Vaccine Adjuvant Activity of Conifer-derived Oil-in-Water Nanoemulsions
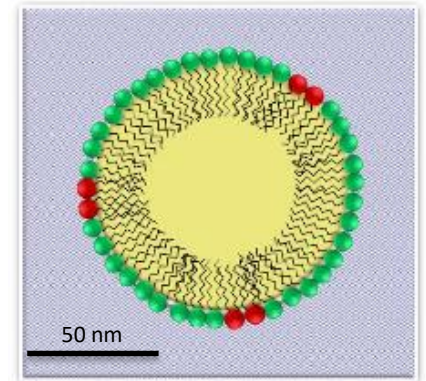

I D R I
TRANSFORMING SCIENCE INTO
GLOBAL HEALTH SOLUTIONS

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Outline

- Introduction to vaccine adjuvants including oil-in-water emulsions

- Emulsion oil structure and source considerations

- Evaluation of conifer-derived polyprenol emulsions as novel adjuvant formulations

- Future directions

What are Adjuvants?

- Substances added to vaccines to improve the immune response
    - Molecular mimics of pathogen molecular patterns
    - Geometric mimics of pathogen physical properties (particle size, etc.)
    - Help deliver vaccine, increase cell uptake, etc.
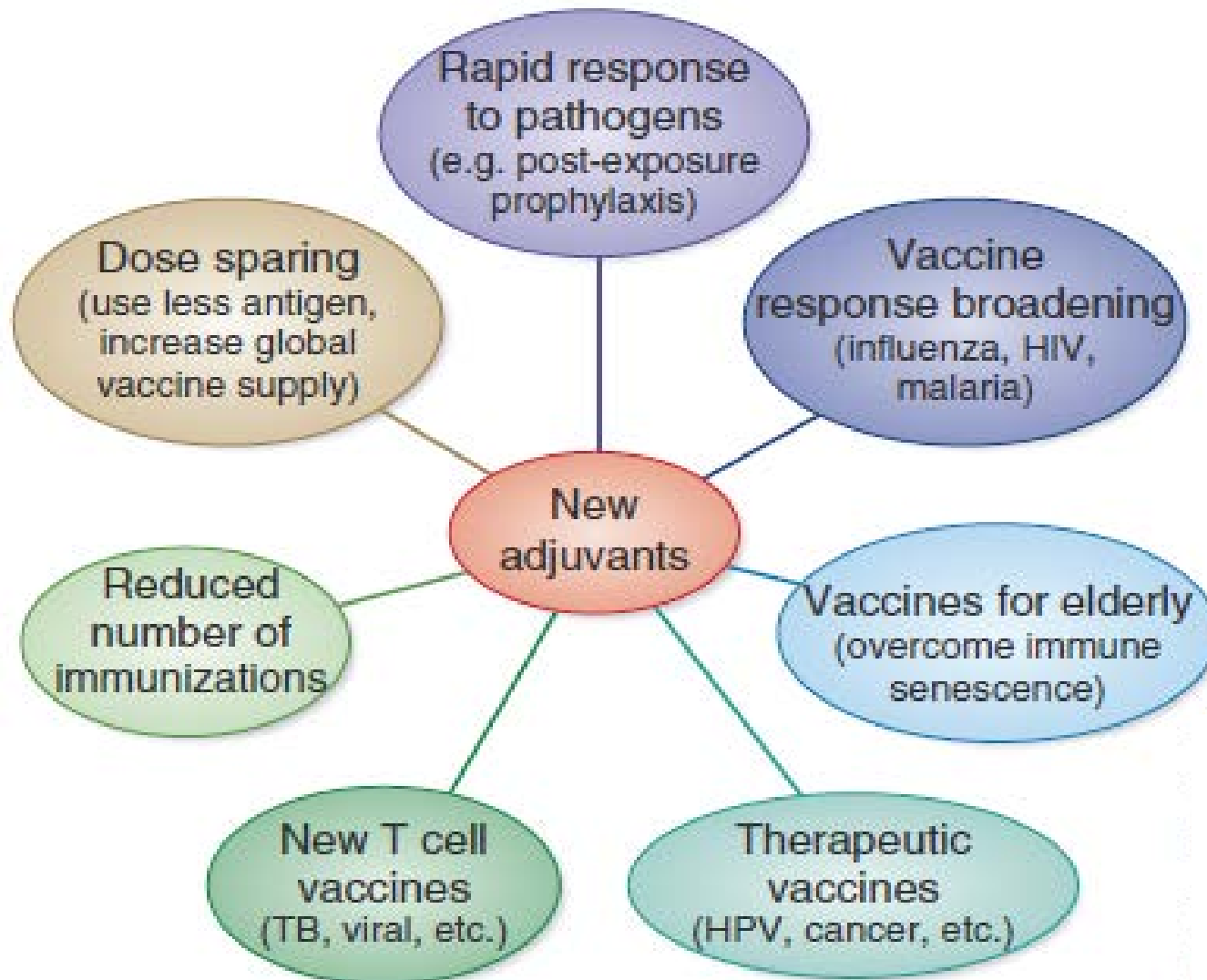   - Most adjuvants are a combination of the above
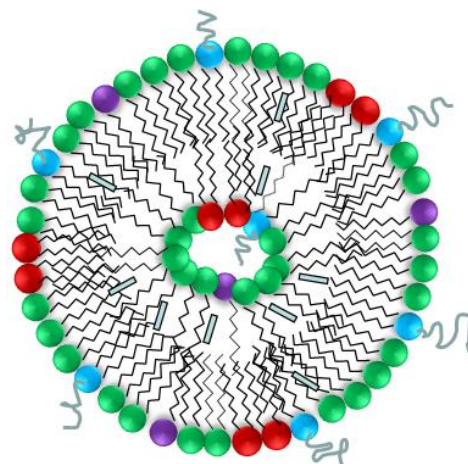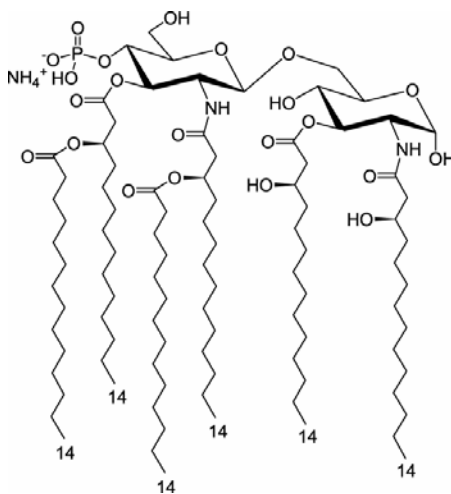
How Adjuvants Work

Reed et al. Nature Med 2013, 19:1597
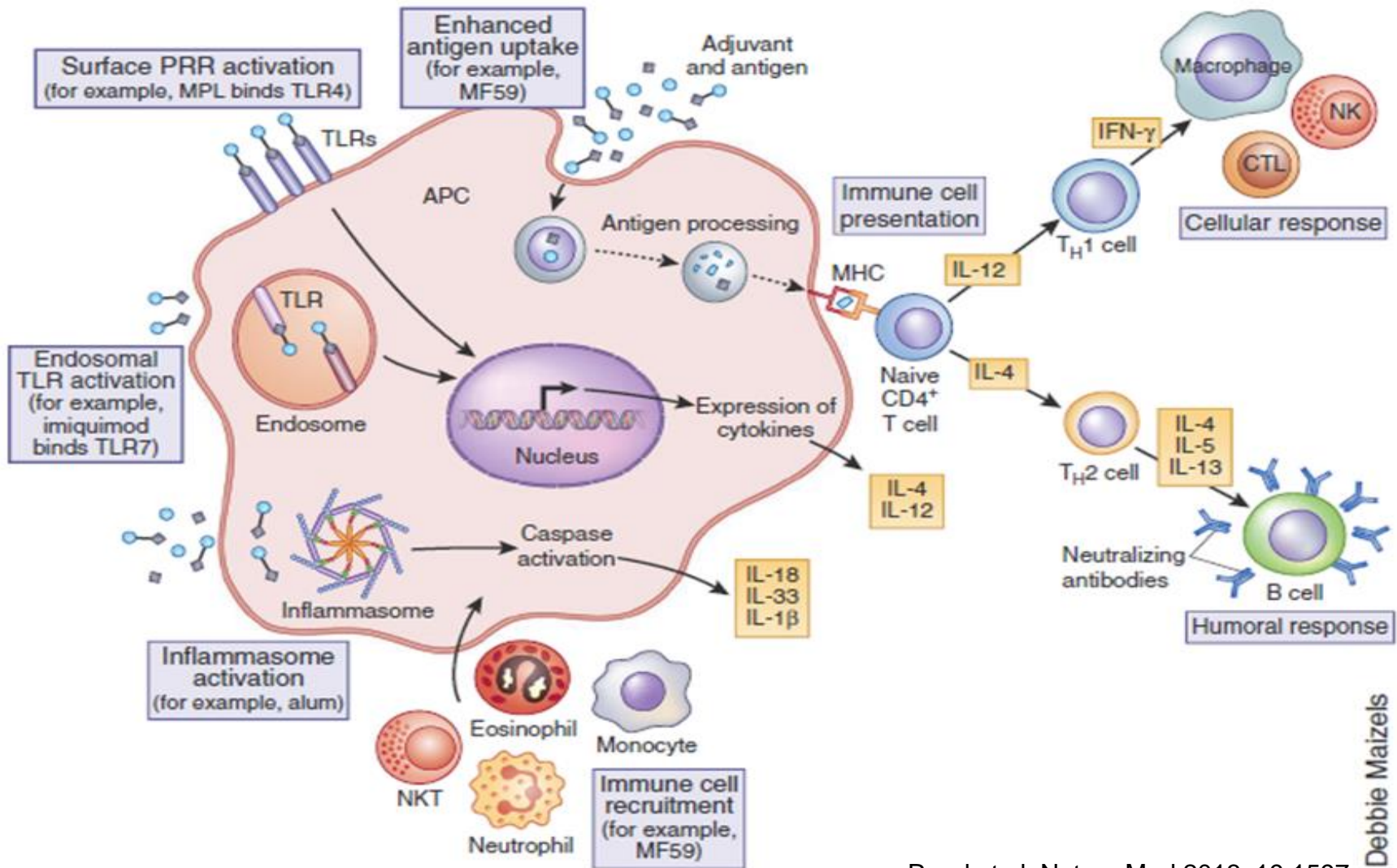
Adjuvant Benefits

IDRI Adjuvant Pipeline

|	PRECLINICAL	PHASE 1	PHASE 2	PHASE 3	PARTNERS
ADJUVANTS					
SE	Leish		Flu		BARDA, Sumaya
GLA-SE	Leprosy, Leish, Schisto, Malaria, Cancer. Thermostable TB		Flu, TB		Orygen, Quratis
GLA-AF	HIV, Flu				
GLA-Alum	Schisto, Hookworm				Sabin
GLA-LSQ	Malaria, TB				NIH, EVI
SLA-SE	ETEC, West Nile	Zoster, Leish, TB			MOGAM
SLA-LSQ	Zoster, ETEC West Nile, Anthrax	TB			
3M-052-SE*	Flu				Medicago
3M-052-Alum*	TB, HIV, Malaria				BMGF
3M-052-LS*	Flu, HIV				
GLA-3M-052-LS*	Amebiasis				
GLA-Imiquimod	Malaria, HIV				
Nano Alum	Flu, Schisto, TB. Pertussis				
NLC	Zika, TB				
Peptide Liposome	HIV				*Collaboration with 3M

O/W Emulsion Adjuvants

- ~200M human doses of influenza vaccines containing o/w emulsions administered to date
- Enhanced responses in elderly and young children
- Antigen dose sparing for pandemic flu

Antigen dose sparing

Increase in efficacy attributable to o/w emulsion adjuvant in young children (6-72 months)

Analysis*	Efficacy Against All Strains	
	Cases/ Vaccinated	VE % (2-sided 95% CI)
FLUAD vs. Non-influenza controls	13/1937 vs. 48/993	86 (74 - 93)
TIV vs. Non-influenza control	50/1772 vs. 48/993	43 (15 – 61)
FLUAD vs. TIV	13/1937 vs. 50/1772	75 (55 - 87)

H5N1+AS03
H5N1
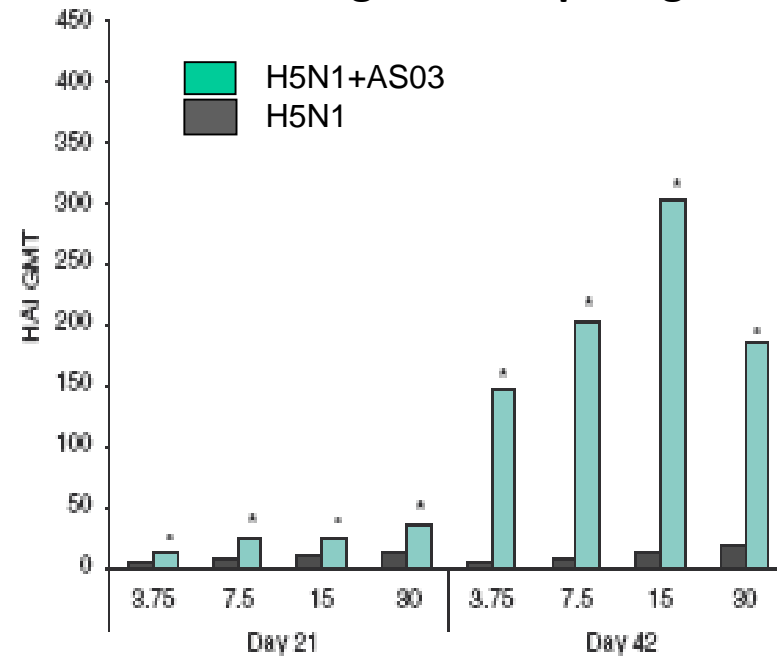
IDRI's O/W Emulsion (SE)
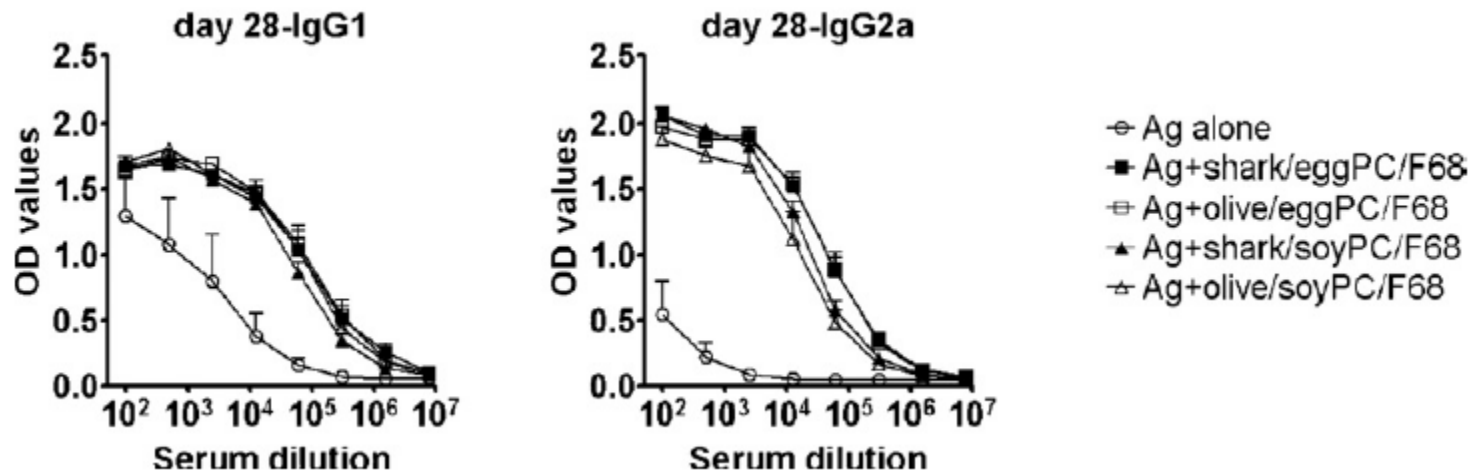
- Squalene-based oil-in-water emulsion (excipients include DMPC, poloxamer 188, glycerol, antioxidant, buffer)
- Manufactured at 4% oil for 1:1 v:v mixing with antigen
- Particle size stability maintained 5 years at 2-8°C
- Multiple cGMP batches produced for Phase 1/2 clinical trials
- May be employed to formulate various TLR ligands (e.g. GLA)

Size Distribution by Intensity

200 nm

Why Squalene?

- First generation emulsion adjuvants based on high amounts of mineral oil were too reactogenic

- Researchers in the 1970-80s sought for more biocompatible oils and lower oil content

- Squalene was shown to facilitate emulsion stability, biocompatibility, and adjuvant activity

- Source for pharmaceutical squalene is shark liver, where it is present in high concentrations

- No structure-function study of squalene adjuvant activity has yet been reported

Plant-derived Squalene

- Emulsions made w/squalene from olives provides comparable adjuvant activity as emulsions made w/squalene from sharks

- Squalene content in plant sources is relatively low, different impurity profiles, no cost savings

Oil Structural Class vs. Adjuvant Activity

- Squalene emulsion induces Ag-specific long-lived plasma cells

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Polyprenol source

- Prenolica sustainably extracts polyprenols from pine/fir trees for pharmaceutical therapeutics

squalene

polyprenol

brambleberry.com

Polyprenol	Source	Purity	Density (g/cm3)	c50	c55	c60	c65	c70	c75	c80	c85	c90	c95	c100
Picea abies	Norway Spruce	96.2	0.899	0.4	0.5	0.9	2.5	15.6	34.8	27.8	10.5	3.2	0.8	0.1
Abies sibirica	Siberian Fur	95.3	0.897	0.3	0.1	0.2	0.3	1.1	8	32.5	38.2	11.9	0.7	
Pinus sibirica	Siberian Pine	90.8	0.901	0.4	0.5	0.2	0.9	2.8	10.7	25.9	27.5	10.3	3.9	3
Ropren (25% Picea abies/75% veg oil)	Norway Spruce + Veg Oil	See PP1 (25%), the rest is triglyceride (similar to grapeseed)												

- Polyprenol emulsion physicochemical characteristics and stability similar to squalene emulsion

Emulsion Physical Characteristics

Description	Visual appearance	Particle diameter (Z-ave, nm)	Size polydispersity index (PdI)
P. abies nanoemulsion	Homogeneous, milky-white	114 +/- 1	0.13 +/- 0.01
A. sibirica nanoemulsion	Homogeneous, milky-white	104 +/- 1	0.06 +/- 0.02
	Homogeneous, milky-white	98 +/- 1	0.05 +/- 0.01
P. sibirica nanoemulsion	Homogeneous, yellow	103 +/- 1	0.06 +/- 0.03
Ropren® nanoemulsion	Homogeneous, milky-white	94 +/- 0	0.05 +/- 0.02
Squalene nanoemulsion**	Homogeneous, milky-white	121 +/- 1	0.04 +/- 0.04
	Homogeneous, milky-white	87 +/- 2	0.05 +/- 0.02
Grapeseed nanoemulsion	Homogeneous, milky-white	91 +/- 1	0.06 +/- 0.02
A. sibirica nanoemulsion + GLA	Homogeneous, milky-white	106 +/- 1	0.08 +/- 0.02
Squalene nanoemulsion + GLA**	Homogeneous, milky-white	80 +/- 0	0.07 +/- 0.02
	Homogeneous, milky-white	84 +/- 1	0.05 +/- 0.02

**Manufactured at 10% oil concentration; all other nanoemulsions manufactured at 4% oil concentration.
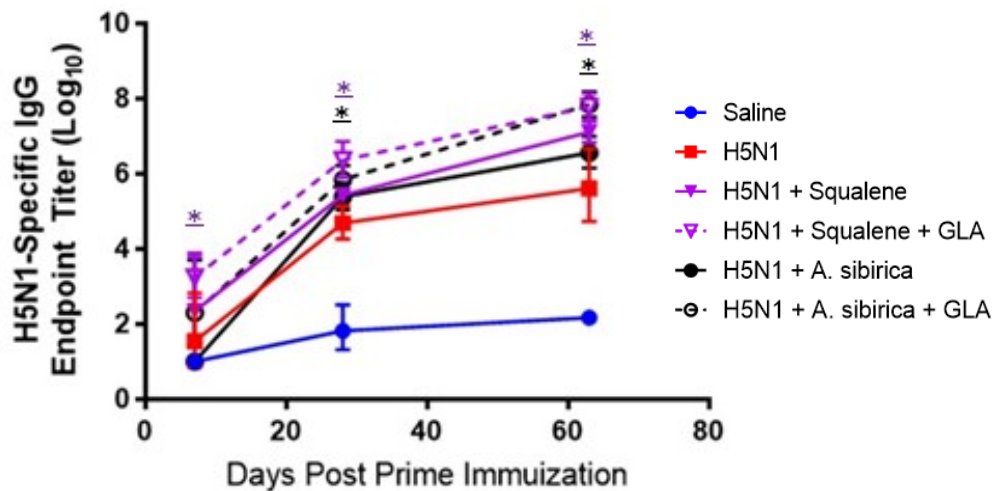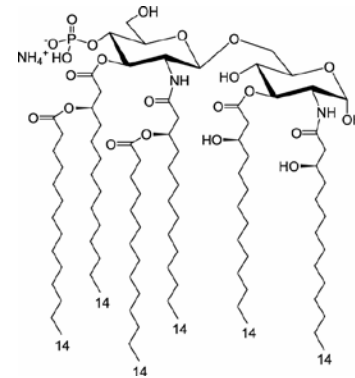
Polyprenol Emulsion Adjuvant Activity in Mouse Model

- Mice were immunized twice intramuscularly with split inactivated H5N1 antigen alone or in combination with emulsion; serum IgG and long-lived plasma cells quantified
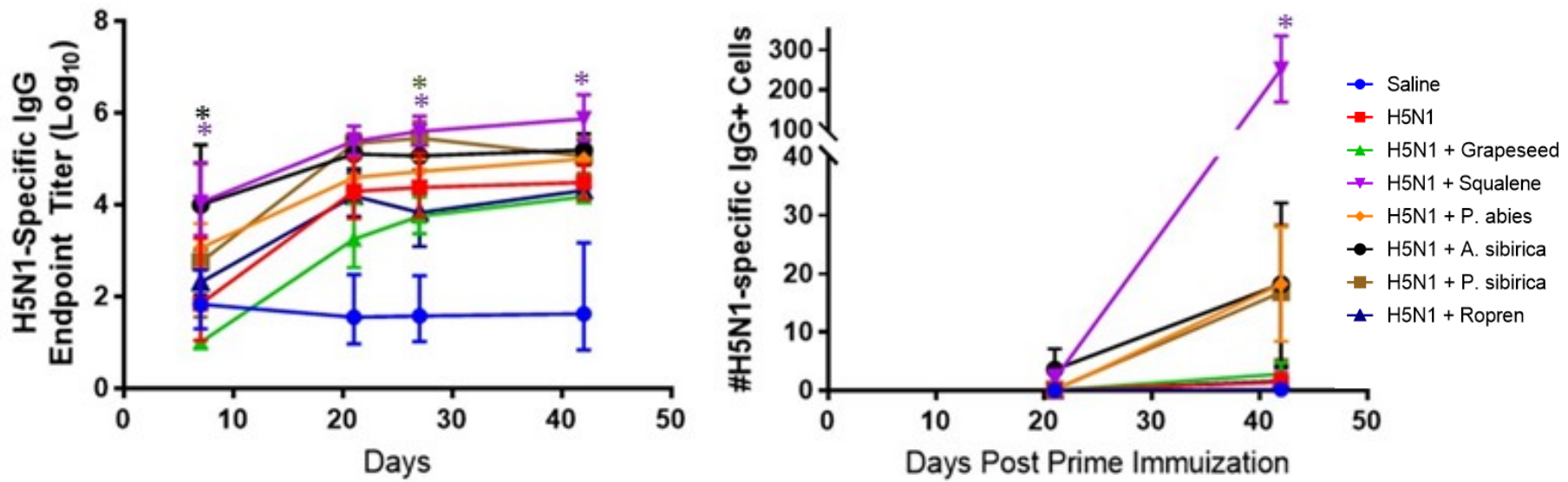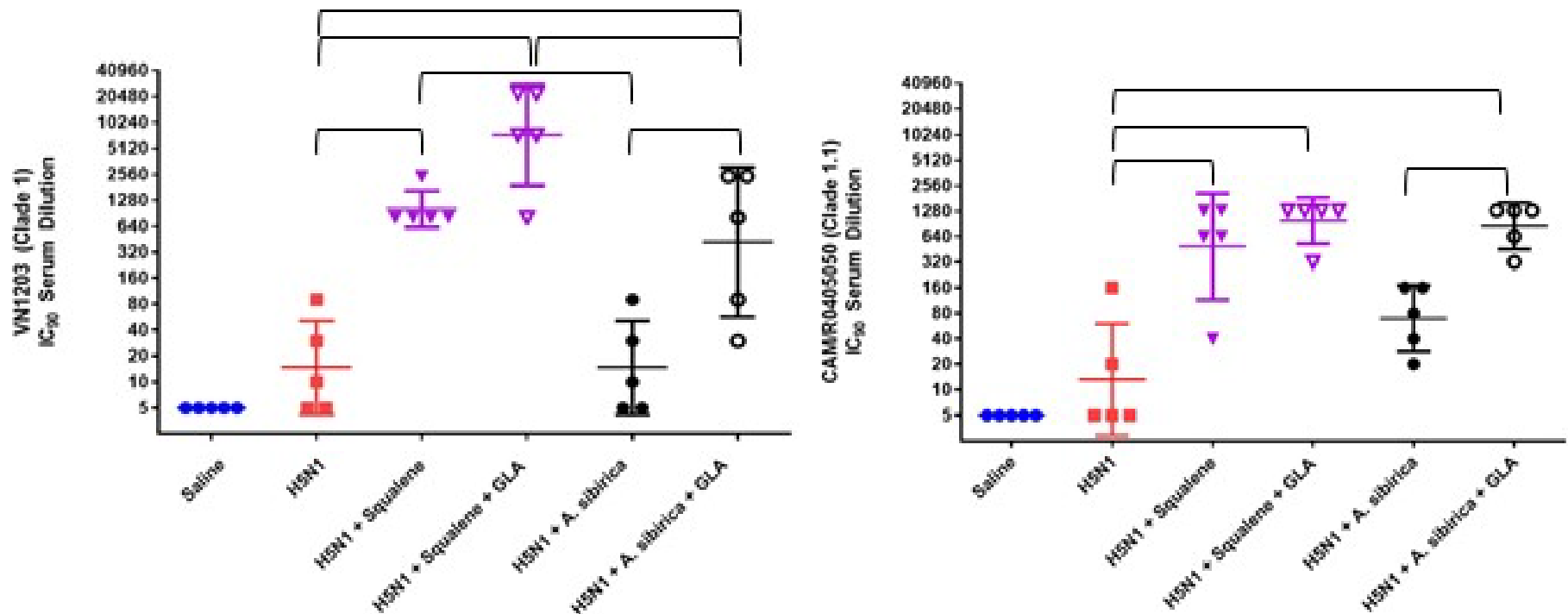
Inclusion of TLR4 Ligand Enhances Antibody Responses

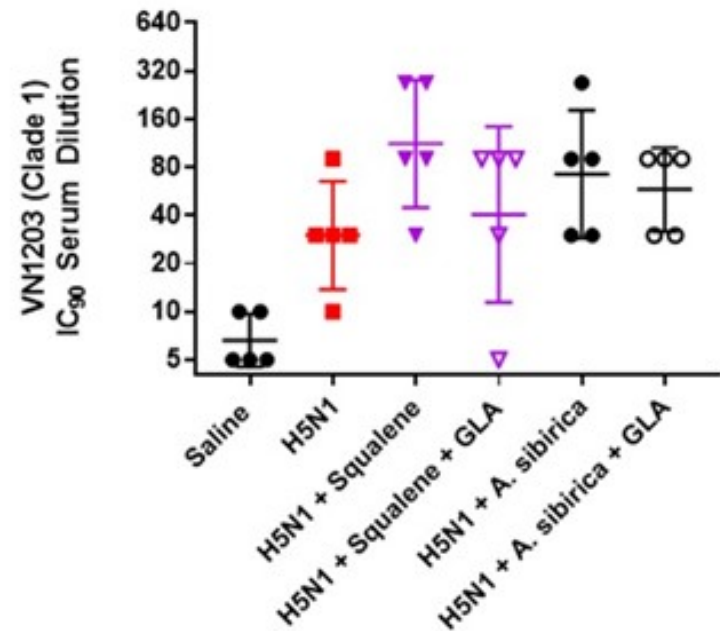- Mice were immunized twice intramuscularly with split inactivated H5N1 antigen alone or in combination with emulsion +/ GLA; serum IgG and long-lived plasma cells quantified
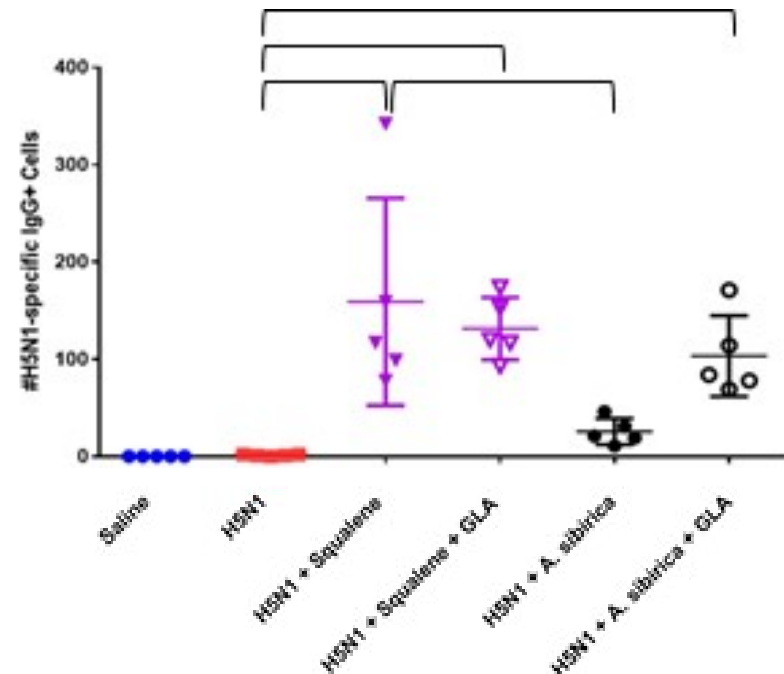
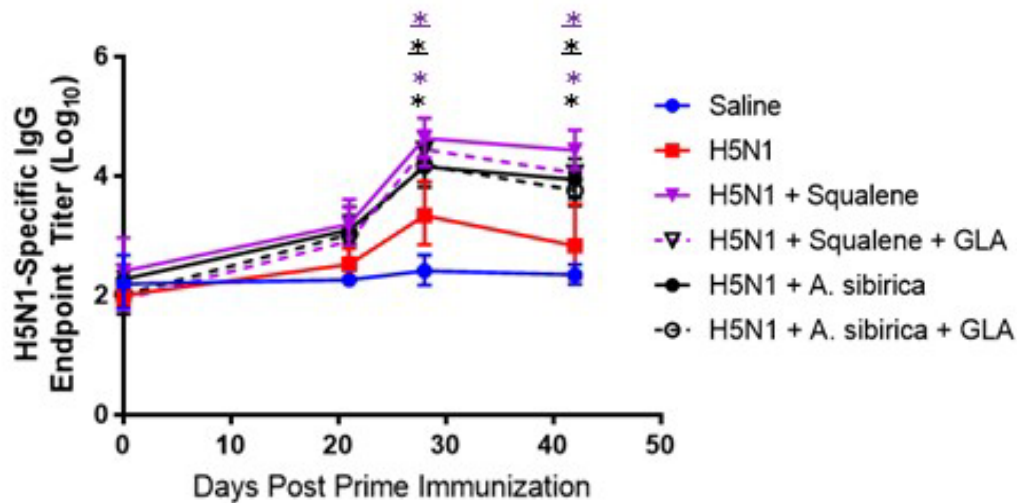# Inclusion of TLR4 Ligand Broadens Functional Antibody Responses

- Mice were immunized twice intramuscularly with split inactivated H5N1 antigen alone or in combination with emulsion +/- GLA; hemagglutination inhibition titers against homologous and heterologous strains quantified

# Polyprenol Emulsion Adjuvant Activity in Pig Model

- Pigs were immunized twice intramuscularly with split inactivated H5N1 antigen alone or in combination with emulsion +/- GLA; serum IgG and hemagglutination inhibition titers against homologous strain quantified

Polyprenol Emulsion Adjuvant Activity in Ferret Challenge Model

- Ferrets were immunized once intramuscularly with split inactivated H5N1 antigen alone or in combination with emulsion +/- GLA, challenged with homologous virus 21 days later; survival and virus titer assessed
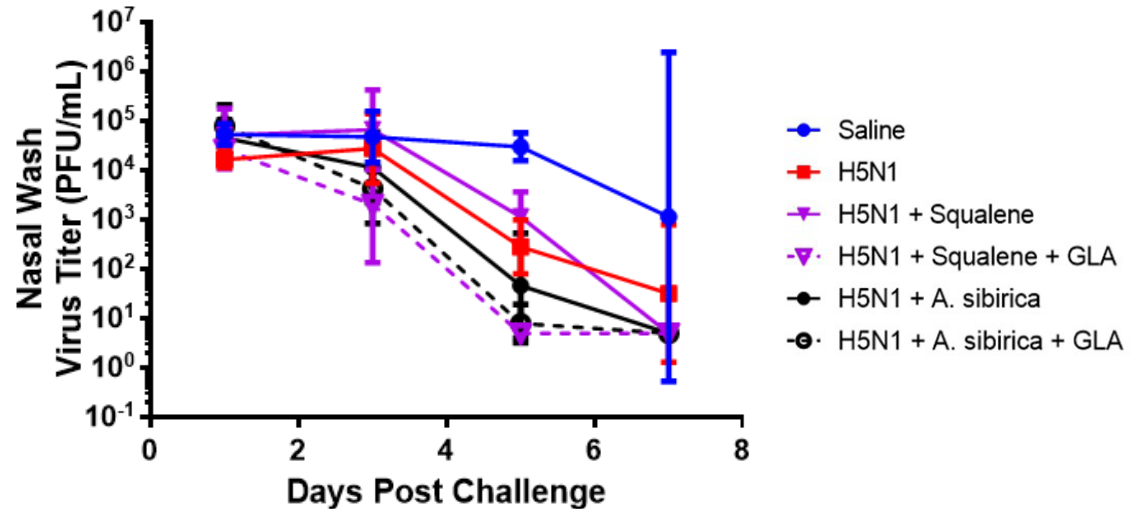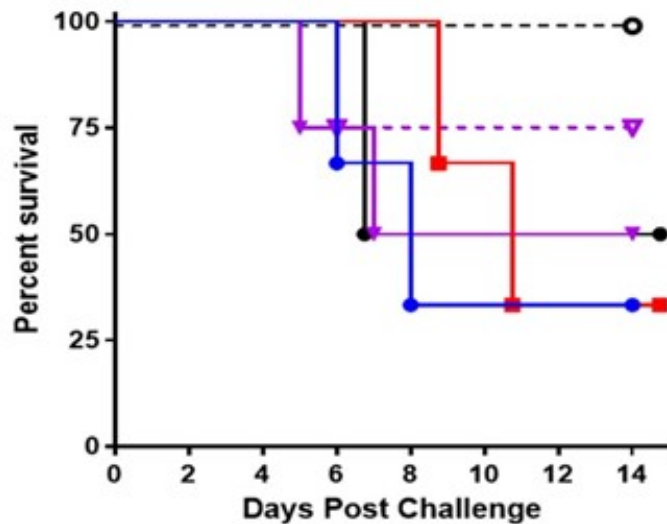
Plant-derived Polyprenol Emulsion Summary

- Emulsions made with polyprenols from conifers demonstrated comparable physical characteristics and stability compared to squalene emulsion

- Emulsions made with polyprenol from Siberian fir demonstrate somewhat less adjuvant activity than squalene emulsion, but more than triglyceride emulsion, in mouse model (depending on vaccine antigen and immune readout)

- Emulsions made with polyprenol from Siberian fir demonstrate comparable adjuvant activity as emulsions made with squalene in pig and ferret challenge models

- Inclusion of TLR4 ligand significantly enhances polyprenol emulsion adjuvant activity in mouse and ferret, but not pig, animal models

Future Directions

- IDRI has partnered with the Derek Irvine Lab at the Univ Nottingham to produce and evaluate synthetic isoprene-based polymers as emulsion adjuvant components

- NIH R01 grant award to pursue and expand this research, including structure-function studies
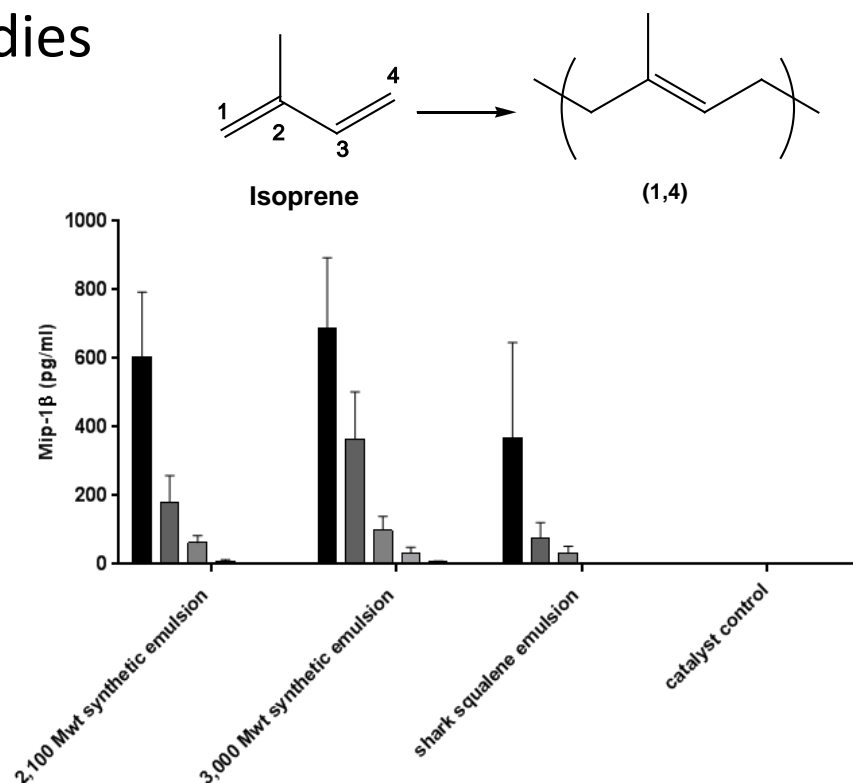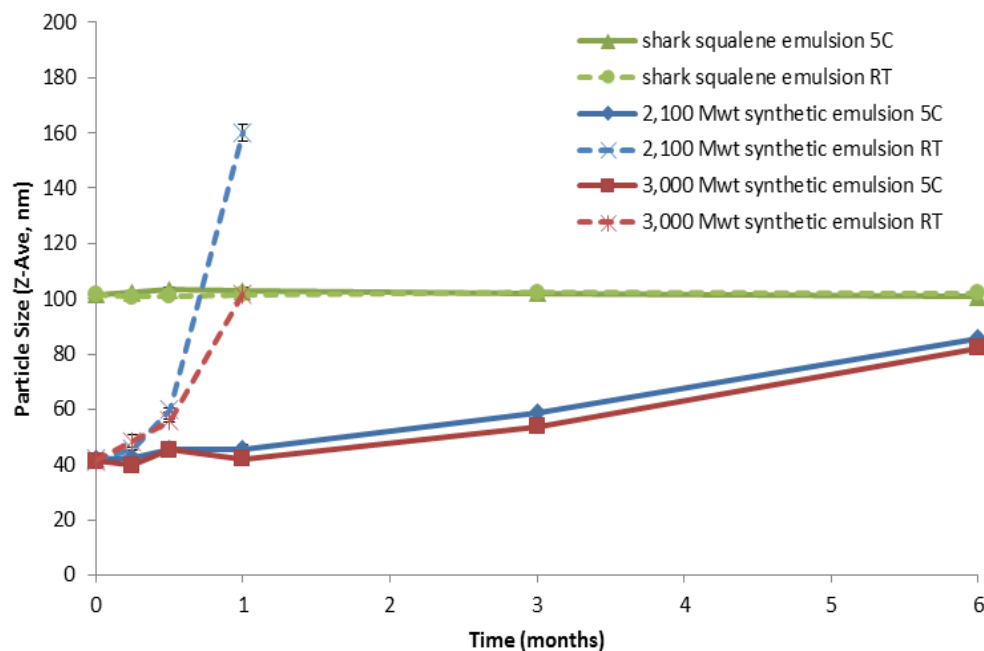
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