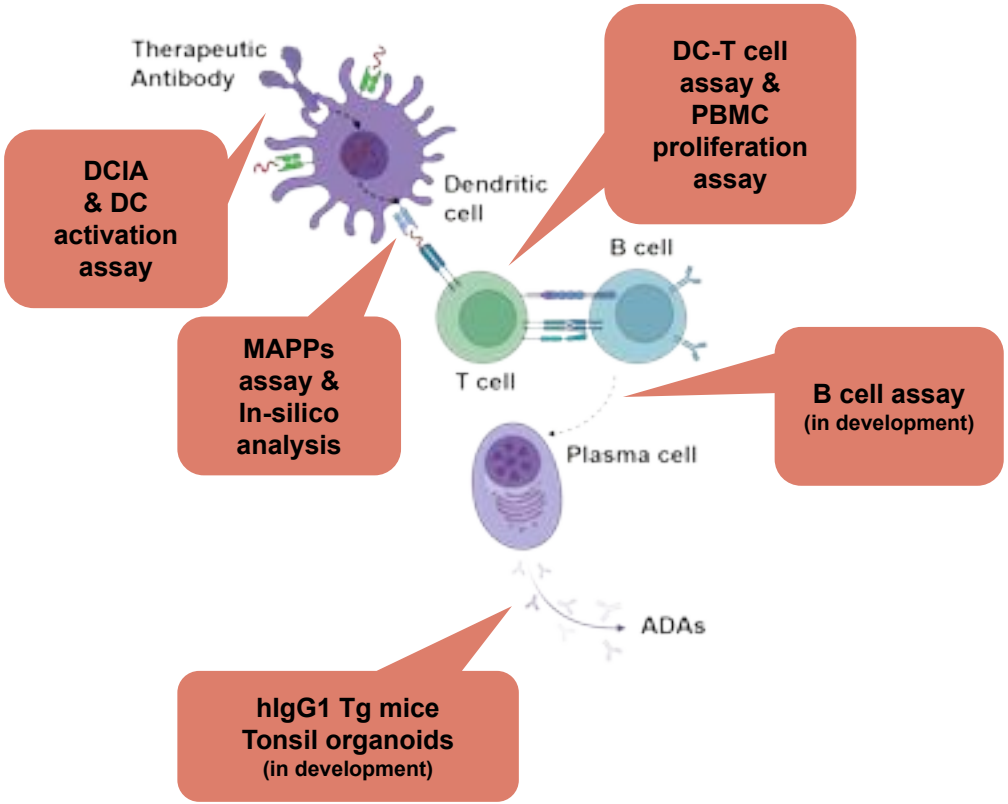
Internalization of therapeutic antibodies into Dendritic cells as a risk factor for immunogenicity

Michel Siegel, postdoctoral Scientist, Roche pharmaceutical Research and Early Development (pRED), Pharmaceutical Sciences (PS), Basel



Roche pRED immunogenicity assay tool box

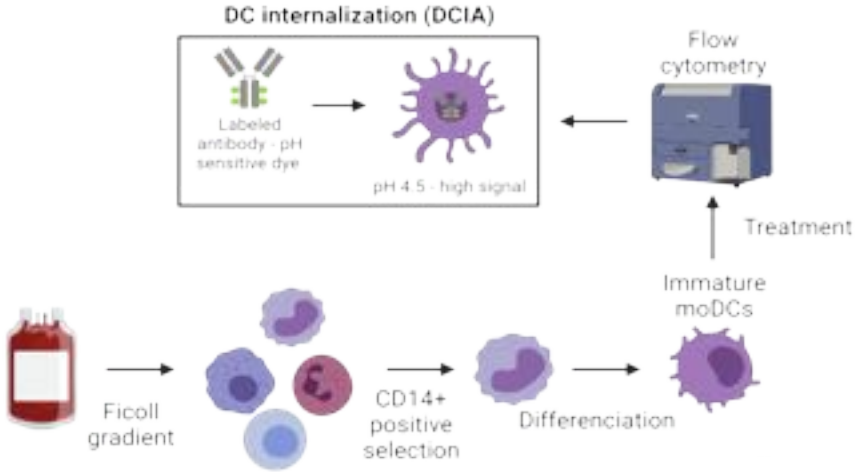


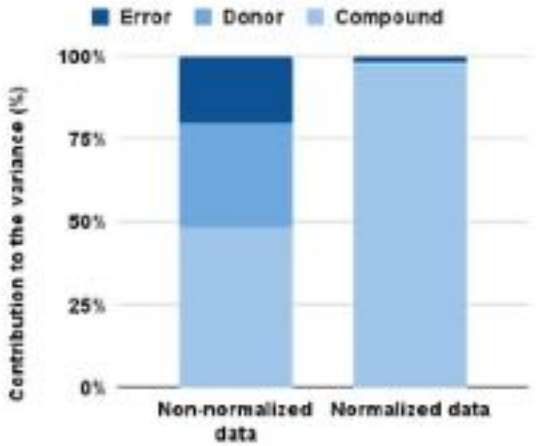
A single assay cannot anticipate clinical immunogenicity

- How does each step of this immune response affect the subsequent one?
- How do the biophysical properties of therapeutic antibodies affect their immunogenicity profile and alter assay outcomes?

Roche pRED DC internalization assay

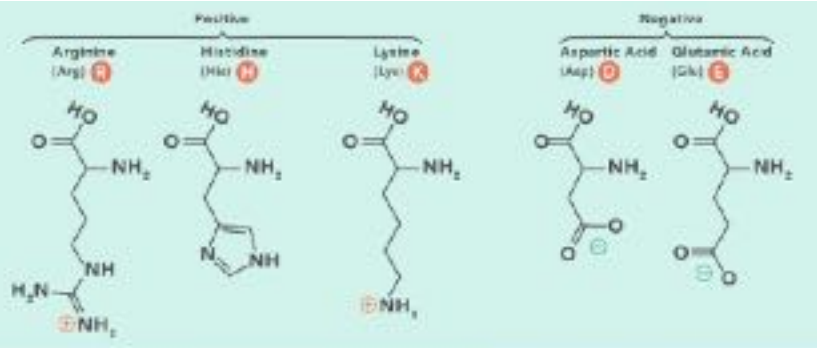
Experimental overview and characterization dataset



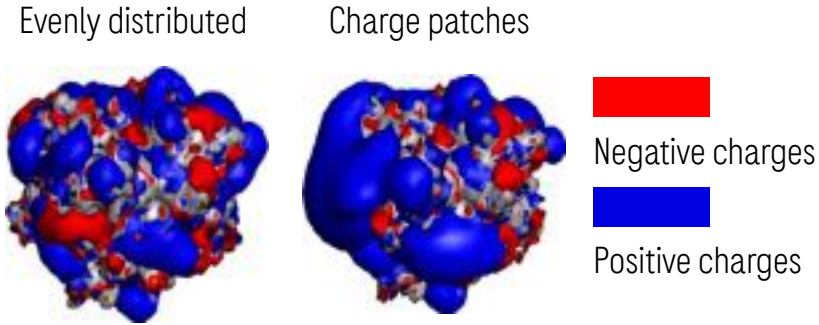


The DC internalization assay gives valuable insight into how rapidly a therapeutic antibody accumulates in the lysosomes

Tool molecules and antibody charge patches

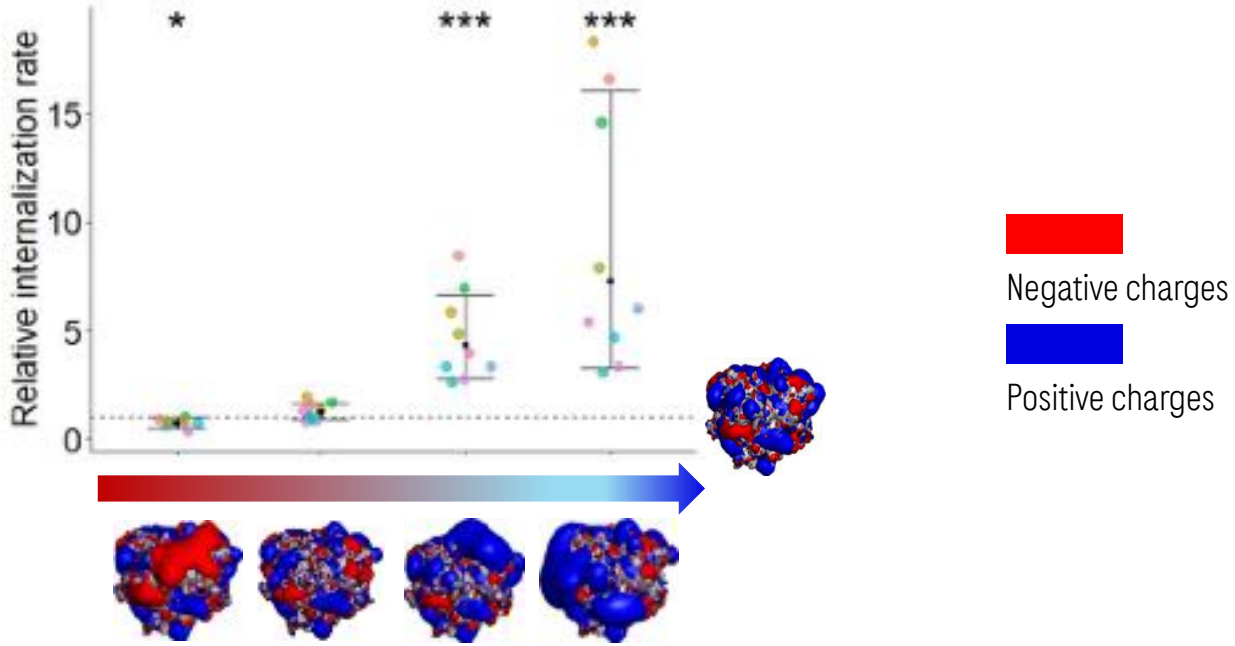


Amino acids with electrically charged side chains



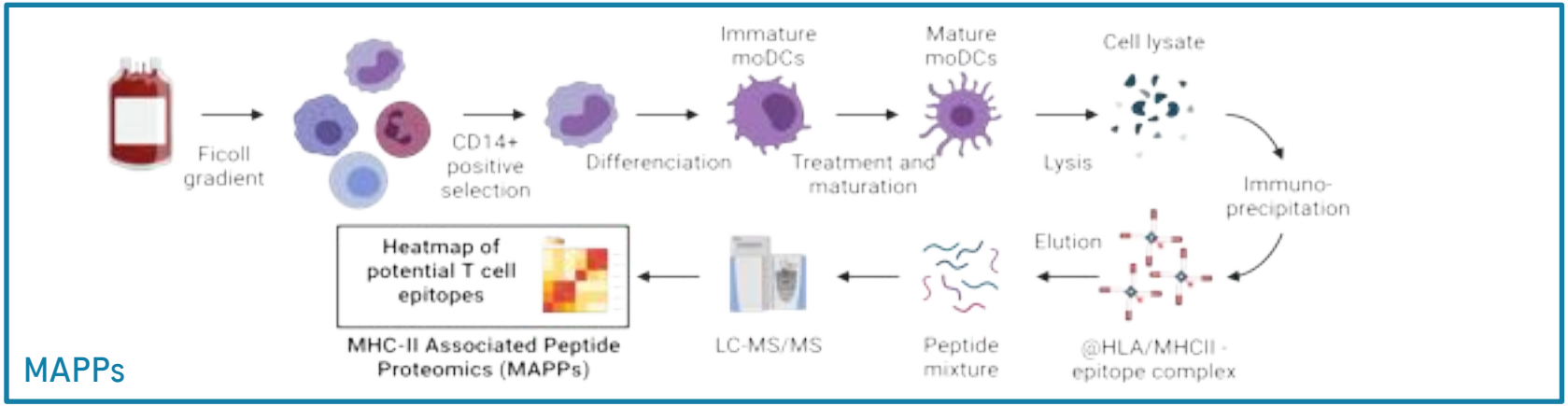
The isopotential surfaces of one of each antibodies' Fabs (viewed from the top)

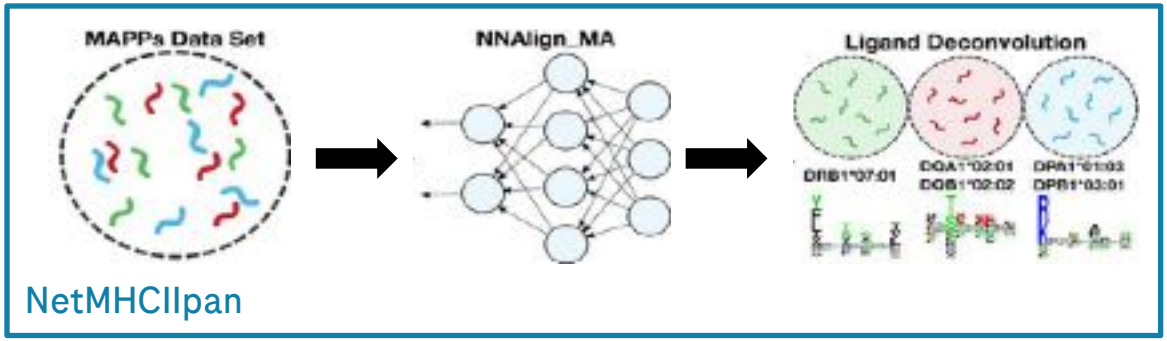
Positive charge patches and DC internalization



Positive charge patches lead to an increased internalization into moDCs

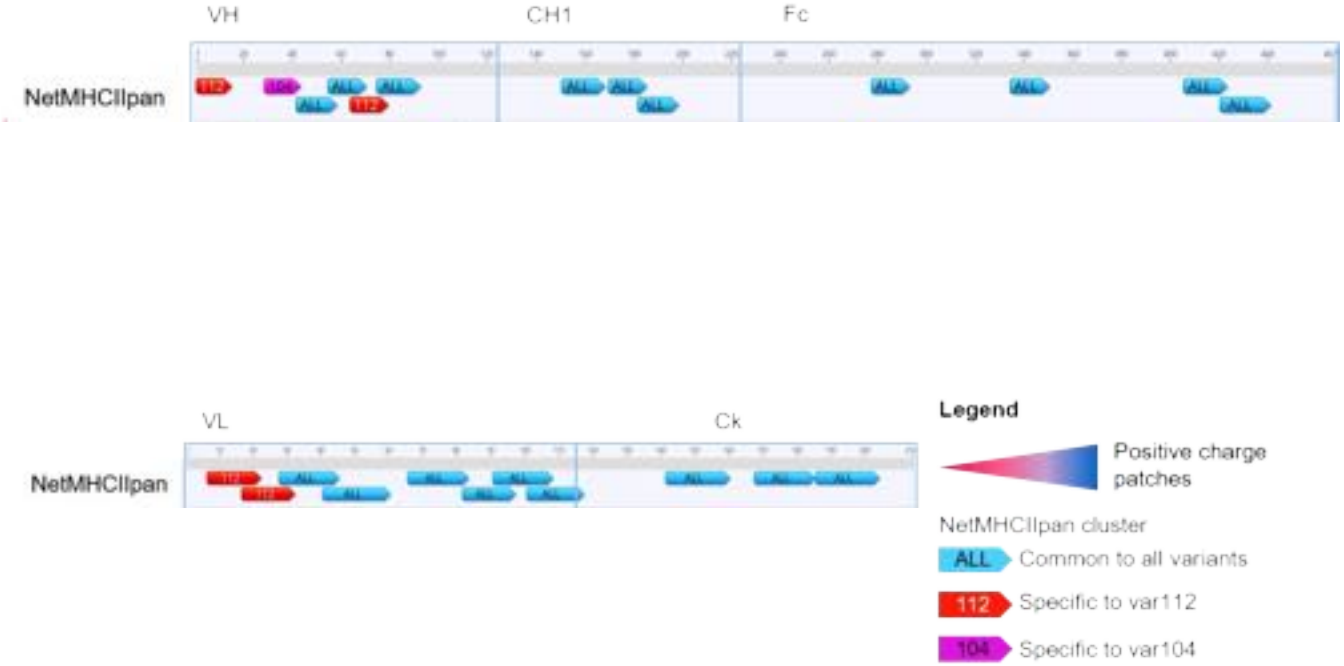
In silico prediction and MAPPs for T cell epitope assessment





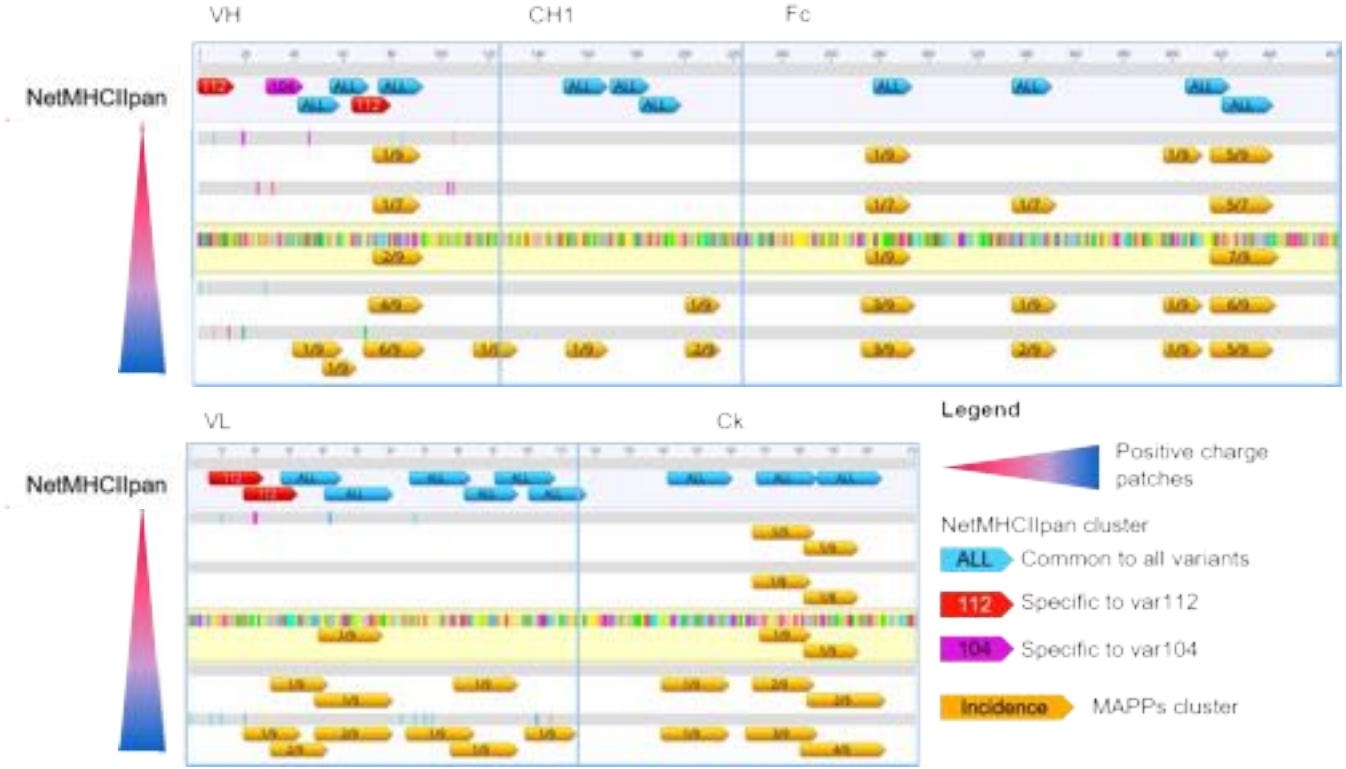
- Top 2 percent epitope hits
- Filter out WT epitope
- Patch induced epitopes

In silico prediction and MAPPs for T cell epitope assessment



The observations are not driven by additional T cell epitopes

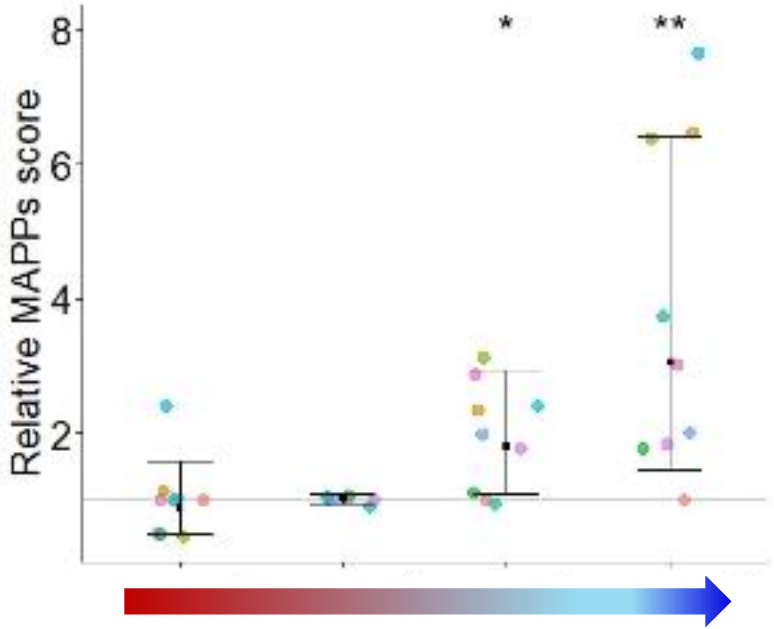
In silico prediction and MAPPs for T cell epitope assessment

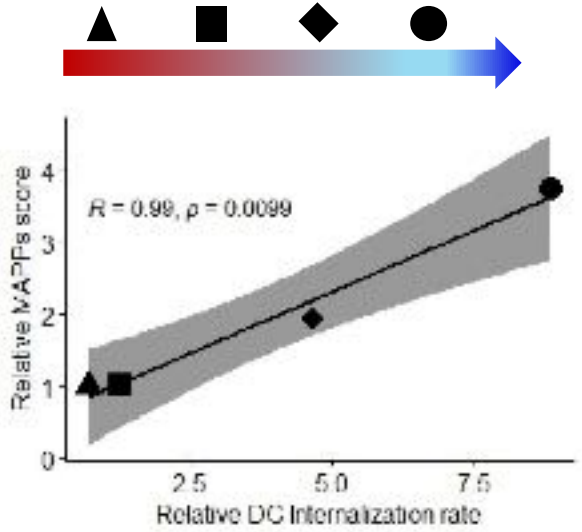


Increased internalization leads to an increased T cell epitope presentation

Epitope presentation and correlation with DC internalization

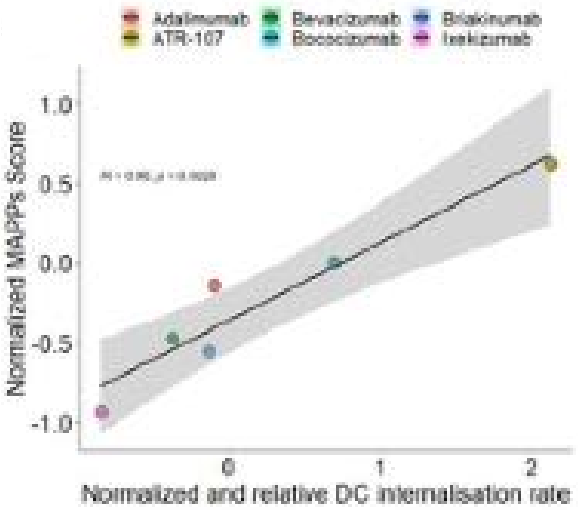
MAPPs score = $N_{epitopes}$ x $mean_{signal}$
Relative MAPPs score = MAPPs score / $MAPPs\ score_{var1}$



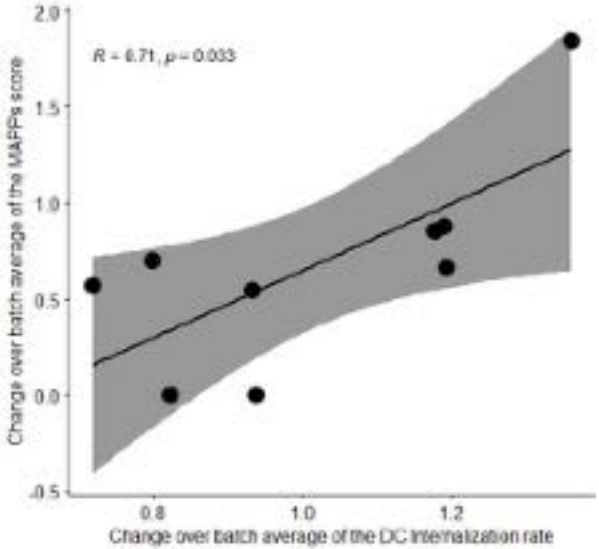


Increased internalization leads to an increased T cell epitope presentation

Epitope presentation and correlation with DC internalization

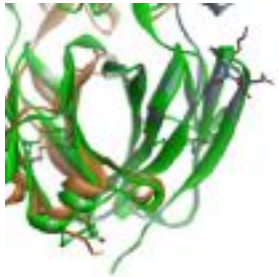


This correlation is also true for a set of therapeutic antibodies

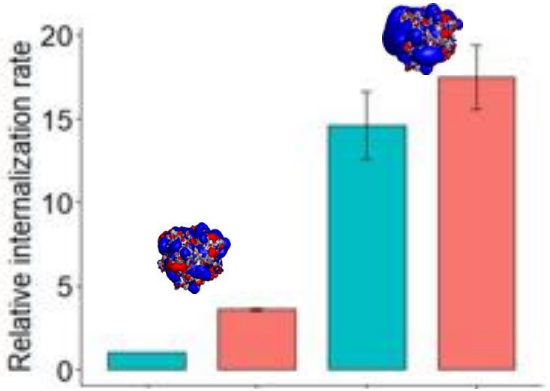


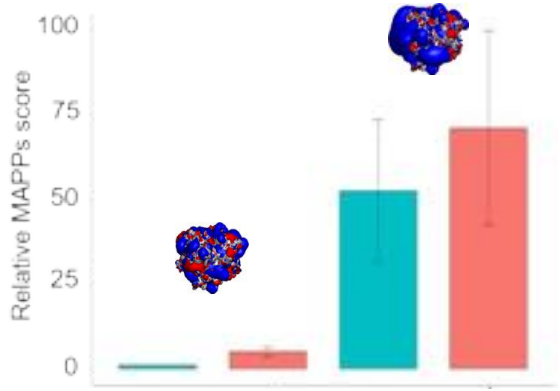
A donor propensity for faster cellular accumulation leads to an increased peptide presentation in MAPPs

Generation of ova CD4+ T cell epitope containing Ab variants



Design of OVA-peptide containing mAbs





Parental Ab

OVA-Ab

Legend

Positive charge patches

NetMHCIIpan cluster

ALL Common to all variants

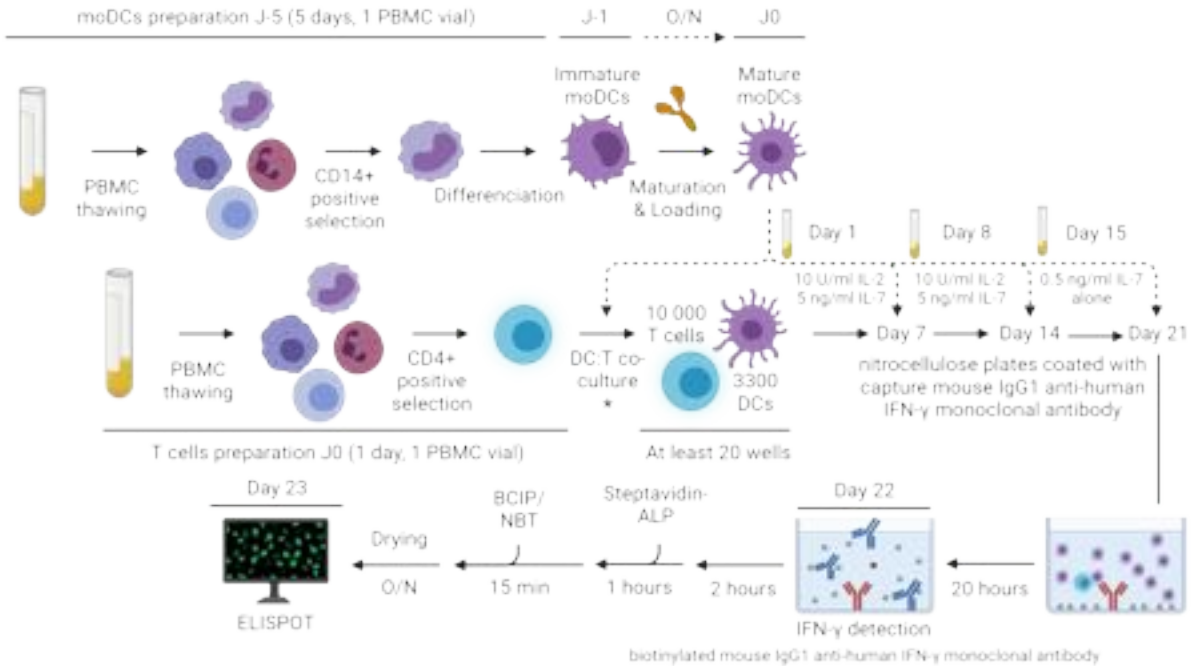
MAPPs cluster

Incidence MAPPs cluster for the initial variants

Incidence MAPPs cluster for the OVAp variants

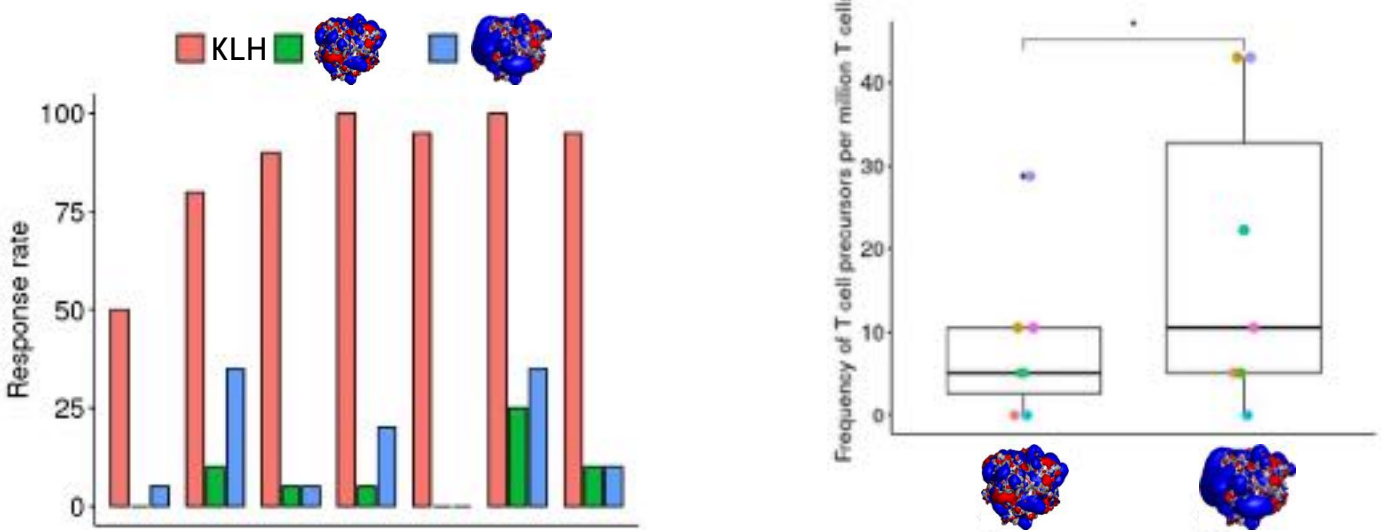
The insertion of the OVA CD4+ T cell epitope does not significantly alter the difference between the charge patches variants

Expansion of specific CD4+ T cells



Adapted from Delluc et. al. 2011

Positive charge patches and T cell activation



Positive charge patches increase the likelihood of expanding OVA-specific CD4+ T cells

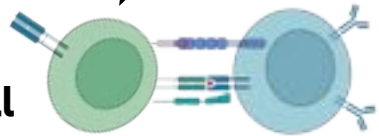
Relationship between DC internalization and immunogenicity

DC

Antibody positive charge patches increase unspecific clearance and DC **internalization**

Increased **DC internalization** leads to an **increased presentation** of potential T cell epitope on MHC-II receptors

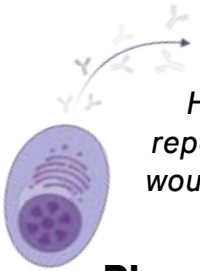
T cell



B cell

Specific T cell

Subsequently, specific and functional **CD4+ T cells are increased** in presence of positive charge patches



ADAs

Having an increased repertoire of naïve T cells would increase the risk for immunogenicity

Plasma cell

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Doing now what patients need next