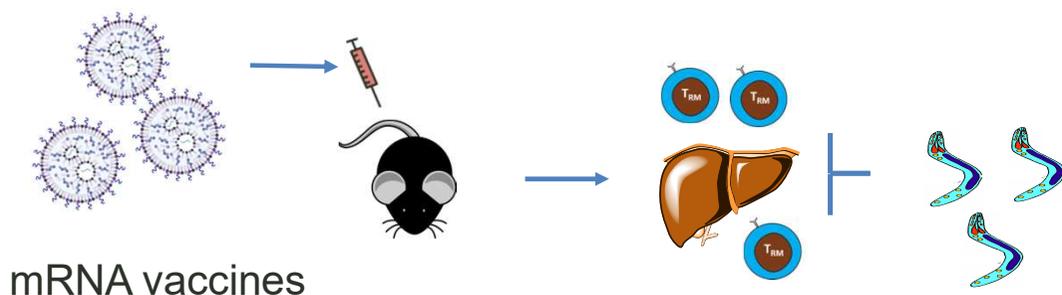


Lessons learned from *P. berghei* vaccine development: an mRNA vaccine adjuvanted with an NKT-cell agonist against liver-stage malaria



Te Kāuru
Ferrier Research Institute

Prof. Gavin Painter, October 2023
WHO/MPP mRNA Technology Transfer Programme,
Bangkok Oct 31 - Nov 1



Plasmodium Berghei



- 4 species described in mice (*P. berhei*, *P. yoelii*, *P. chabaudi*, *P. vinckeu*)
- Structure, physiology and life cycle similar to those that infect humans (in vivo model)
- Good conservation genes between murine and human parasites
- *P. Berghei*; in vitro cultivation, purification of each life cycle stage – accessible
- Originally worked on (Bill Heath) blood-stage infection and this is the only species that causes cerebral malaria



Bill Heath

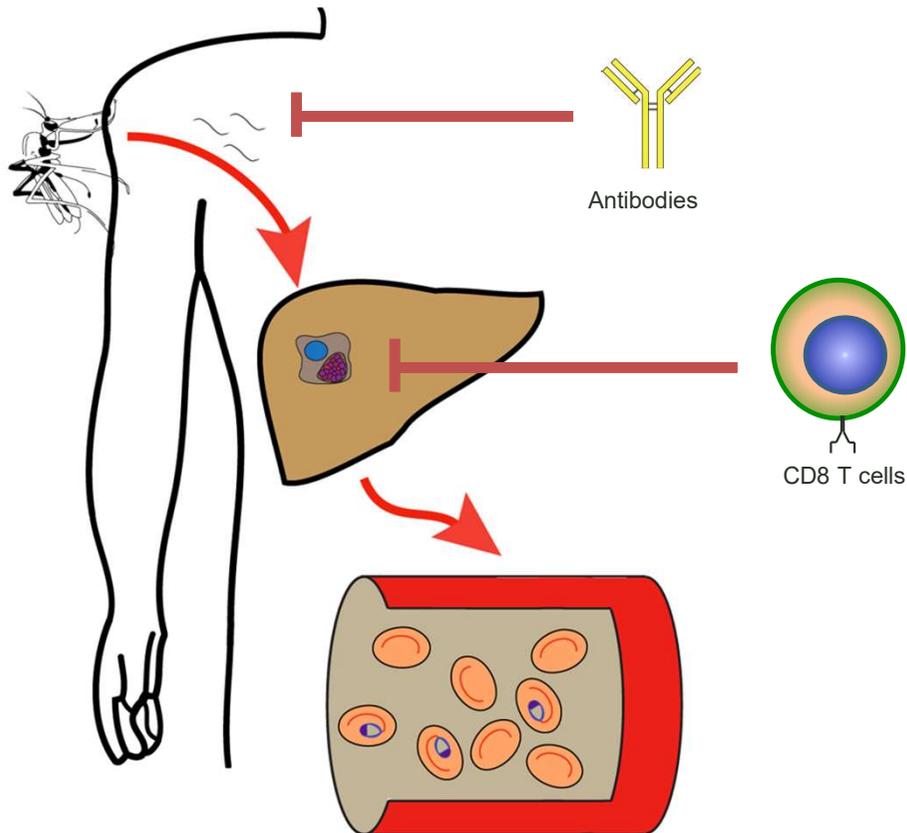


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Geoff McFadden

Malaria life cycle – vaccine design



- Hard to find and kill...limited time
- Need high Ab titres
- RS21 Matrix M - Dattoo, M.S. et al *Lancet* 2021, 397, 1809-18

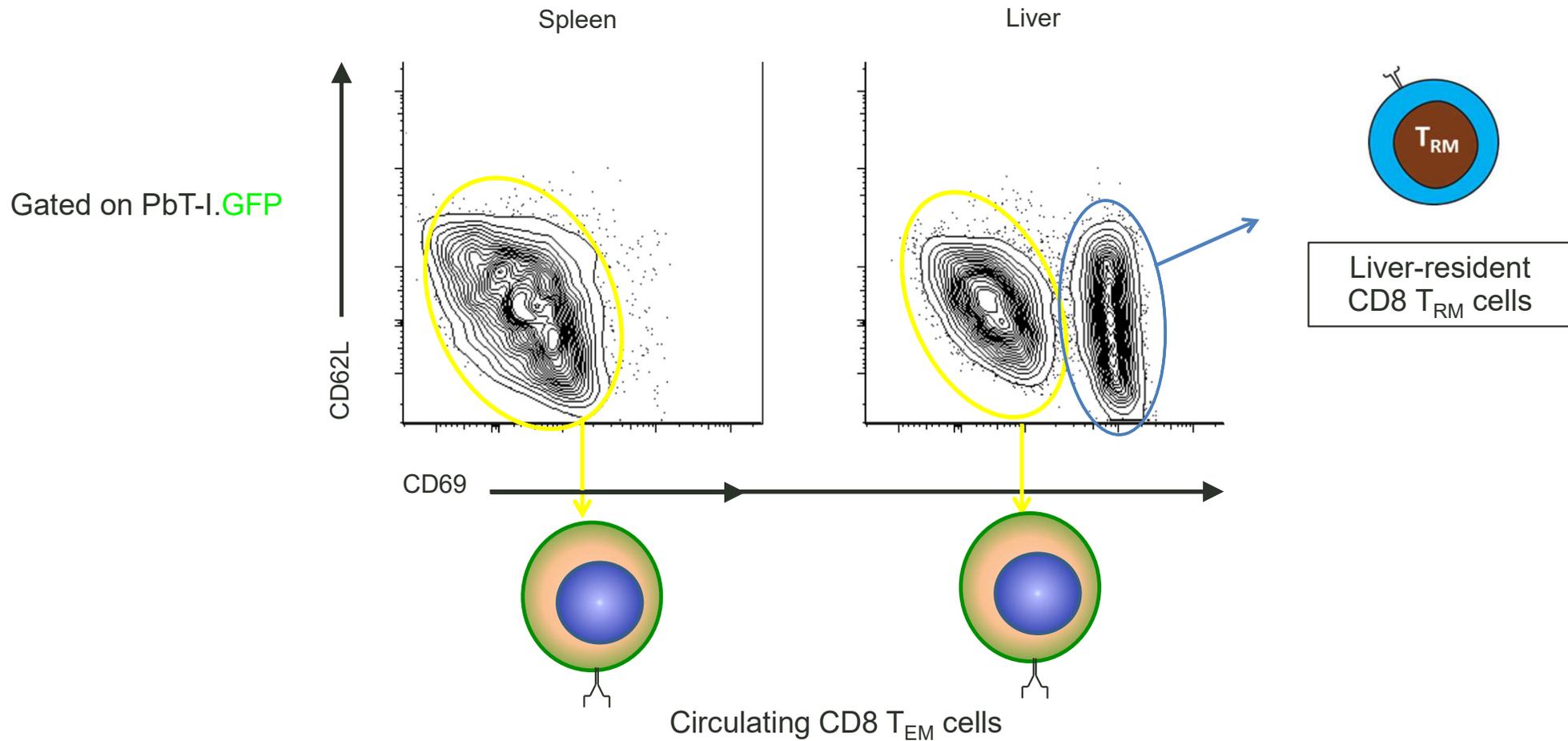
Liver stage:

- Definition of antigens required
- Vaccine generally not designed to make the correct response
- Radiation attenuated sporozoites work but are suboptimal and complicated

Blood stage:

- T cell response unable to access the erythrocytes
- Disease symptoms ensuing
- High antigenic variability (Ab responses)

RAS vaccination induces liver CD8 T_{RM} cells

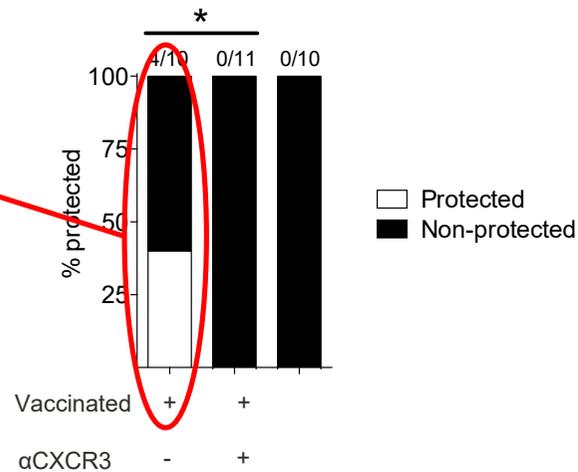


Fernandez-Ruiz et al., Immunity 45: 889, 2016

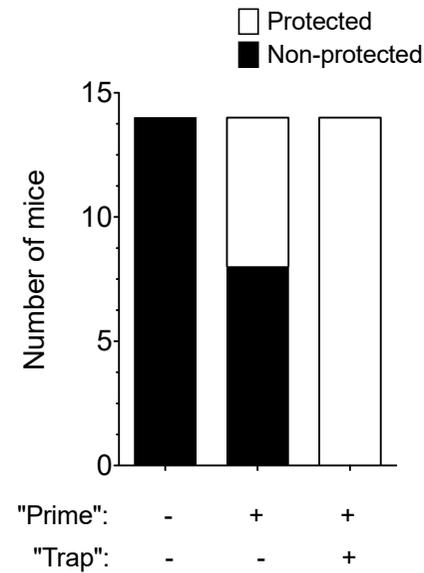
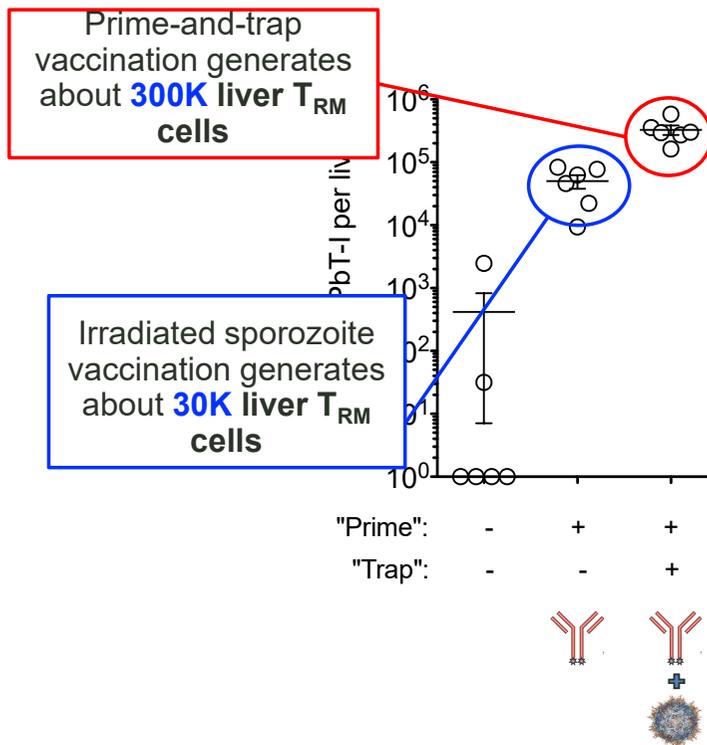
Depletion of liver T_{RM} cells ablates protection



Irradiated sporozoite vaccination generates about **30K liver T_{RM} cells**

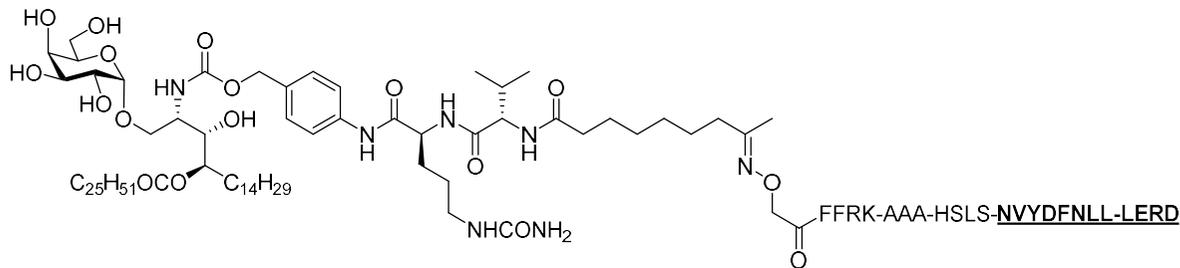
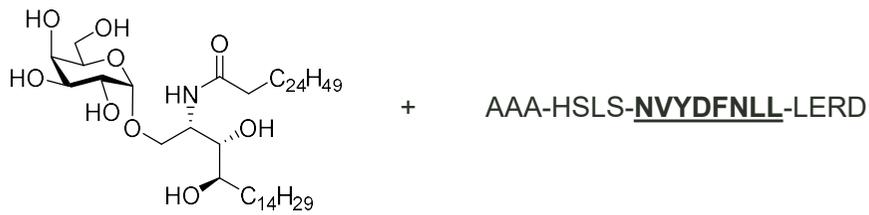


“Prime-and-trap” vaccination protects against sporozoite challenge

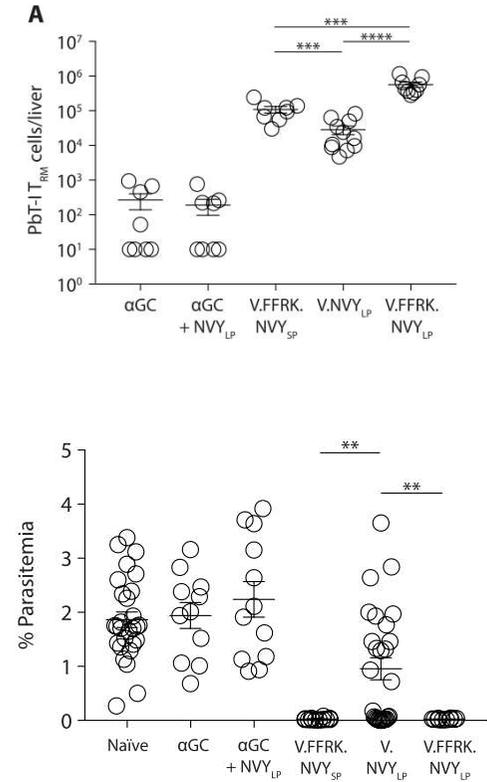


Long-term protection
Against high spz dose
But, complex
and cannot prime-boost

Glycolipid peptide (GLP) Conjugates Induce Liver T_{RM}



Glycolipid peptide (GLP) conjugate



Conclusions

- NKT cells can drive liver Trm and protect
- Peptide antigens need to be conjugated to the adjuvant
- Co-delivery/biodistribution likely required

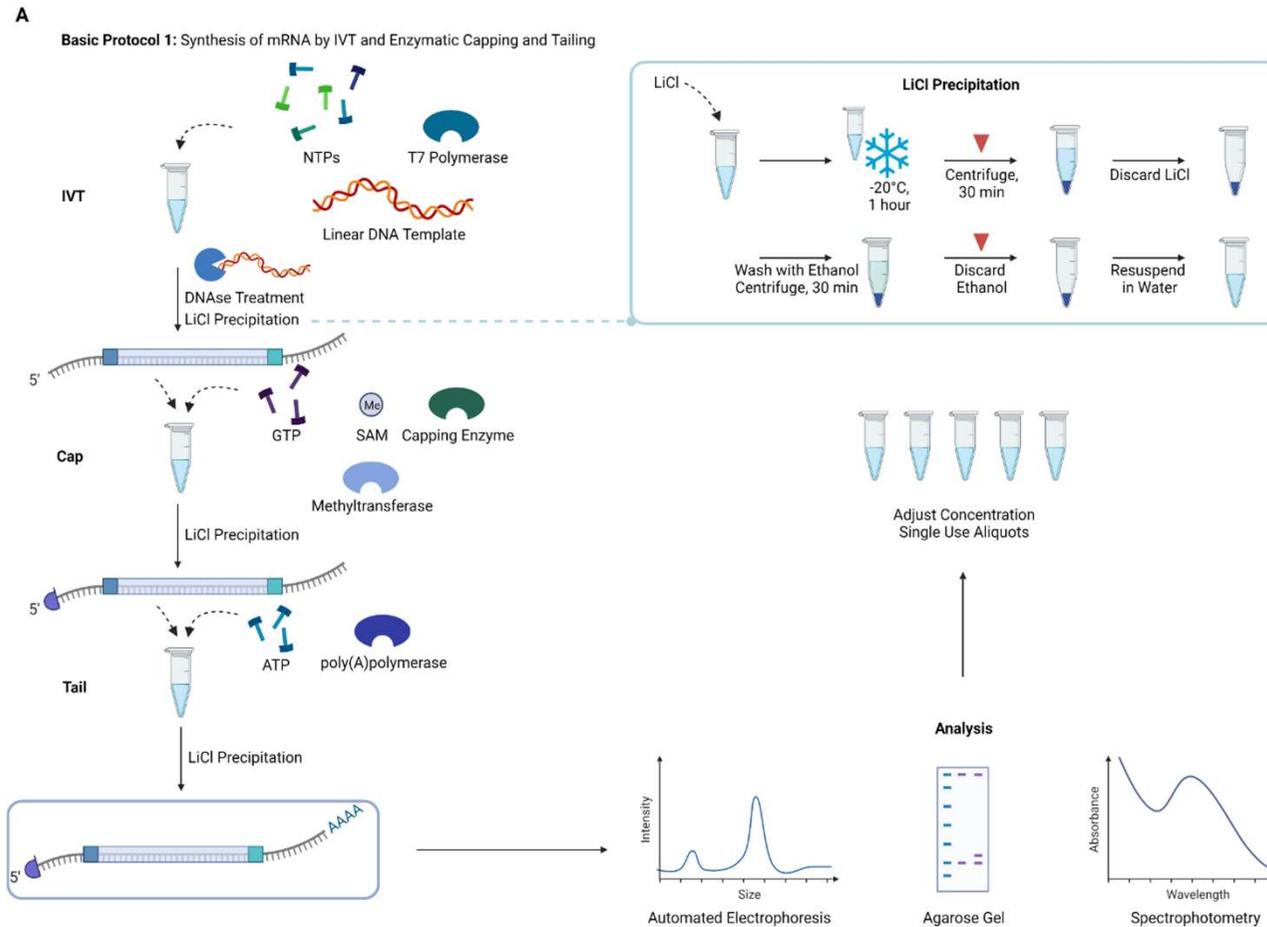
Peptide vaccines require epitope characterization

2668 *P. falciparum* genomes from 15 countries (Alyssa Barry, Myo Naung)



Peptide vaccines require full characterisation of antigen targets

Developing an mRNA Vaccine for Malaria



mRNA Synthesis and Encapsulation in Ionizable Lipid Nanoparticles

Rebecca Elizabeth McKenzie,^{1,2} Jordan James Minnell,^{1,3} Mitch Ganley,^{3,4} Gavin Frank Painter,^{3,4} and Sarah Louise Draper^{3,4,5}

¹Malaghan Institute of Medical Research, Wellington, New Zealand

²Hugh Green Cytometry Centre, Malaghan Institute of Medical Research, Wellington, New Zealand

³Ferrier Research Institute, Victoria University of Wellington, Lower Hutt, New Zealand

⁴Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, New Zealand

⁵Corresponding author: Sarah.draper@vuw.ac.nz

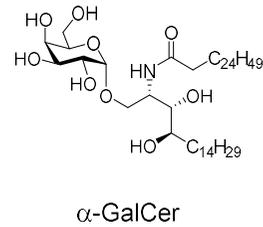
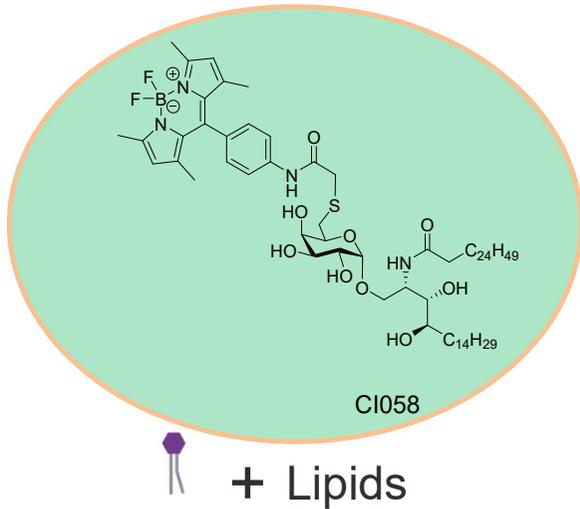


Only requires the protein antigen to be characterised

McKenzie, R. et al *Current Protocols* 2023

Developing an mRNA Vaccine for Malaria

NKT cell agonists



α -GalCer

Liposome development

Rotary evaporation



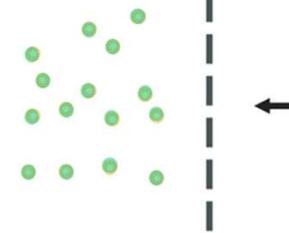
Hydration



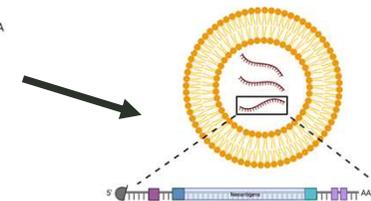
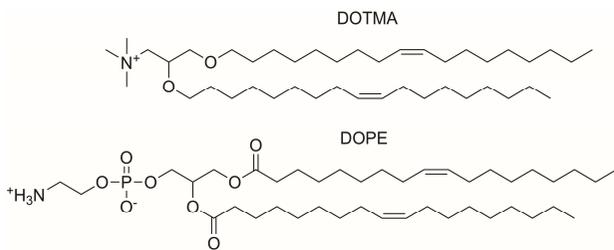
Sonication



Extrusion



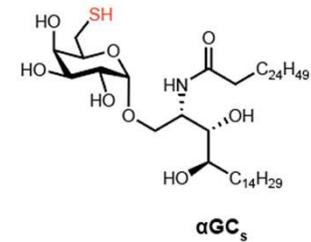
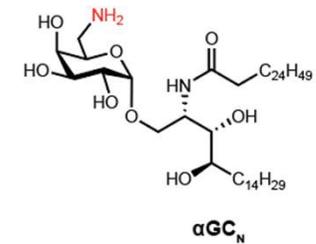
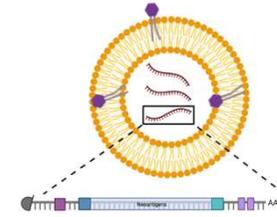
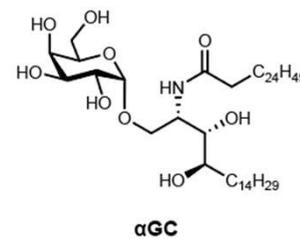
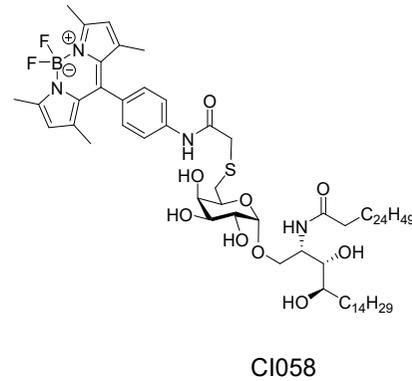
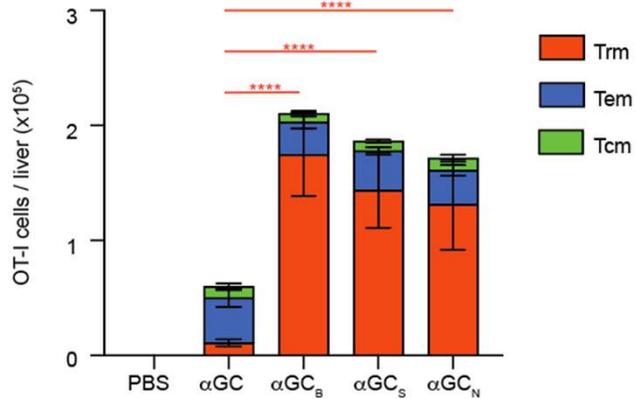
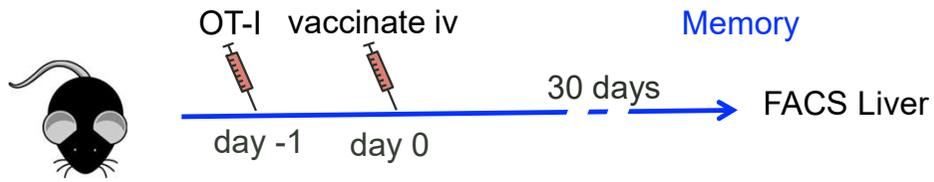
200 nm membrane



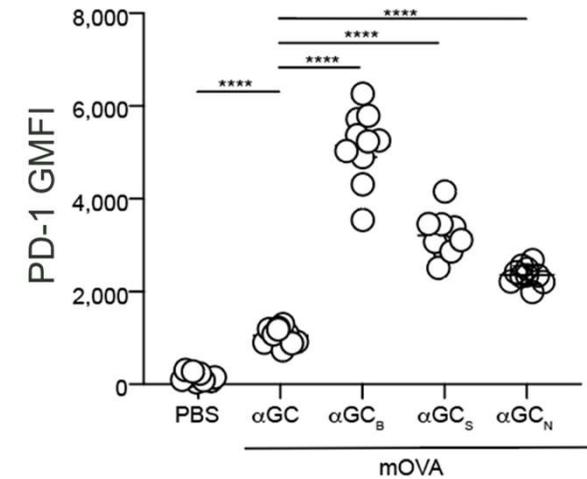
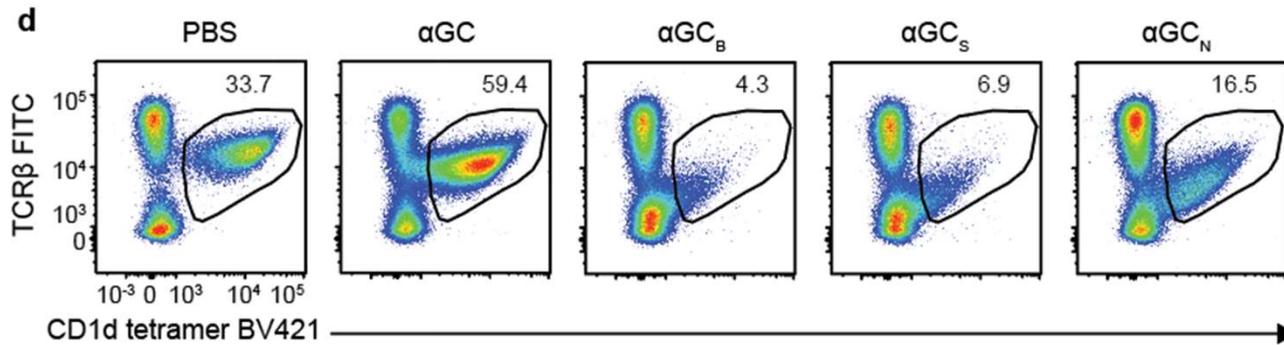
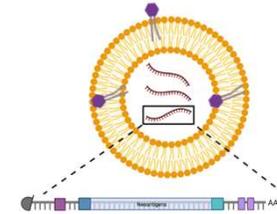
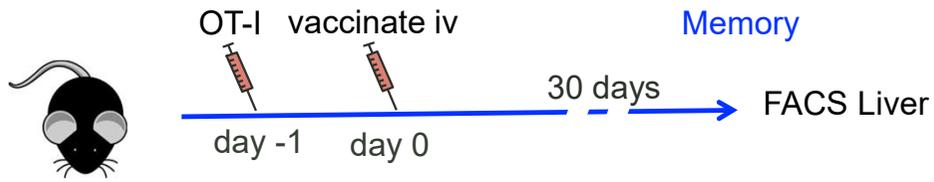
Lipoplexes (LPX)

Ugar Sahin, et al *Nat.* 2020

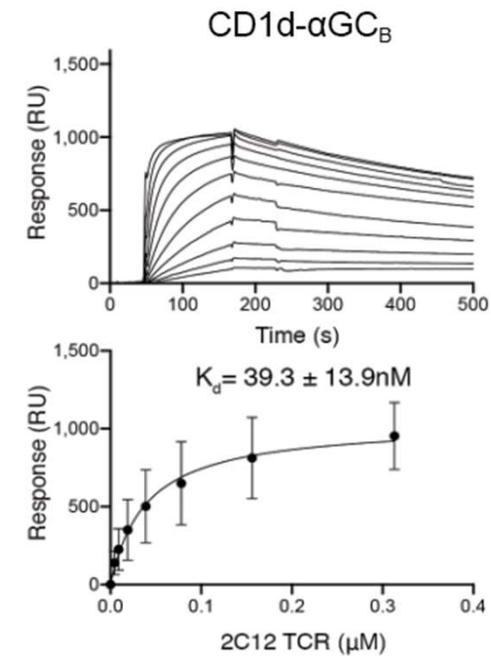
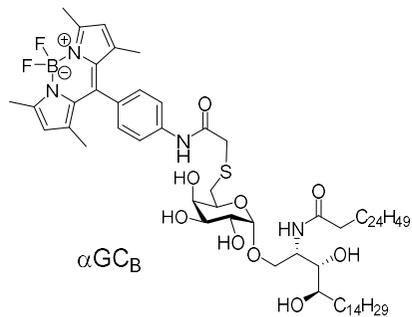
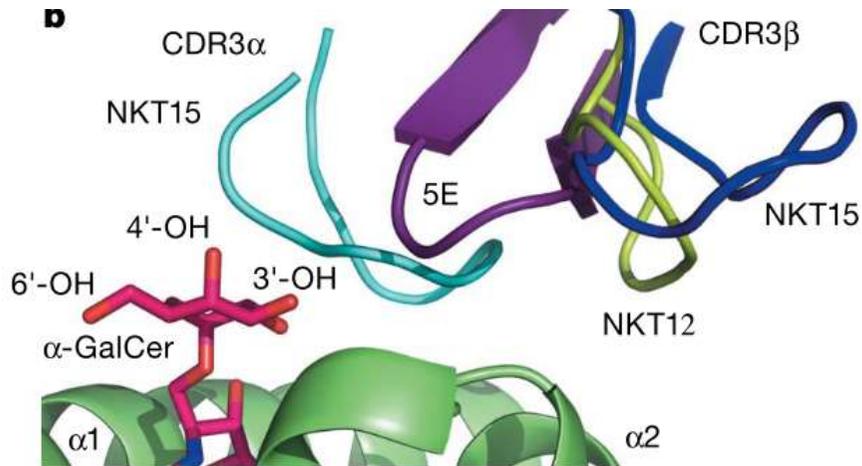
Developing an mRNA Vaccine for Malaria



Effective NKT cell agonists induce stronger NKT activation



CD1d-ligand-TCR binding affinity?

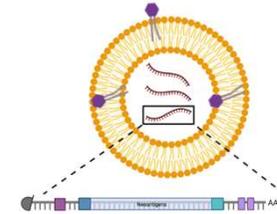
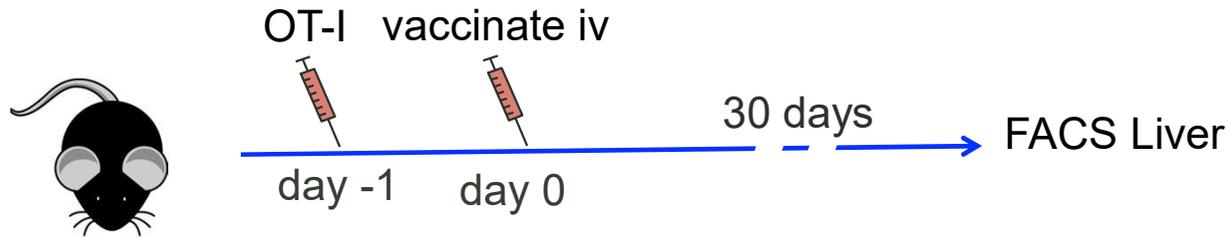


Affinity of binding of NKT TCR 2C12

CD1d- α GC ($K_d = 102 \text{ nM}$)

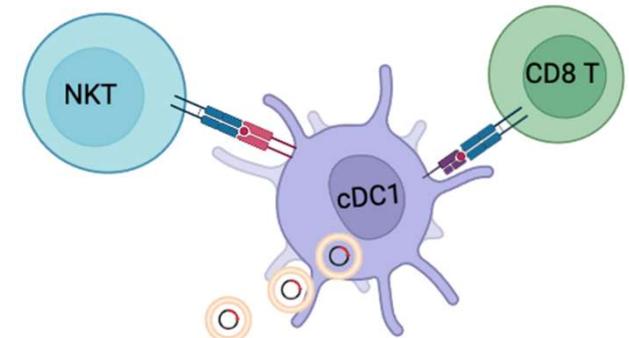
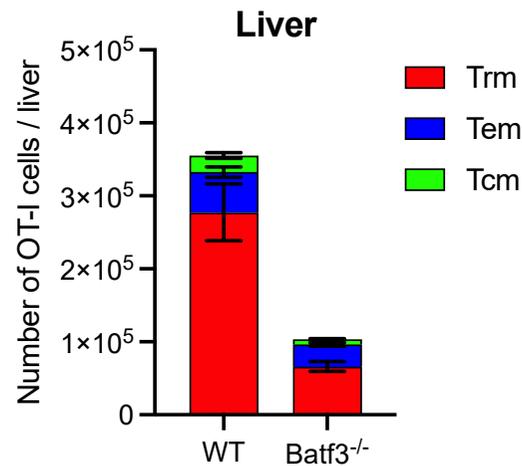
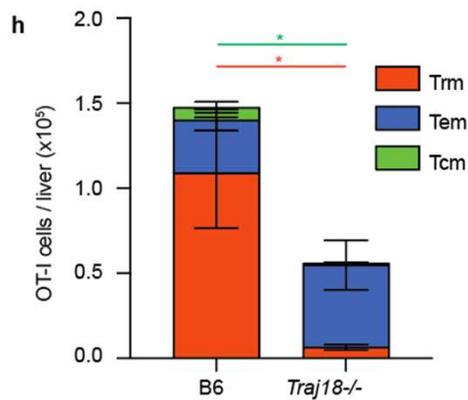
CD1d- α GC_B ($K_d = 39 \text{ nM}$)

NKT cells are required for induction of liver Trm to mRNA vaccine



B6 vs *Tra18^{-/-}*

B6 vs *Batf3^{-/-}*



Effective NKT cell agonists induce stronger NKT activation

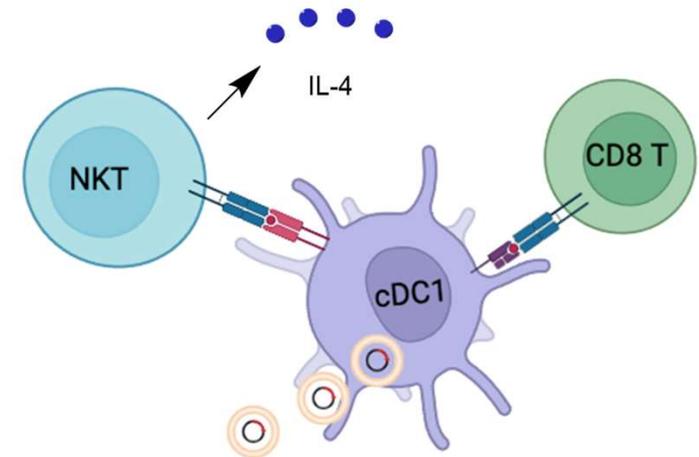
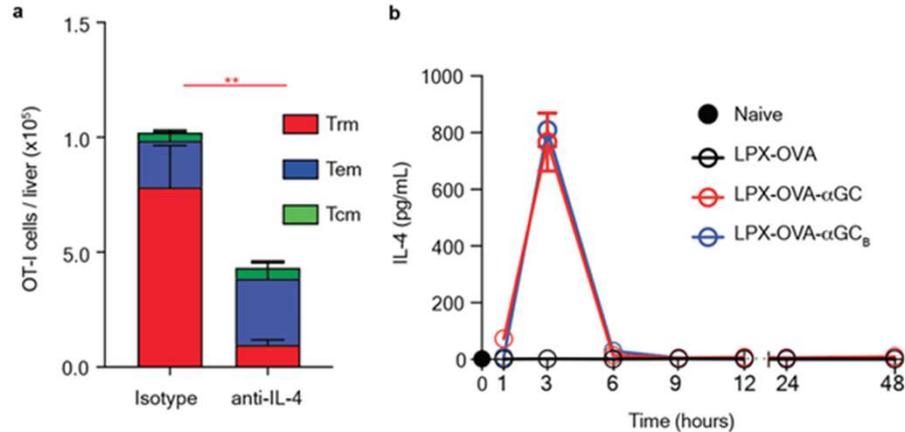
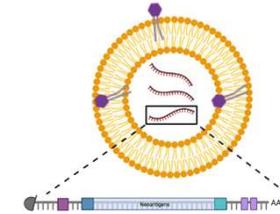
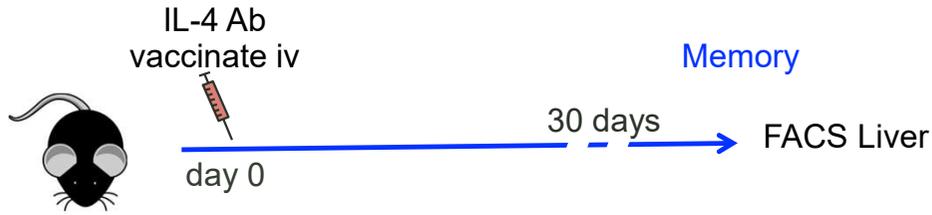
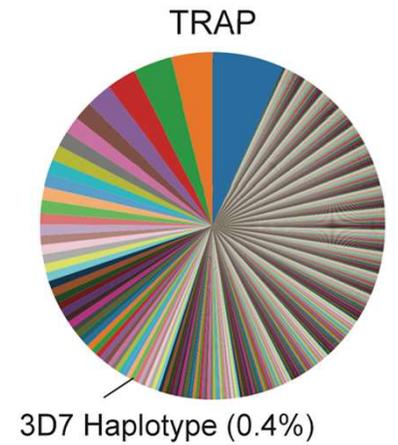
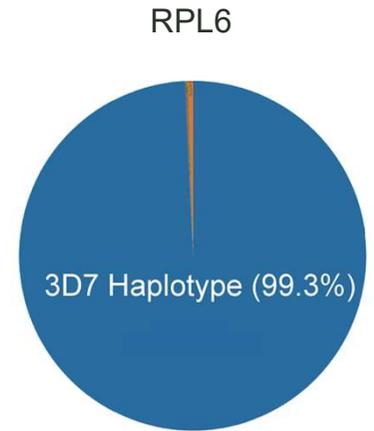
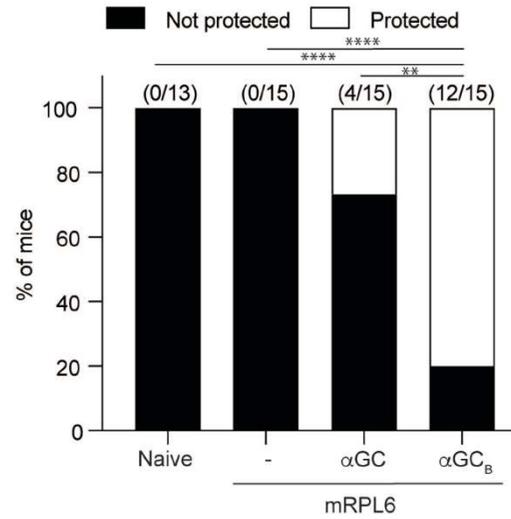
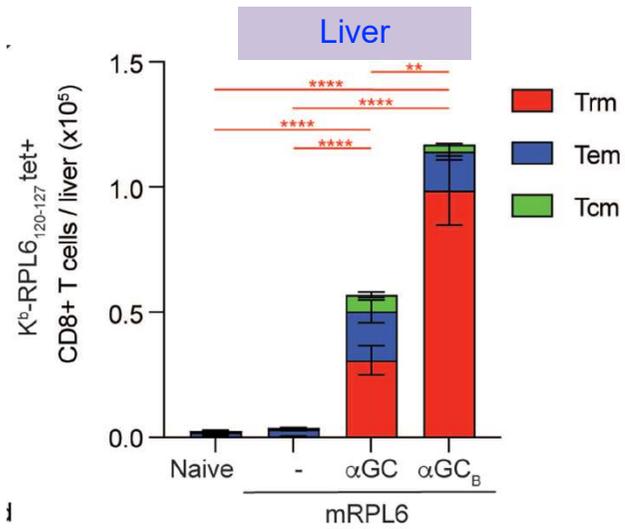
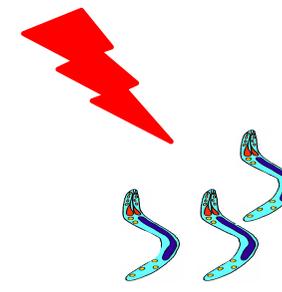
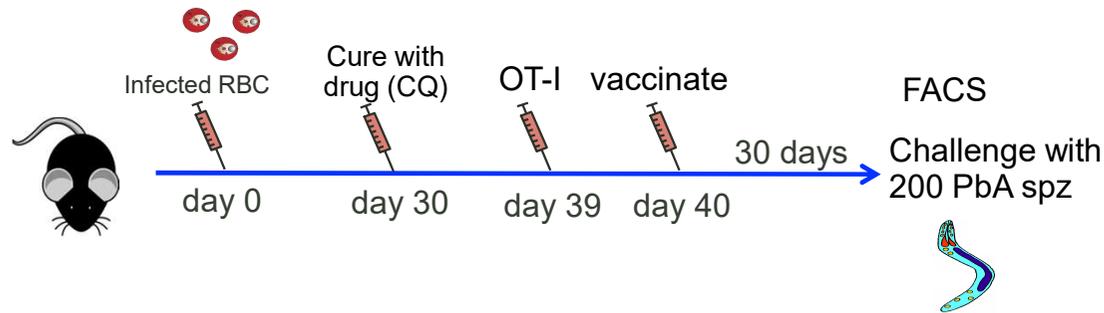


Figure 5. IL-4 is important for liver Trm cell generation. (a) OTI liver Trm cell responses to LXP-mOVA-αGC_β were inhibited by exposure to anti-IL-4 mAb during priming. (b) IL-4 is detected in the serum after vaccination with LPX mRNA vaccines containing NKT cell agonists.

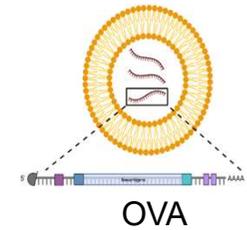
Authentic malaria antigens work (RPL6)



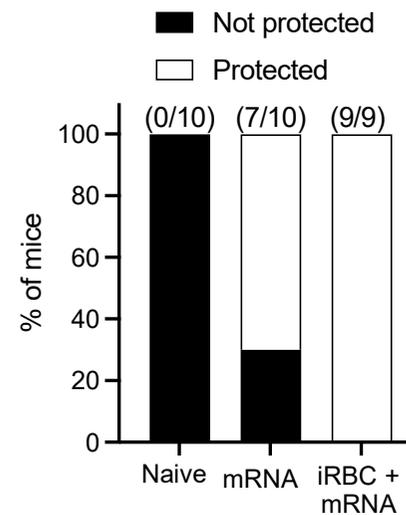
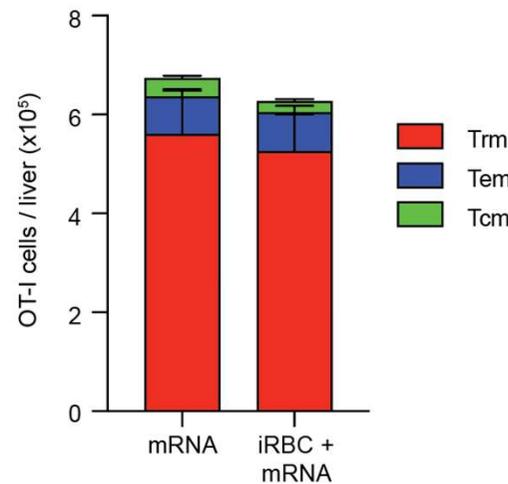
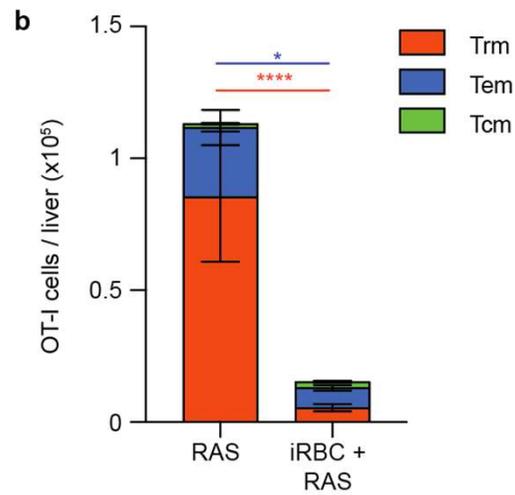
Previous exposure to blood stage malaria does not impair mRNA vaccination



Radiation attenuated sporozoites-OVA



mRNA + aGC_B



M. Ganley et al *Nat Immunol* 2023

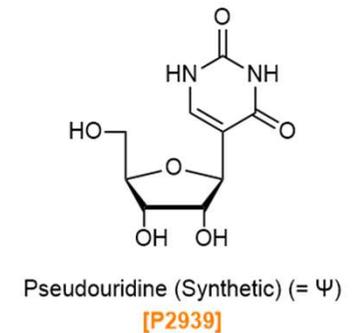
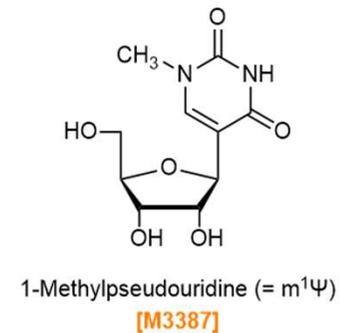
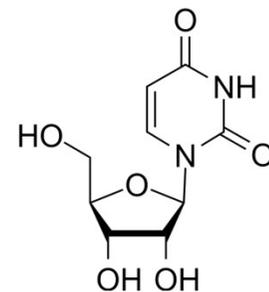
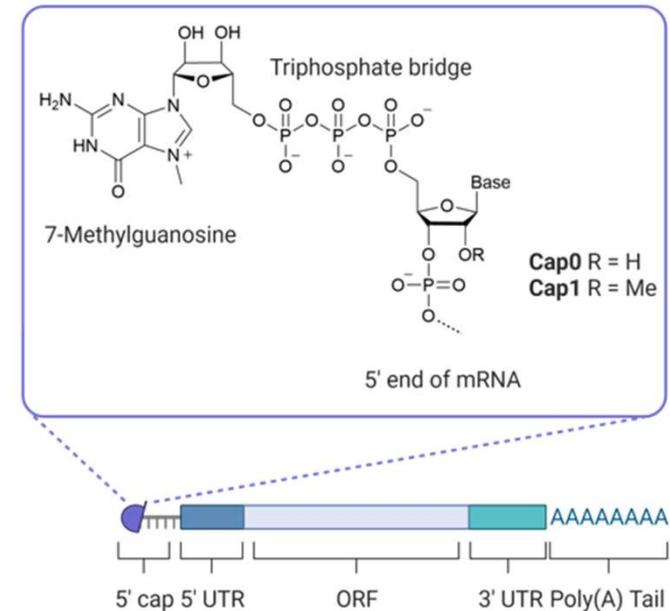
Transition to an Industry Standard LNP-mRNA Vaccine Format – mRNA Design

mRNA design

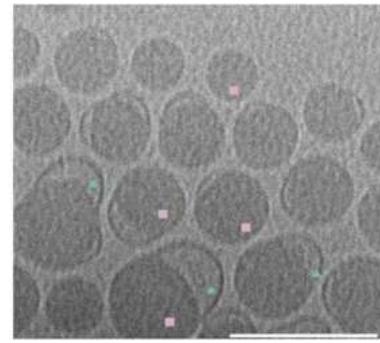
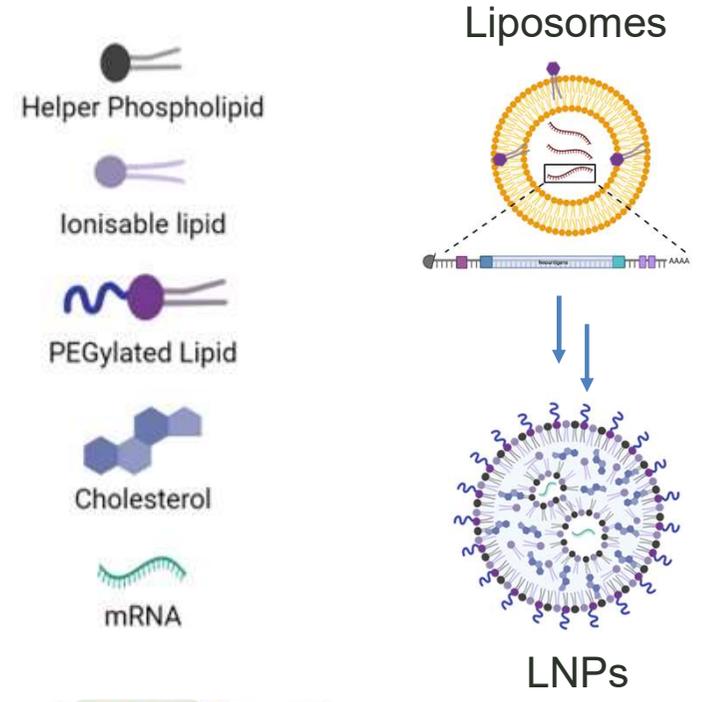
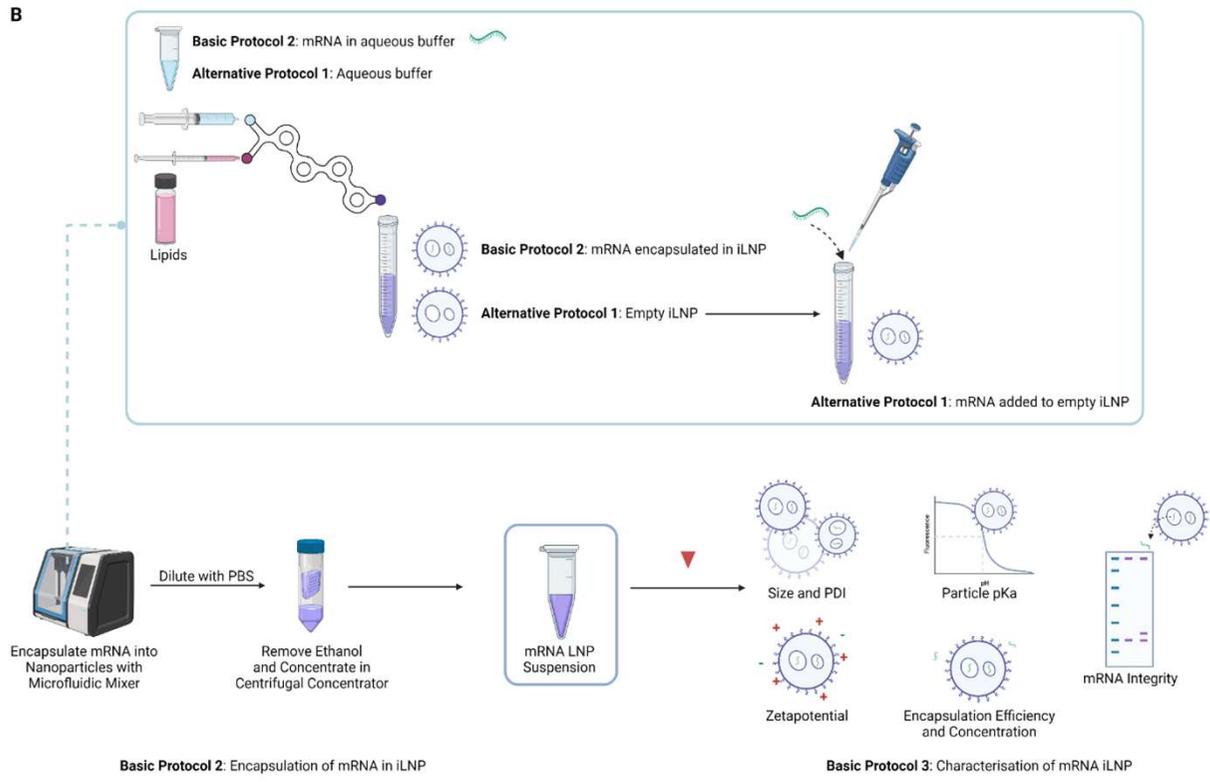
- Modified bases
- Inclusion of UTRs
- Capping (co-transcriptional capping/clean-cap)
- Poly-A vector encoded tail

Purification

- ds-RNA removal (cellulose chromatography)
- FPLC, oligo-dT affinity, ion-exchange
- Analytical method development

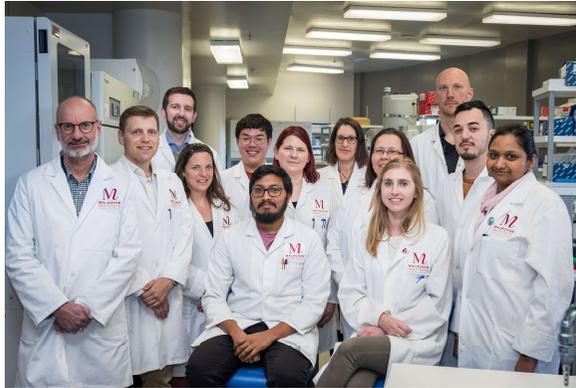
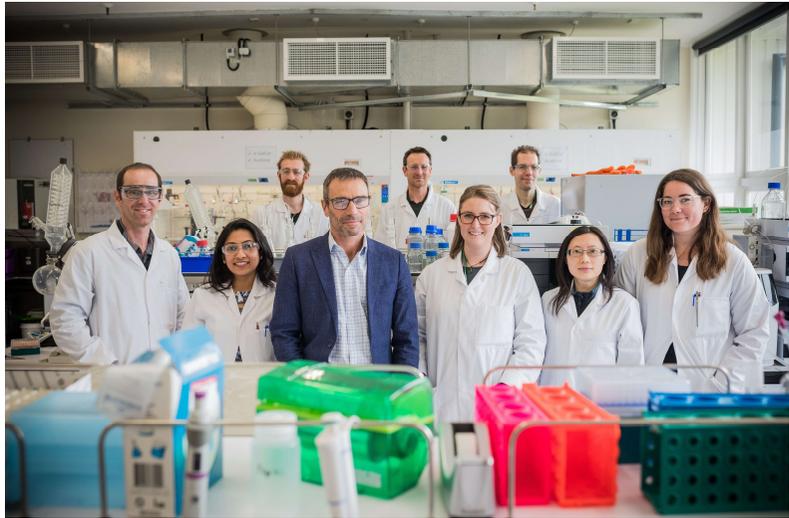


Transition to an Industry Standard LNP-mRNA Vaccine Format – The Formulation



McKenzie, R. et al *Current Protocols* 2023

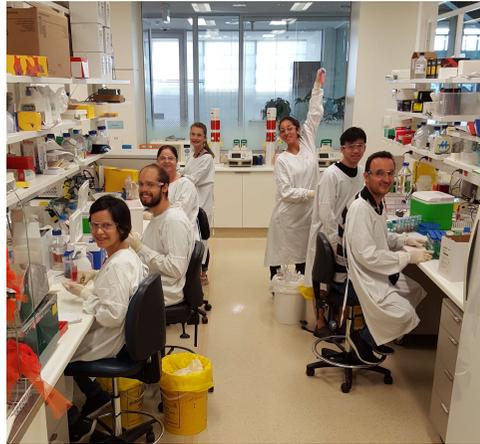
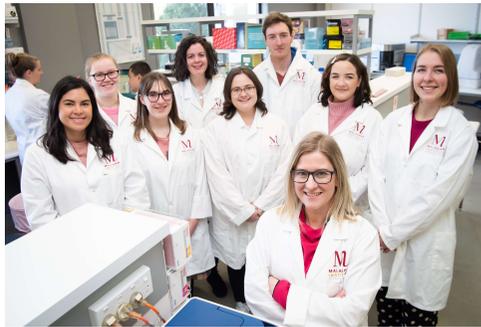




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