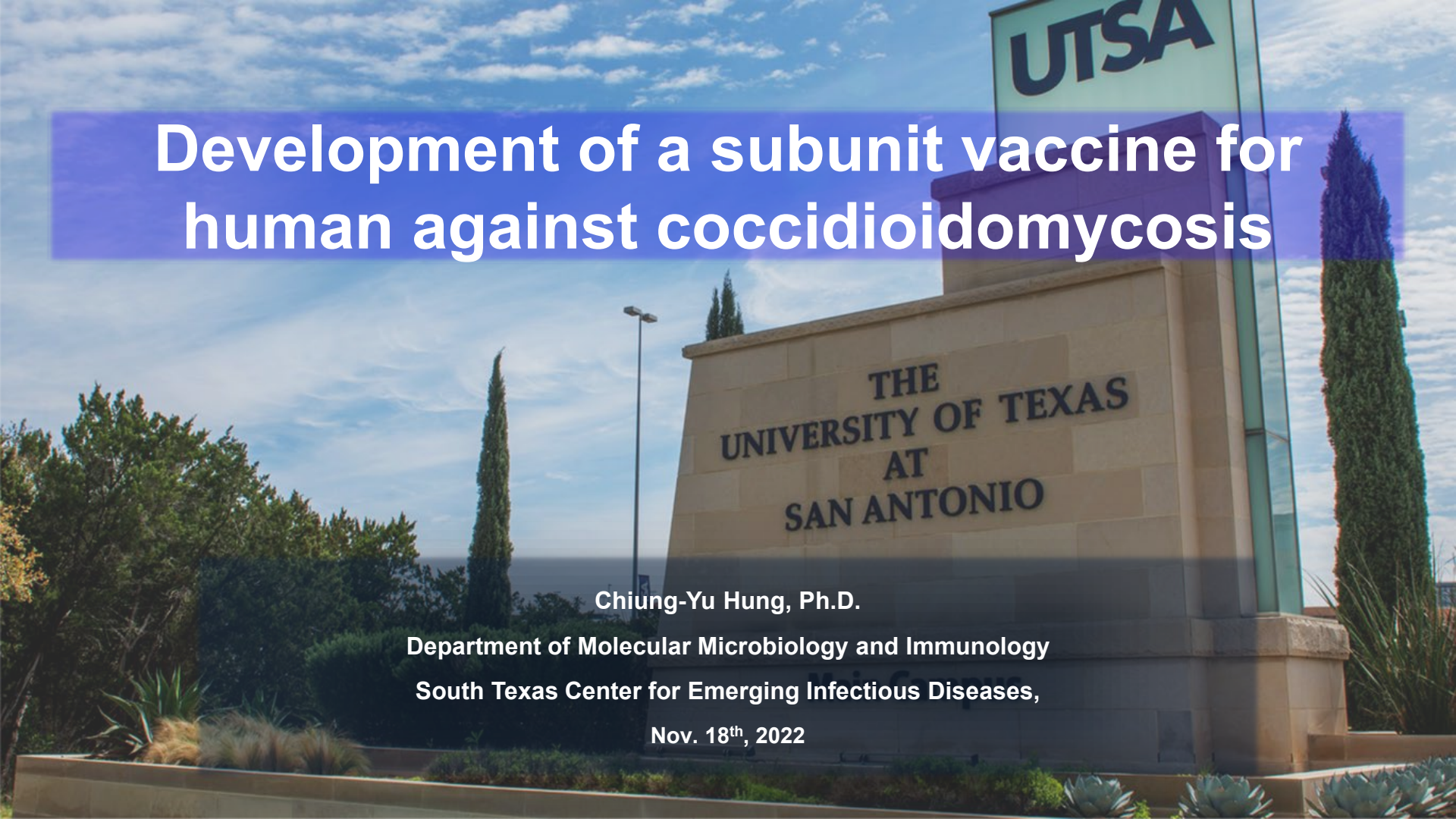


Development of a subunit vaccine for human against coccidioidomycosis

Chiung-Yu Hung, Ph.D.

Department of Molecular Microbiology and Immunology

South Texas Center for Emerging Infectious Diseases,

Nov. 18th, 2022

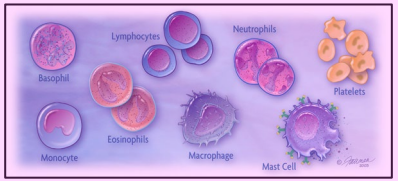
Rationale design of a safe subunit vaccine for human

Step#1: What are the protective mechanisms and the immune protection correlates?

Step#2: Select vaccine antigens

Step#3: Select an effective adjuvant delivery system

Immune mechanisms



Protective correlates

Create a safe vaccine that can be approved for clinical usage

Protective Vaccine

Ag

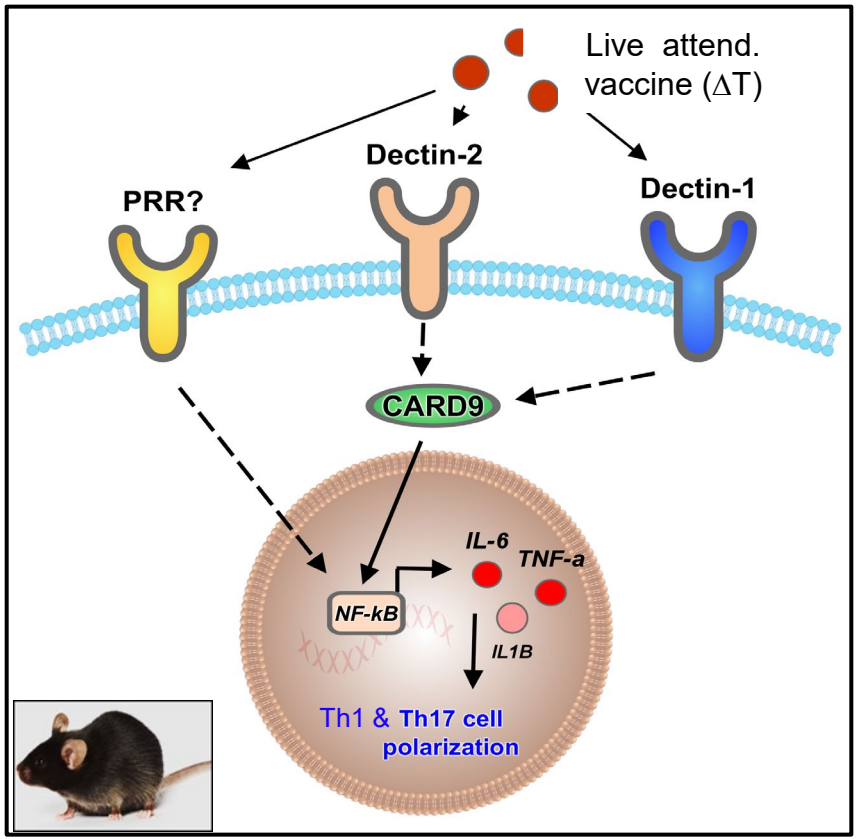
MHC II presentation

Immune modulation

Antigen

Adjuvant / Delivery system

Step#1: What is protective immunity against VF?



- Campuzano, *et al*. 2020, J Immunol. 15:204
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- Wang and Klein et al, 2016. PLoS Pathog 12(8): e1005787
- Hung et al., 2016. Infect. Immun., 84:906-916
- Viriyakosol and Fierer et al., 2013
- Hsu and Holland et al., JCI *insight.* 2022. https://doi.org/10.1172/jci.insight.159491.

Th1- and Th17-mediated responses are essential immunity against pulmonary *Coccidioides* infection

Step#2: Discover coccidioidal antigens

Criteria:

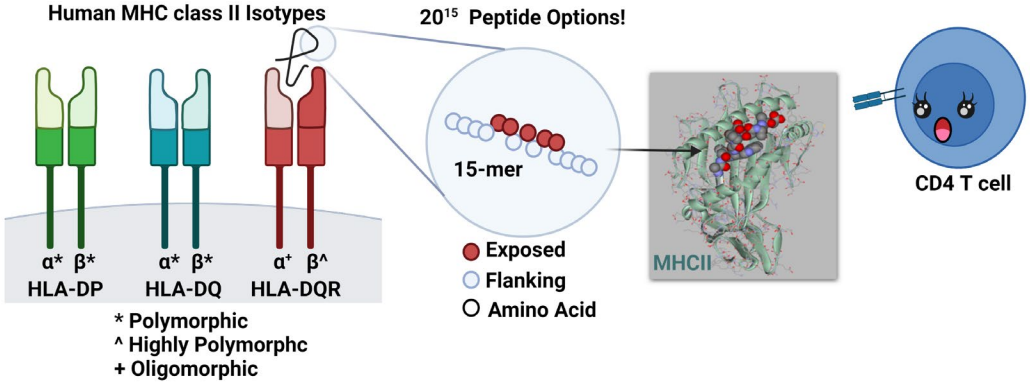
1. Immunogenic to stimulate and activate $CD4^+$ T cells
2. Conserved coccidioidal proteins recognized by humans
3. Expressed during the parasitic life cycle of *Coccidioides* growth as well as *in vivo* inside the host
4. No homology to human proteins
5. Acceptable cellular toxicity

Discovery methods:

1. Immunological chemistry and functional assays
2. Prediction by immuno-bioinformatics methods
3. Combination of both technologies

In silico epitope prediction

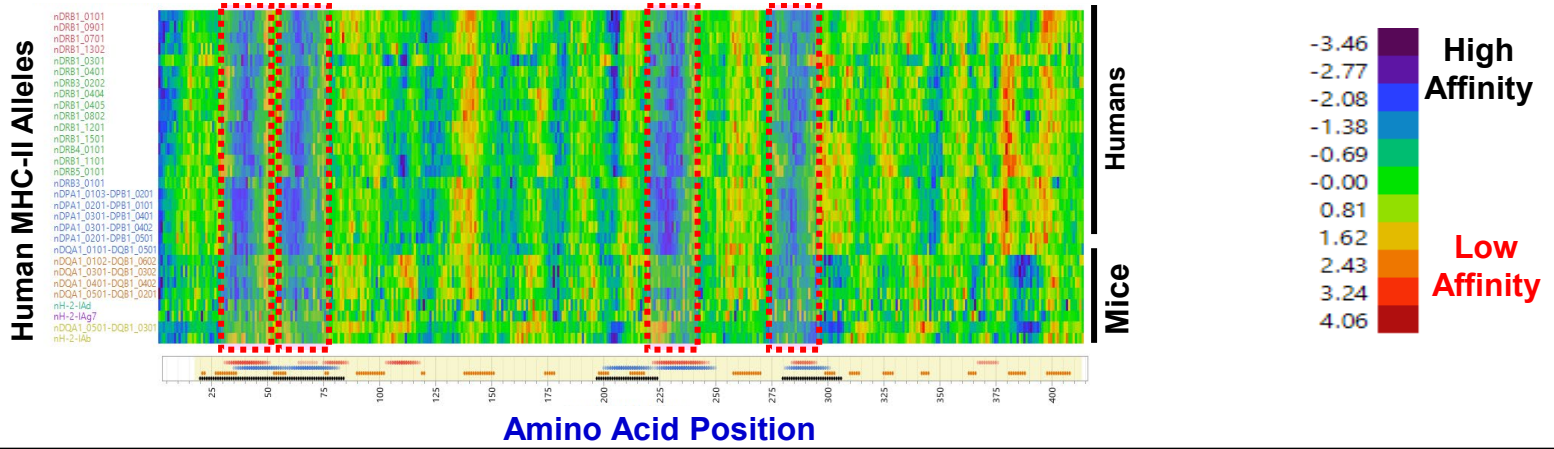
MHC II molecules (HLA alleles) present epitopes to CD4⁺ T cells



MHC II Hierarchical Plot

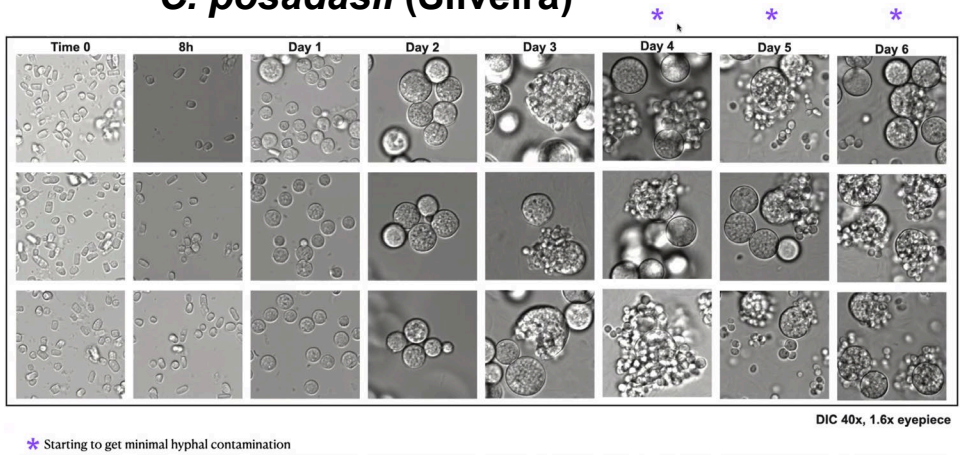
Dr. Jane Homan, IoGenetics

(each colored pixel represents probability of binding affinity between a peptide and an HLA allele)



Example of *in silico* epitope prediction

RNAseq data from *C. posadasii* (Silveira)



No significant similarity with human

133 genes

Med-High expression (≥ 2k)

Cp vs Ci Identity >98%

Identity to mice = 0%

Top 23 candidates

In silico

Predict binding affinity to different human MHC alleles

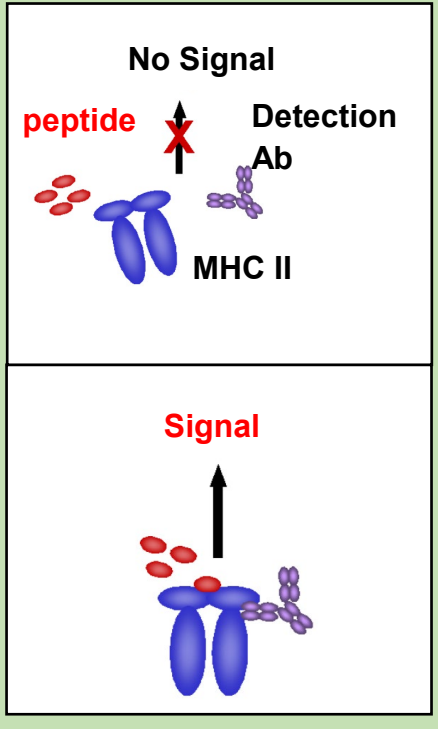
IoGenetic AI software

Synthesize peptides predicted to bind strongly to multiple HLA molecules

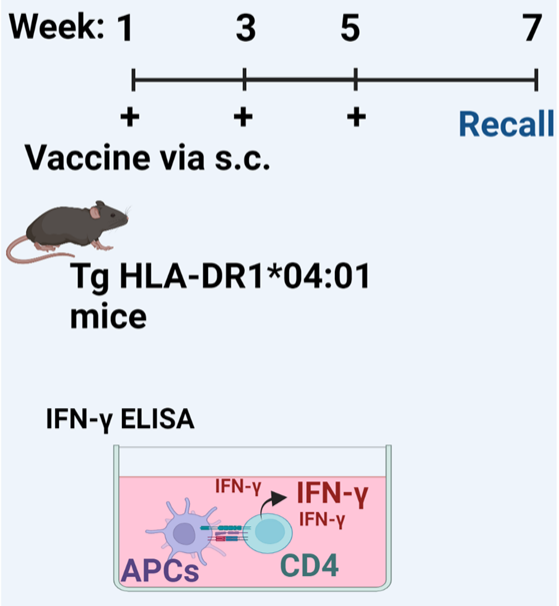
Dr. Anita Sil
Dr. Christina Homer

Immunological evaluation of epitopes

1 Immunochemical Binding Assay



2 *In vivo* Evaluation

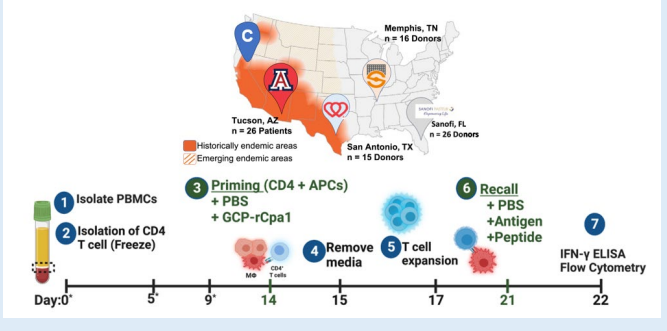


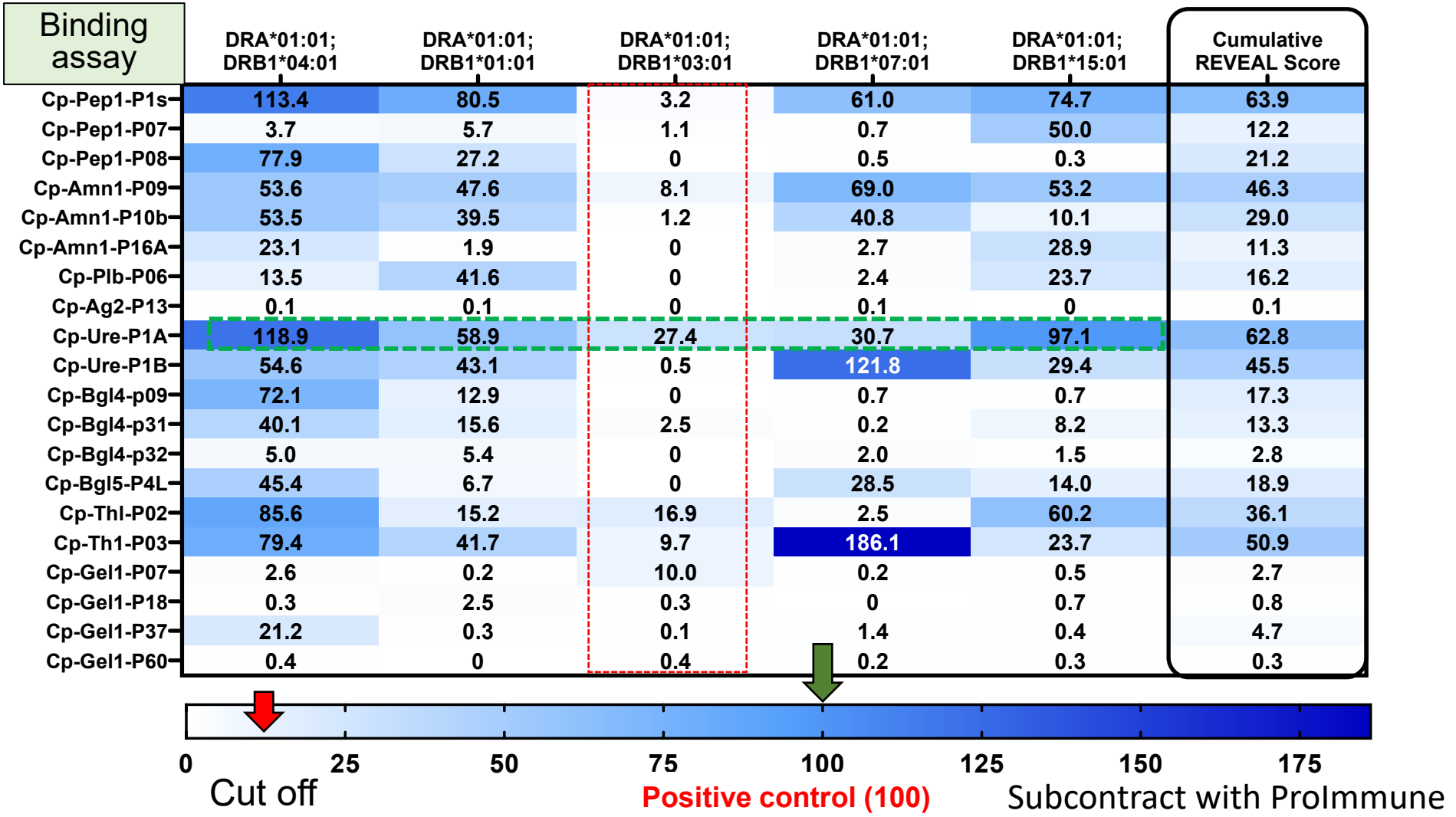
3 *Ex vivo* evaluation

T-cell recall assay using human APCs and T cells derived from PBMCs

1. Healthy donors
2. Recovered patients

Cytokine ELISA
Flow cytometry




Binding assay
DRA*01:01; DRB1*04:01
DRA*01:01; DRB1*01:01
DRA*01:01; DRB1*03:01
DRA*01:01; DRB1*07:01
DRA*01:01; DRB1*15:01
Cumulative REVEAL Score
Cp-Pep1-P1s 113.4 80.5 3.2 61.0 74.7 63.9
Cp-Pep1-P07 3.7 5.7 1.1 0.7 50.0 12.2
Cp-Pep1-P08 77.9 27.2 0 0.5 0.3 21.2
Cp-Amn1-P09 53.6 47.6 8.1 69.0 53.2 46.3
Cp-Amn1-P10b 53.5 39.5 1.2 40.8 10.1 29.0
Cp-Amn1-P16A 23.1 1.9 0 2.7 28.9 11.3
Cp-Plb-P06 13.5 41.6 0 2.4 23.7 16.2
Cp-Ag2-P13 0.1 0.1 0 0.1 0 0.1
Cp-Ure-P1A 118.9 58.9 27.4 30.7 97.1 62.8
Cp-Ure-P1B 54.6 43.1 0.5 121.8 29.4 45.5
Cp-Bgl4-p09 72.1 12.9 0 0.7 0.7 17.3
Cp-Bgl4-p31 40.1 15.6 2.5 0.2 8.2 13.3
Cp-Bgl4-p32 5.0 5.4 0 2.0 1.5 2.8
Cp-Bgl5-P4L 45.4 6.7 0 28.5 14.0 18.9
Cp-Th1-P02 85.6 15.2 16.9 2.5 60.2 36.1
Cp-Th1-P03 79.4 41.7 9.7 186.1 23.7 50.9
Cp-Gel1-P07 2.6 0.2 10.0 0.2 0.5 2.7
Cp-Gel1-P18 0.3 2.5 0.3 0 0.7 0.8
Cp-Gel1-P37 21.2 0.3 0.1 1.4 0.4 4.7
Cp-Gel1-P60 0.4 0 0.4 0.2 0.3 0.3
0 25 50 75 100 125 150 175
Cut off
Positive control (100)
Subcontract with ProImmune

Step#3: Create a subunit vaccine using T-cell epitopes and an adjuvant /delivery system

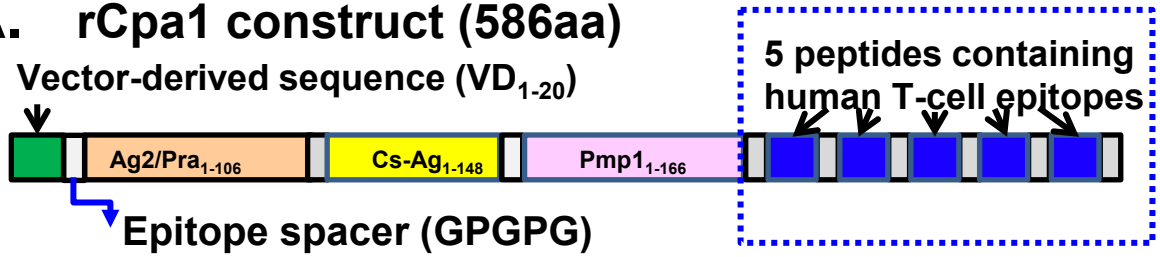
1. Evaluate T-cell memory responses require to prime the immune system with a coccidioidal vaccine and restimulated with a relevant antigen(s) in a recall assay.

2. An adjuvant /delivery system that can stimulate Th1 and Th17 immunity

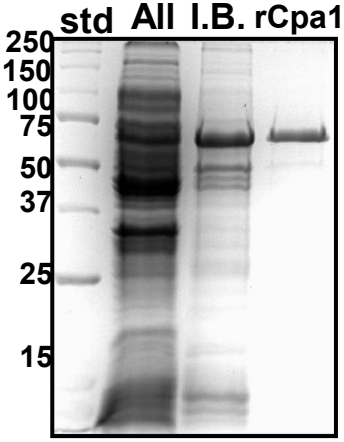
3. Formulation
    a. Recombinant antigens
    b. synthetic peptides
    c. mRNA

Create a *Coccidioides* polypeptide antigen (rCpa1)

A. rCpa1 construct (586aa)



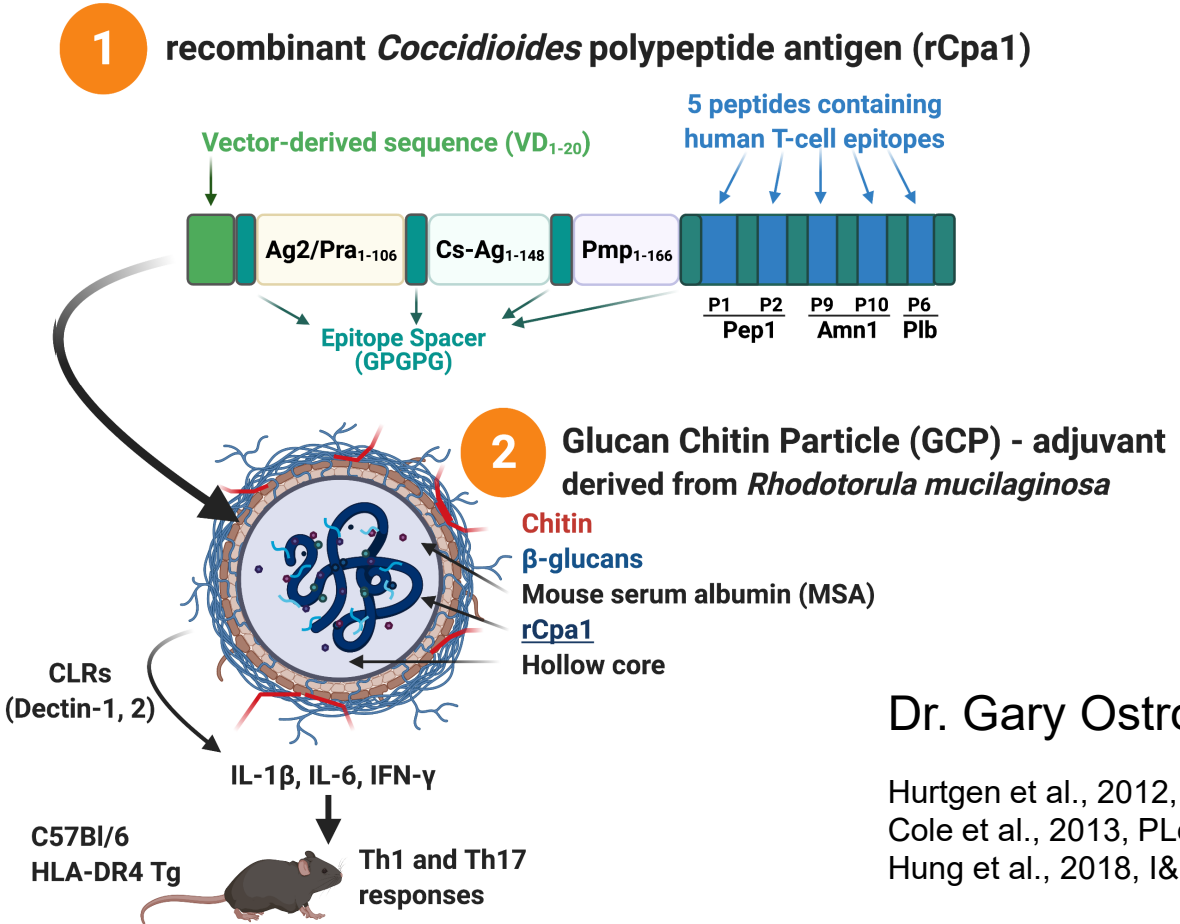
B. rCpa1 production



C. Murine models

US11,413,336B2
Hung et al., 2018

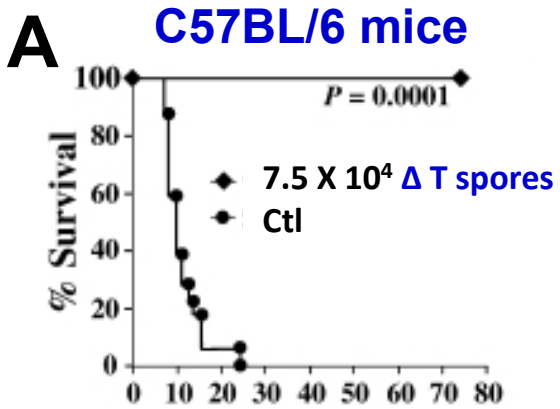
Select an adjuvant and delivery system: GCP

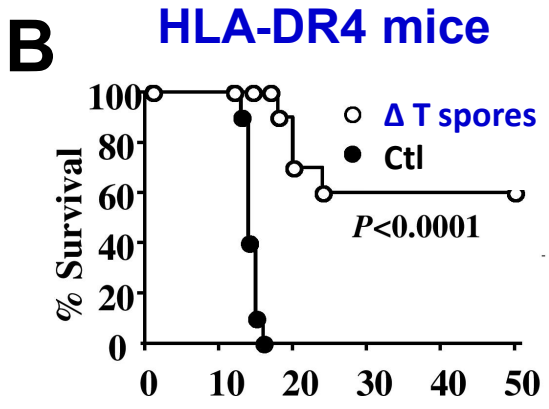


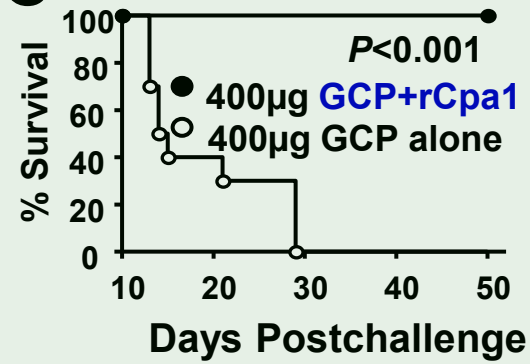
Dr. Gary Ostroff, UMMS

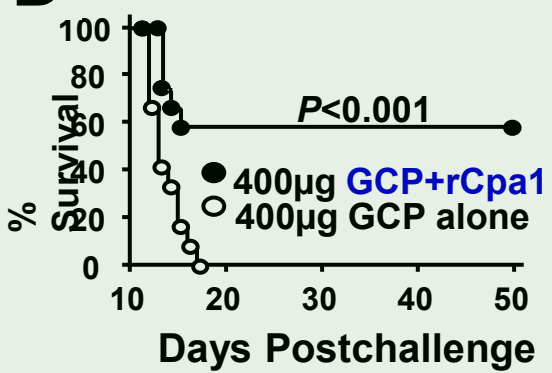
Hurtgen et al., 2012, I&I. 80:3960
Cole et al., 2013, PLoS Pathogens
Hung et al., 2018, I&I. 10.1128/IAI.00070-18

The GCP-rCpa1 vaccine shows comparable protective efficacy to a live attenuated vaccine (ΔT)







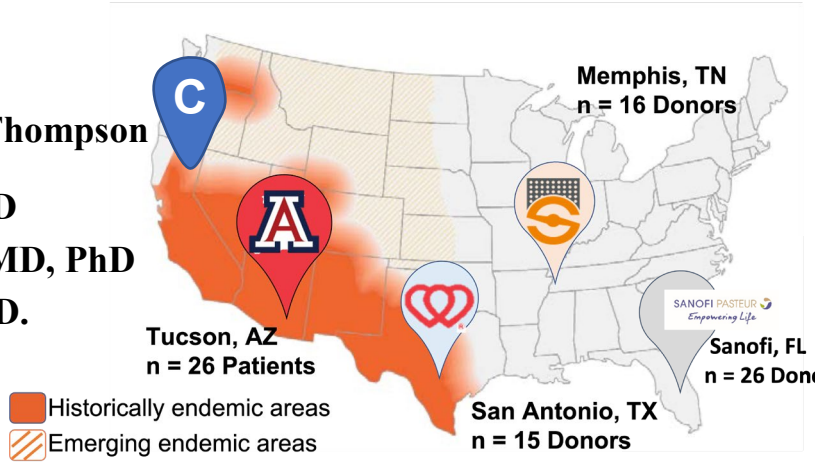


Campuzano, *et al*. 2020, J Immunol. 15:204

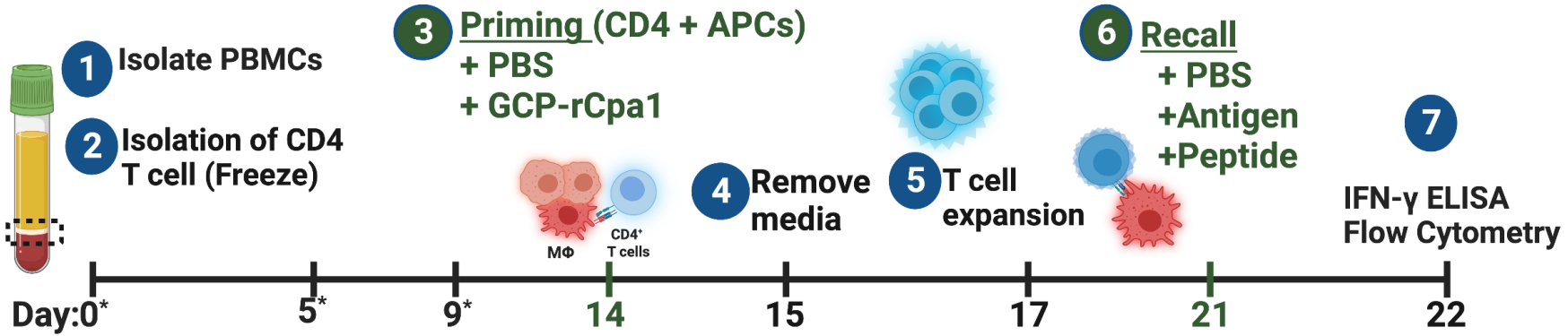
Determination of human T-cell response to *Coccidioides* Ags

Dr. George Thompson

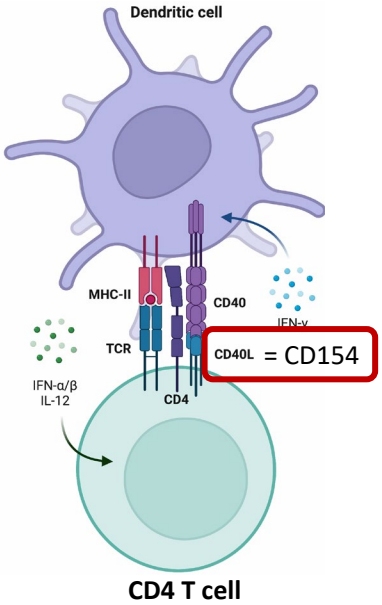
Neil M. Ampel, MD
Fariba Donovan, MD, PhD
John Galgiani, M.D.
Ken S. Knox, MD
Josh Malo, MD

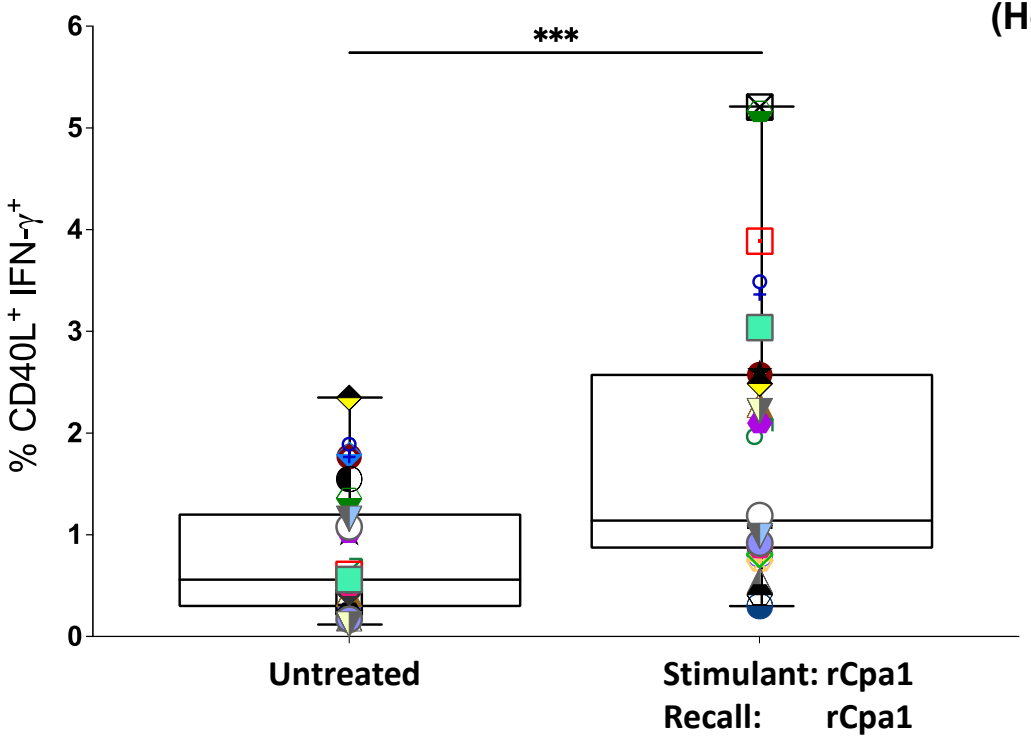


Dr. Chinh Dao



Human CD4⁺ T-cell response to rCpa1 (MIMIC®)





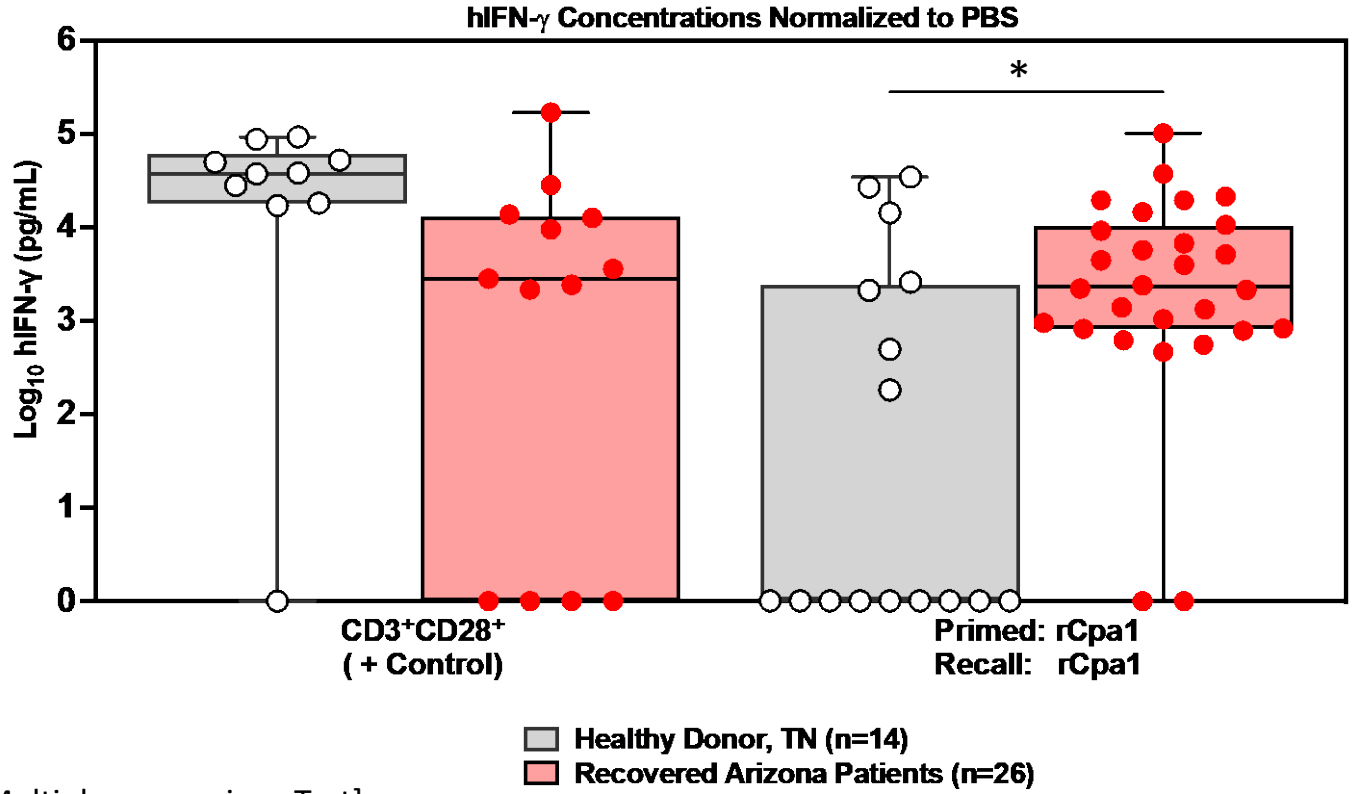
(Healthy donors, FL n=26)

4761 3336
4759 3450
4756 4328
4758 4454
4752 4582
4398 3351
4511 3359
4391 3781
5180 4141
3546 4307
4307 4436
3301 4440
3363 4628

[K-W Paired T Test]

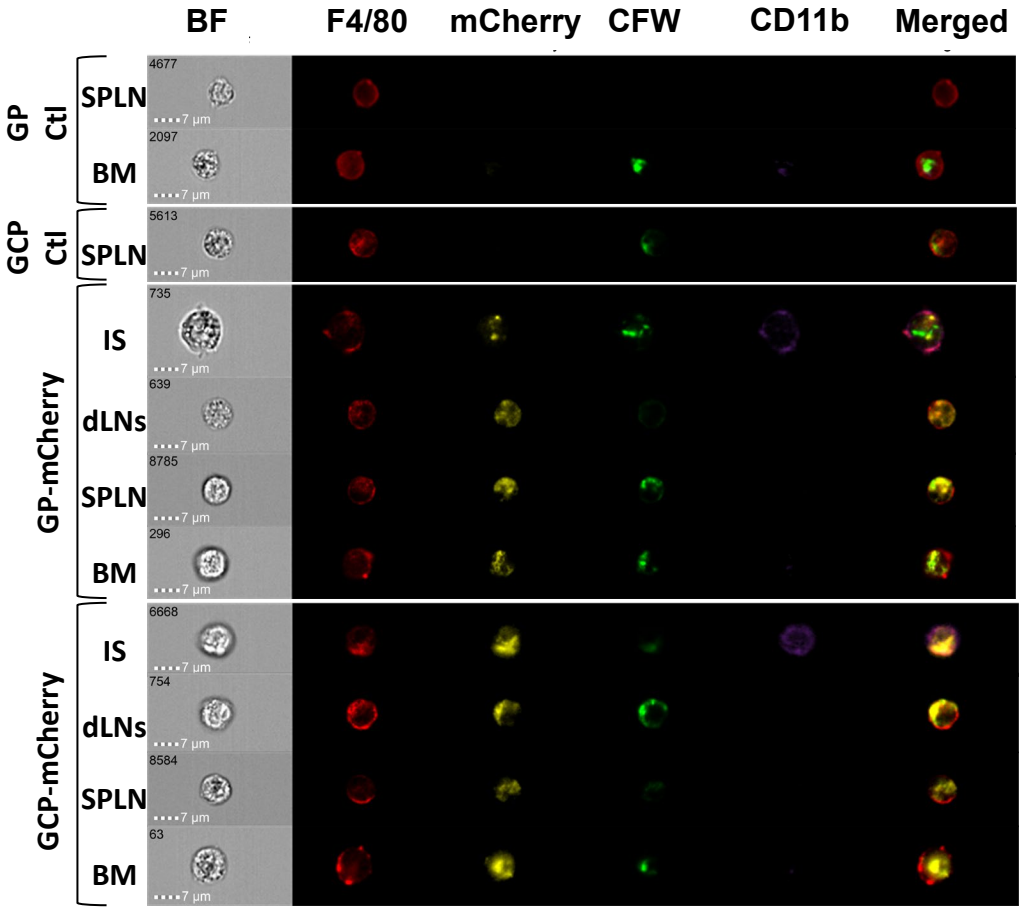
SANOFI PASTEUR
Empowering Life

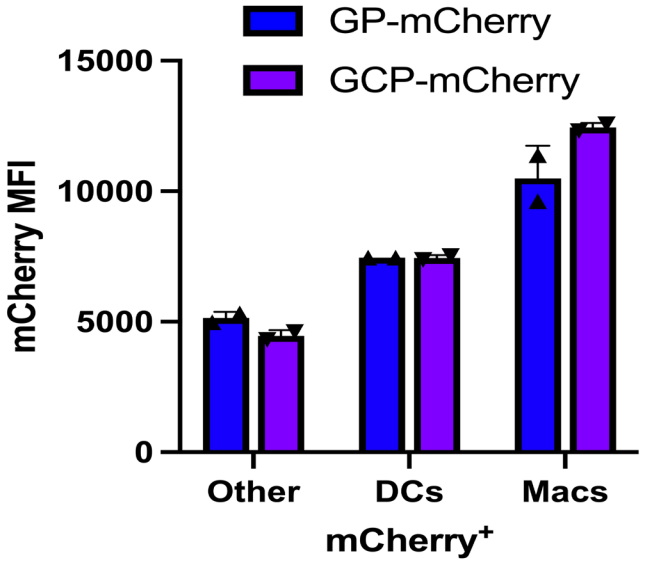
Comparison of CD4⁺ T-cell responses to rCpa1 between recovered Arizona patients and health donor (IFN-γ ELISA)



[Kruskal-Wallis Multiple comparison Test]

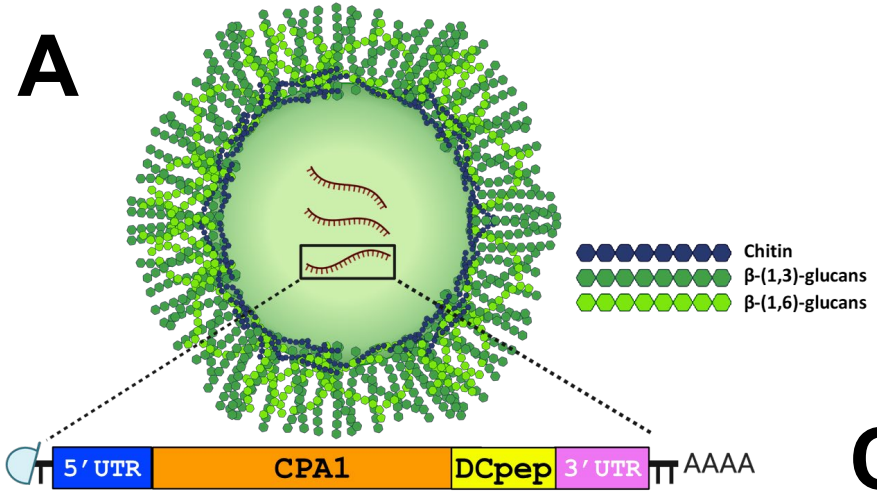
GCPs and GPs efficiently deliver mCherry-mRNA





GCP-CPA1-mRNA vaccine is expressed in the vaccinated mice

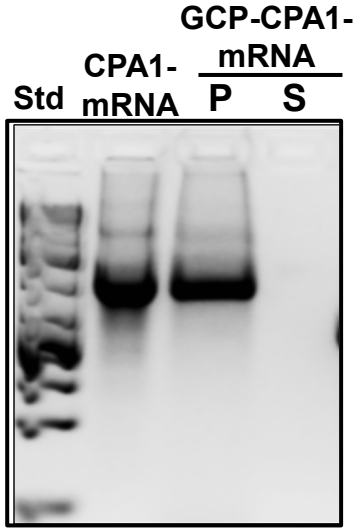
A



Modification of CPA1-mRNA

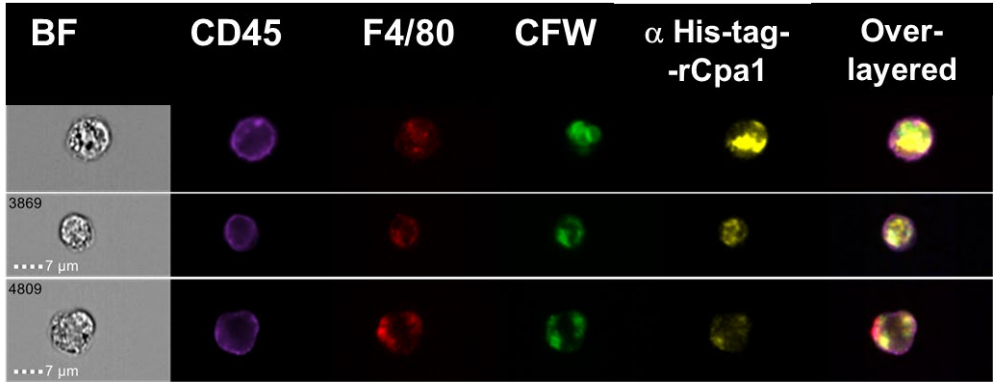
1. 5’ cap
2. 3’ poly A tail
3. Methylpseudouridine
4. His-Tag

B



Successful loading CPA1-mRNA into GCPs

C



Future Direction

1. Continue to discover coccidioidal T-cell epitopes (60+) for designing an optimized vaccine antigen(s)
2. Evaluate protective efficacies and immune correlates of GCP-epitopes and GCP-CPA1-mRNA vaccines
3. Evaluate vaccine adjuvants that can elicit a Th1- and Th17-mixed immunity
4. Preclinical *Ex vivo* T-cell recall assays using human PBMCs

5. Bridge to clinical trials—GMP production of vaccine components and scale up

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University of California Davis

Dr. George Thompson

MiraVista
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IMMY

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