



Need for and development of a protein-based pneumococcal vaccine

William Hausdorff, Fabrice Godfroid, Frederik Fierens, and Vincent Verlant

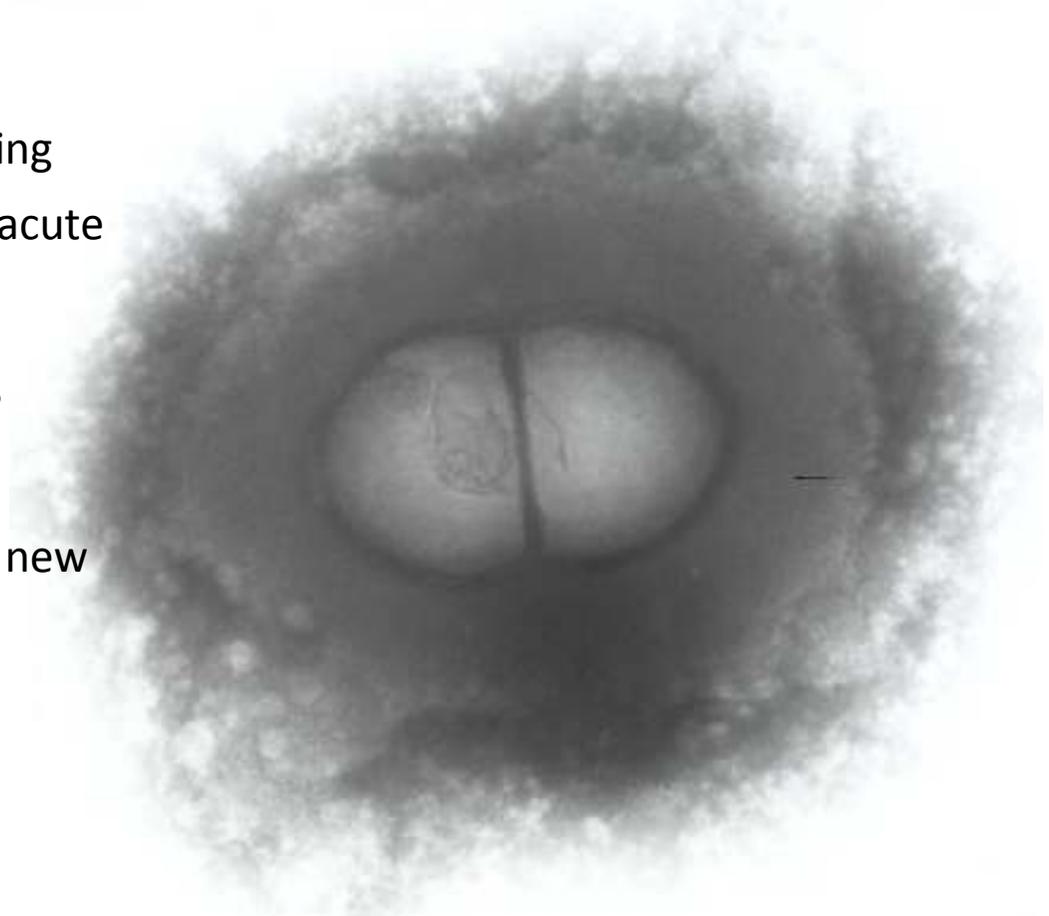
Vaccine Development Leader, Pneumococcal Vaccines
GlaxoSmithKline Biologicals, Wavre, Belgium

Streptococcus pneumoniae (aka Pneumococcus)

- Normal inhabitant of nasopharynx
- A major cause of invasive disease including meningitis as well as of pneumonia and acute otitis media
- Gram-positive encapsulated diplococcus
- **Serotypes defined by capsular polysaccharide:** 94 are known-- but like new elements more discovered every year

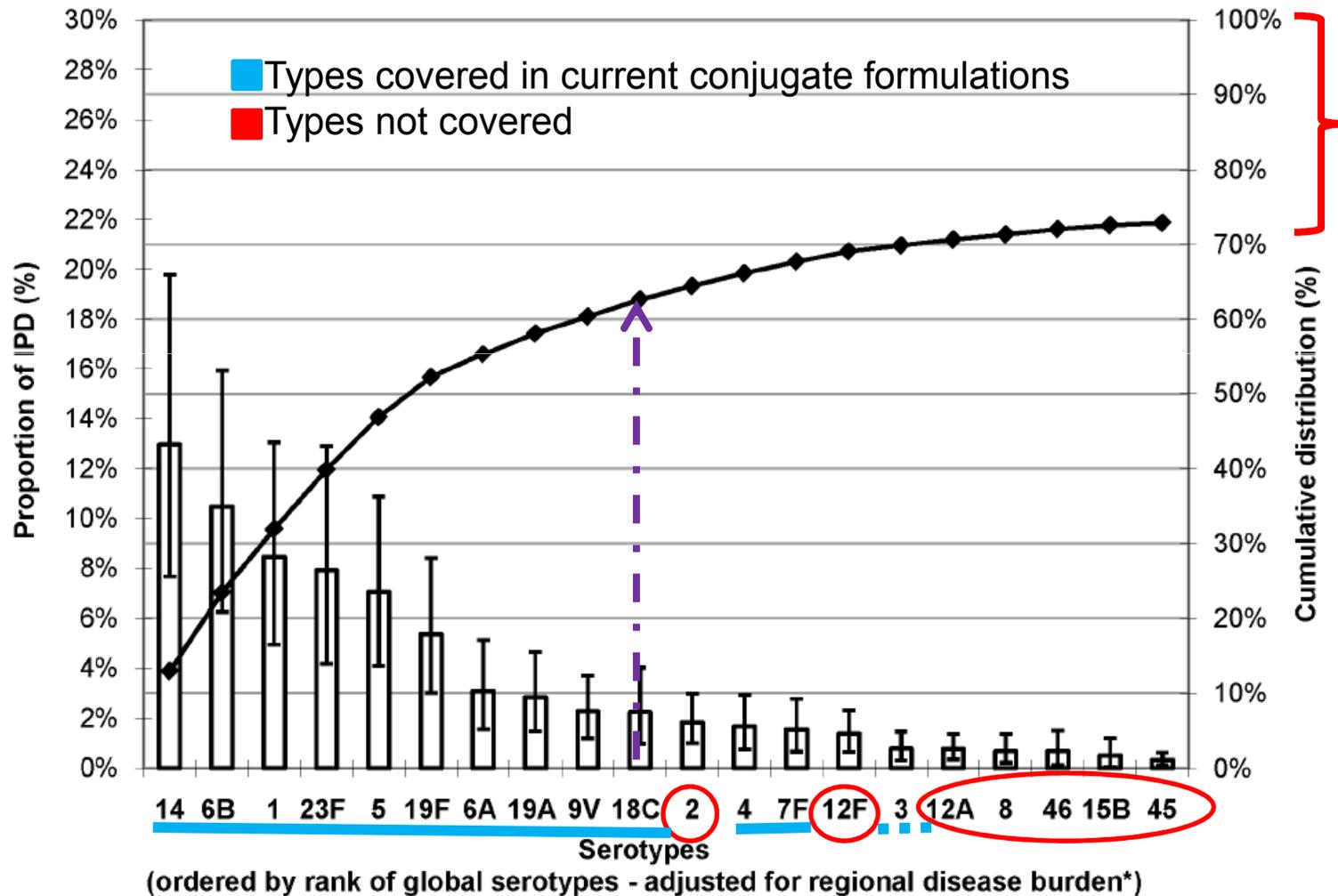
(Welcome 6D!)

- Capsule is target of polysaccharide-protein conjugate vaccines



Serotype 19F; Photograph by Rob Smith

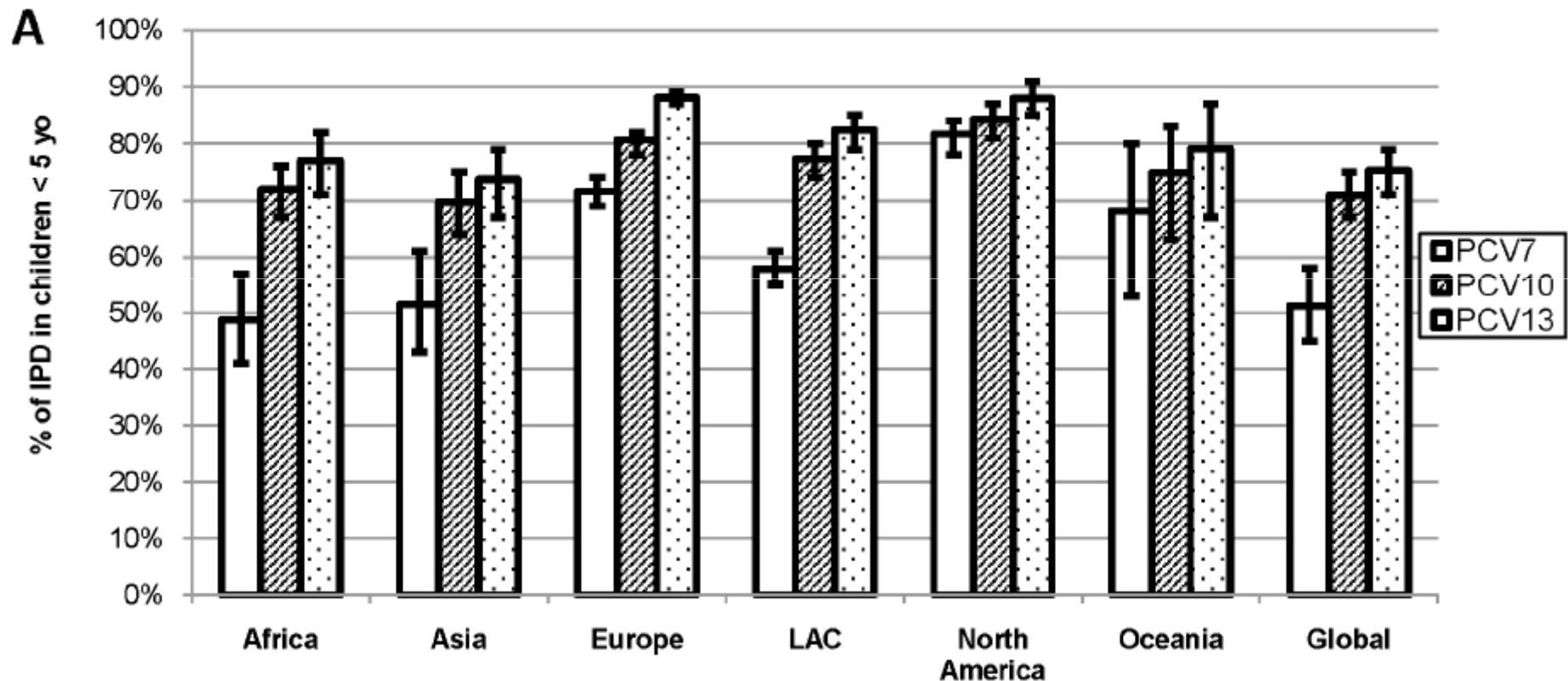
10-15 serotypes cause majority of invasive pneumococcal disease (IPD) in children worldwide—but there are limits



Johnson 2010 PlosMed

*adjusted for regional incidence of cases

Proportion of IPD in young children potentially covered by different vaccine formulations



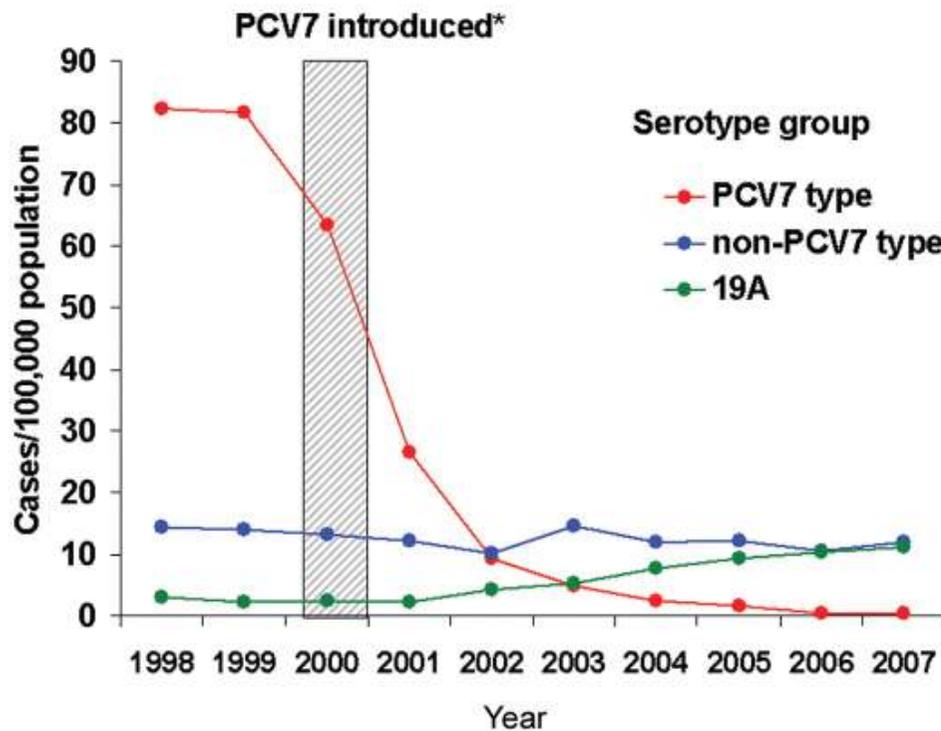
Johnson PLOS Med 2010

In older children & adults, PCV coverage is lower and additional serotypes play important roles

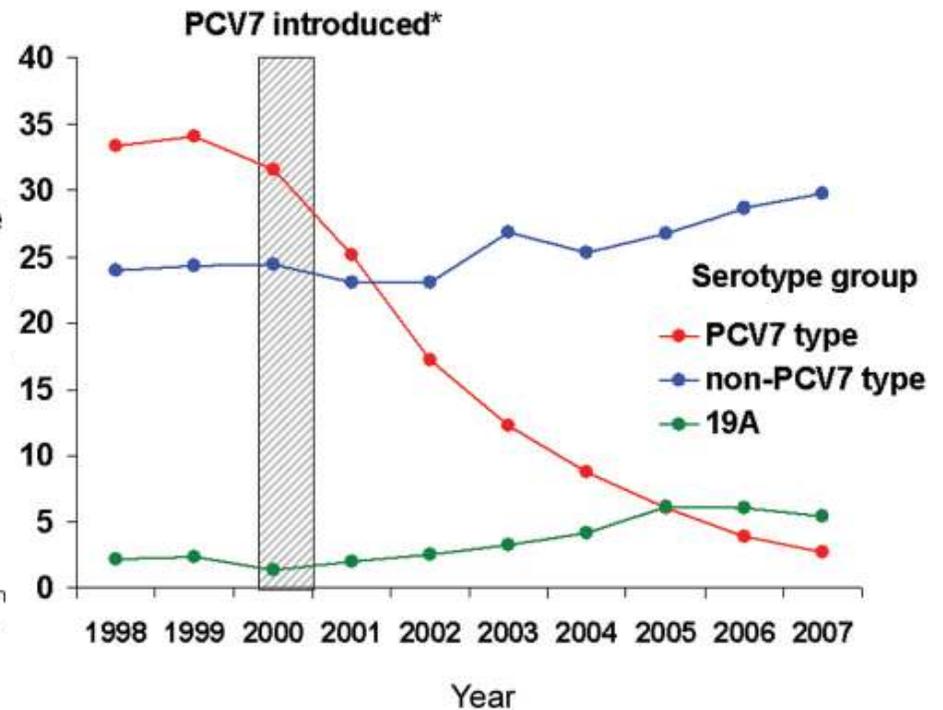
Hausdorff et al CID 2000

Extremely effective vaccines: Major drop in overall IPD incidence after PCV7 introduction in US population

children <5 years



adults ≥65 years



But...situation is dynamic:

PCV7 induces serotype replacement at level of nasopharyngeal carriage

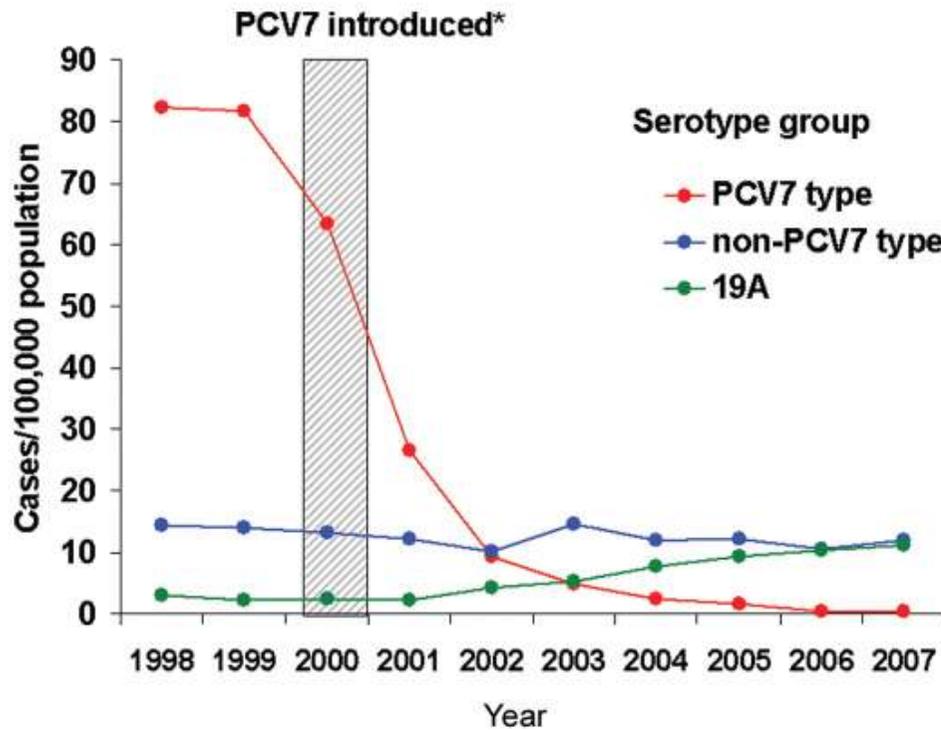
Thus, widespread use of glycoprotein pneumococcal conjugate vaccines might be associated with an alteration in serotype distribution of pneumococcal carriage, and potentially of invasive disease.

However...our preliminary results need to be interpreted with caution.

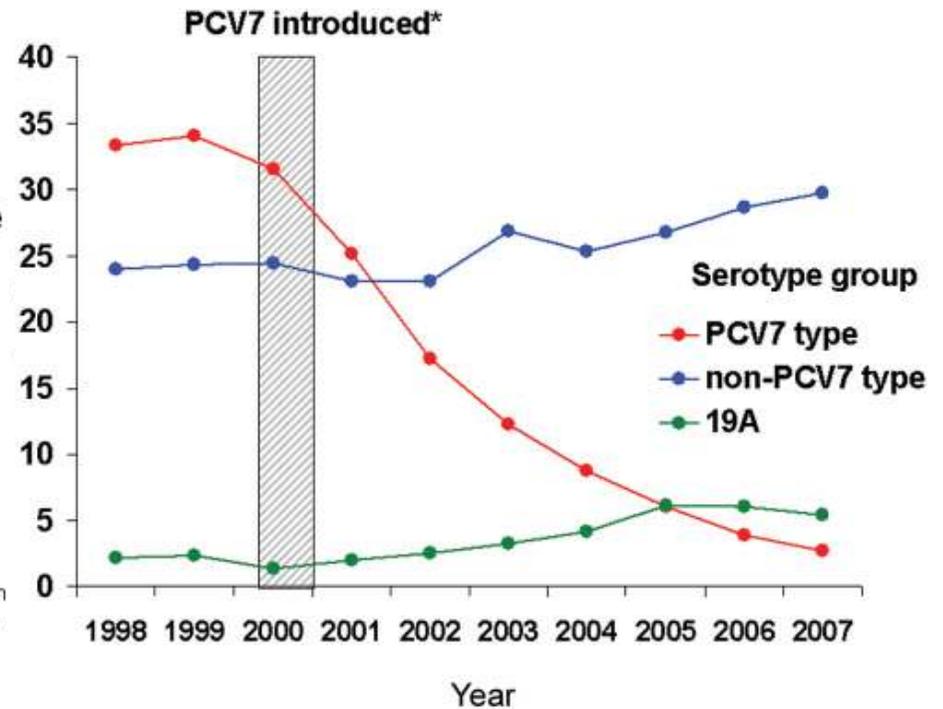
Does PCV7-CRM also cause significant replacement disease?

In the US, limited (but high profile) so far

children <5 years

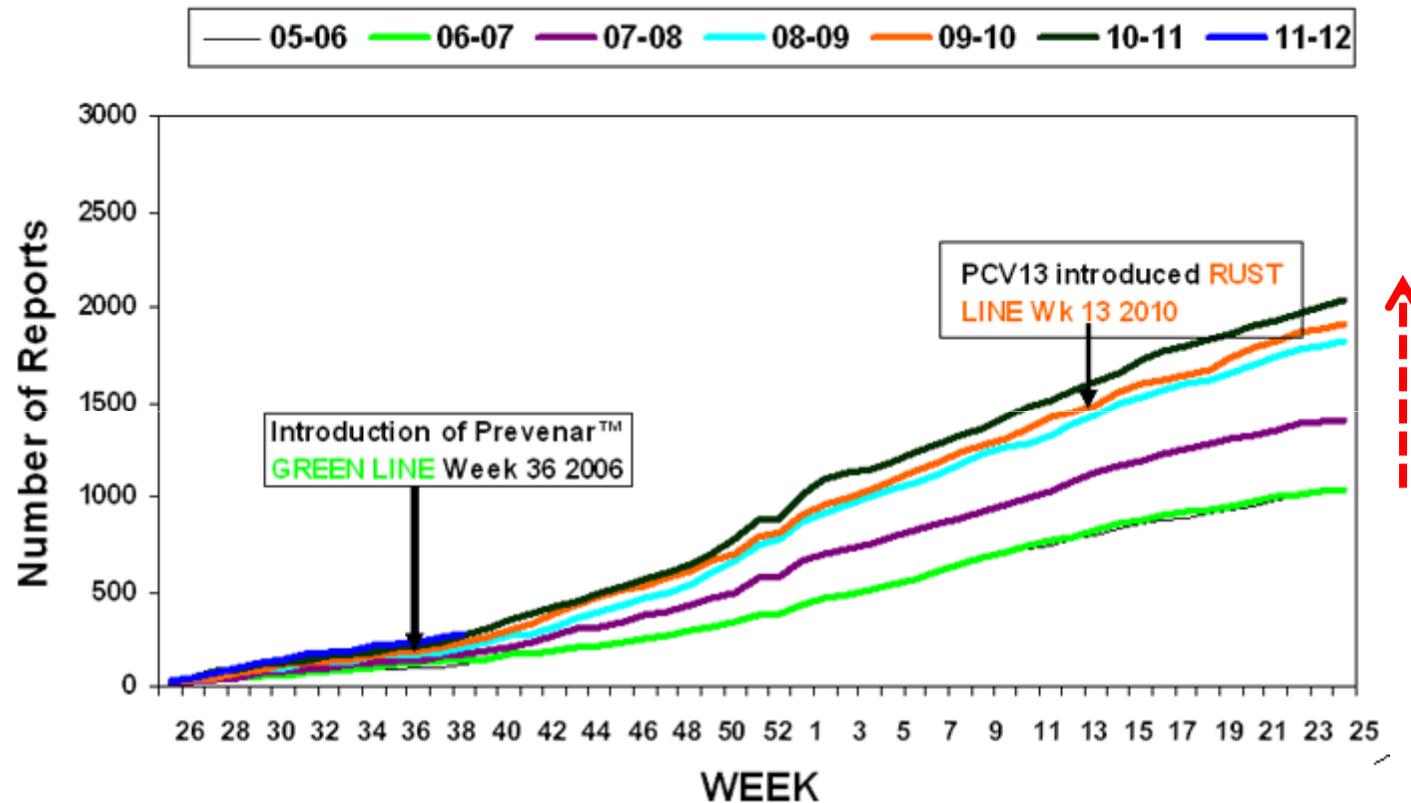


adults ≥65 years



Does PCV7-CRM also cause significant replacement **disease**?

In England and Wales, perhaps a different story?



IPD due to any of the serotypes NOT in Prevenar13™ : Persons aged >5 Years
by Epidemiological Year: July-June (2005-To Date)

What about other diseases e.g. Acute Otitis Media?

PCV7 impact on AOM in Finland (double blind randomized efficacy study)

Acute Otitis Media Endpoint		Vaccine Efficacy (95% CI) FinOM [PCV-7]
Any (confirmed by presence of middle-ear fluid)	→	% 6 (-4 to 16)
Vaccine pneumococcal serotypes	→	% 57 (44 to 67)
Non-vaccine pneumococcal serotype	→	% -33 (-80 to 1)
<i>Haemophilus influenzae</i>	→	(-%11) (-34 to 8)

Negative efficacy values mean an **increased** # of cases in the vaccinated group:
evidence of non-vaccine type replacement for AOM

Unclear whether replacement is inevitable for ALL conjugate vaccines

PCV11 PnPD (vaccine candidate) impact on AOM in Czech & Slovak Republics

(double blind randomized efficacy study)

Acute Otitis Media Endpoint	Vaccine Efficacy (95% CI) POET [11Pn-PD]
Any (confirmed by presence of middle-ear fluid)	→ % 33.6 (20.8 to 44.3)
Vaccine pneumococcal serotypes	→ % 57 (41.4 to 69.3)
Non-vaccine pneumococcal serotype	→ % 8 (-64.2 to 49)
<i>Haemophilus influenzae</i>	→ % 35.6* (3.8 - 57.0)

The Need

- Conjugate vaccines are highly effective. However...
 - many serotypes not covered by current formulations
- In addition, non-conjugate vaccine serotype carriage and disease have increased since PCV7-CRM introduction
 - Magnitude of increases highly variable; confounded by antibiotic use, schedule, age group examined
 - Unknown whether replacement disease is inevitable for all conjugates
 - Carriage, efficacy and effectiveness data with PHiD-CV (10-valent with protein D carrier) and PCV13-CRM just now emerging

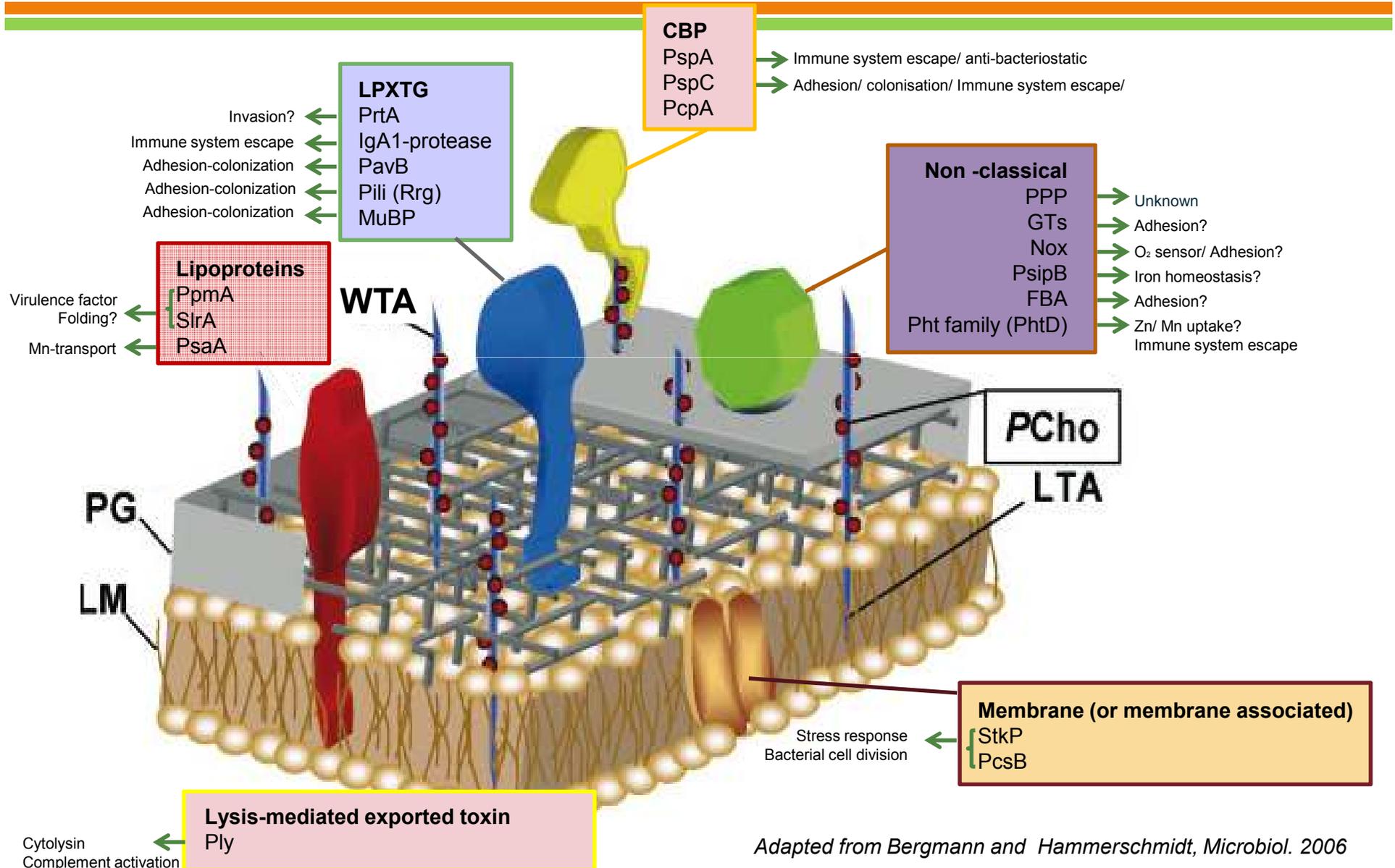
The dream: A protein vaccine that covers all pneumococci

1. Highly protective
2. Minimal reactogenicity
3. Expressed by all strains regardless of serotype
4. Antigenically highly conserved
5. Represent a virulence or growth factor
6. Accessible to immune system
7. Not be susceptible to immune evasion
 - multiple antigens?

The reality: such vaccines exist for pertussis, diphtheria, tetanus, but not yet for other encapsulated bacteria

- Will MenB be the first?

Main *Pneumococcus* surface exposed proteins



Adapted from Bergmann and Hammerschmidt, Microbiol. 2006

Complexities of testing proteins—the hard questions

- On what basis do you move into the clinic?
 - No immune correlate of protection for **pneumococcal** proteins
 - If a protein elicits opsonophagocytic (killing) or bactericidal activity, would that be sufficient?
 - Animal models have unknown predictivity
- A proof of concept clinical study is highly desirable before committing to major efficacy trial investment
- What would constitute an adequate proof of concept study ?
 - What disease endpoint?
 - Could impact on carriage serve as such an endpoint?
 - What kind of effect on pneumococcal carriage do you want to see?

Complexities of testing proteins—more questions

- The design of clinical studies
 - Since PCVs are >90% effective, can you do a placebo controlled study?
 - Since PCVs are >90% effective, can you ever give young infants **only** proteins, or always co-administered with PCVs?
 - If co-administered, can you measure the incremental value over PCVs?
- Pathway to licensure uncharted
 - Limited guidance to date from regulatory agencies

Selected pneumococcal proteins with demonstrable efficacy as immunogens in animal models

Protein family	Examples of antigen	Evidence of protection in animal models versus	
		Invasive disease	Carriage
Choline-binding proteins	PspA	+	+
	CbpA (PspC)	+	+
	PcpA	+	-
	LytA	+	NR
	LytB/C	+	NR
	Pneumolysin	+	-
Sortase and sortase-dependent proteins	SrtA	+	-
	RrgA/B/C	+	NR
	NanA	+	+
	PrtA	+	NR
ABC (ATP-binding cassette) transporter proteins	PiaA and PiuA	+	NR
	PsaA	+	+
	PotD	+	+
	SP 2108	-	+
	SP 0148	NR	+
	ClpP	+	+
Enzymatic proteins	StkP	+	NR
	PhtA/B/D/E	+	+
Histidine triad proteins	PcsB	+	NR
Other	PppA	+	+

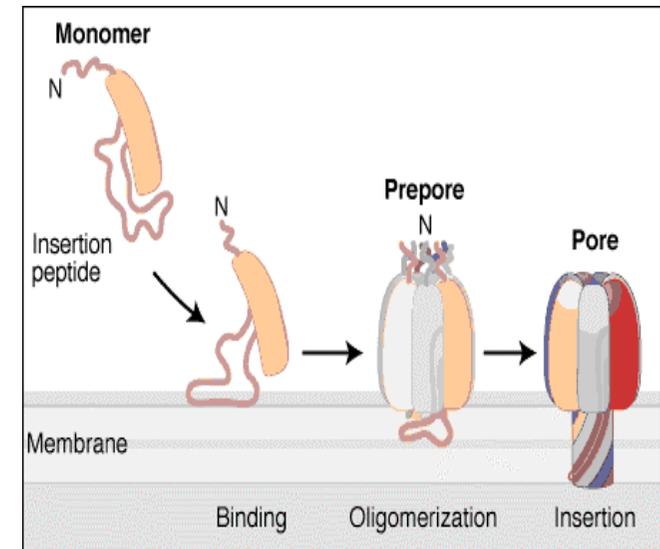
Kristin L Moffitt and Richard Malley. Next generation pneumococcal vaccines, *Current Opinion in Immunology* 2011, 23:407–413

Pneumolysin (Ply)

Length: 471aa – MW: ~53kDa

- Encoded by almost all *S. pneumoniae*¹
 - Highly conserved: sequence variation of 3.3% (>200 isolates)
 - 8 different alleles; most common: alleles 1 (~40%) and 2 (~44%)
- No signal sequence
 - Released after pneumococcal lysis² by an autolysin - mechanism³ and localized at cell wall⁴

- Inhibits cilia beat frequency⁵
- Pore-forming protein leading to epithelial damage and hemolysis⁶
- Activates classical complement pathway (C3 activating factor)⁷, synthesis of pro-inflammatory mediators⁸, and apoptosis of neutrophils and macrophages⁹
- Ply KO *Spn* mutants have reduced ability to colonize mouse nasopharynx and lungs¹⁰



1. Jefferies (2007), J. Infect. Dis.; Jefferies (2010), J. Med. Microbiol. 2. Johnson (1977), FEMS Microbiol. Lett., 3. Balachandran (2001), J. Bacteriol., 4. Price (2009), J. Bacteriol., 5. Hirst (2000), Ped. Res., 6. Palmer (2001), Toxicon, - Tiley (2005), Cell, - Rossjohn (1998), J. Mol. Biol., 7. (1984), Infect. Immun., 8. Cockeran (2002), J. Infect. Dis., 9. Cockeran (2002), Curr. Opin. Infect. Dis., 10. Kadioglu (2000), Infect. Immun., Kadioglu, 2002.

The pneumococcal histidine triad proteins (Phts)

- Characterized by a **histidine triad motif HxxHxH** conserved and repeated 5 to 6 times
- Restricted to genus *Streptococcus*, four members of family:: PhtA, PhtB, PhtD and PhtE.
 - **PhtD**, PhtE, PhtB and PhtA genes are present in **100**, 97, 81 and 62% of the strains
 - **PhtD** is **well conserved** across pneumococcal strains (**94 to 100 % identity**).
- Detectable on the **surface** of intact bacteria.
- Pht proteins bind Zn
- Quadruple *pht* KO mutants grow poorly in Zn/Mn-limited medium. Growth can be restored by Zn/Mn
 - Hypothesis: Pht proteins may regulate Zn/Mn
Pht's may be involved in C3b cleavage

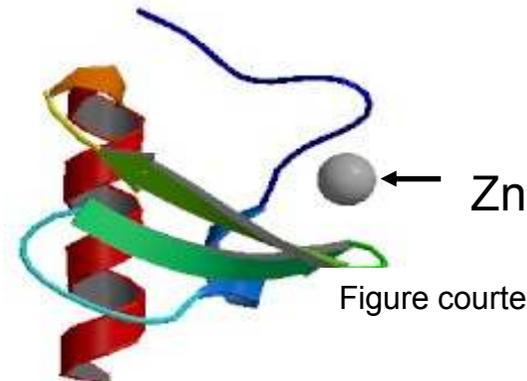
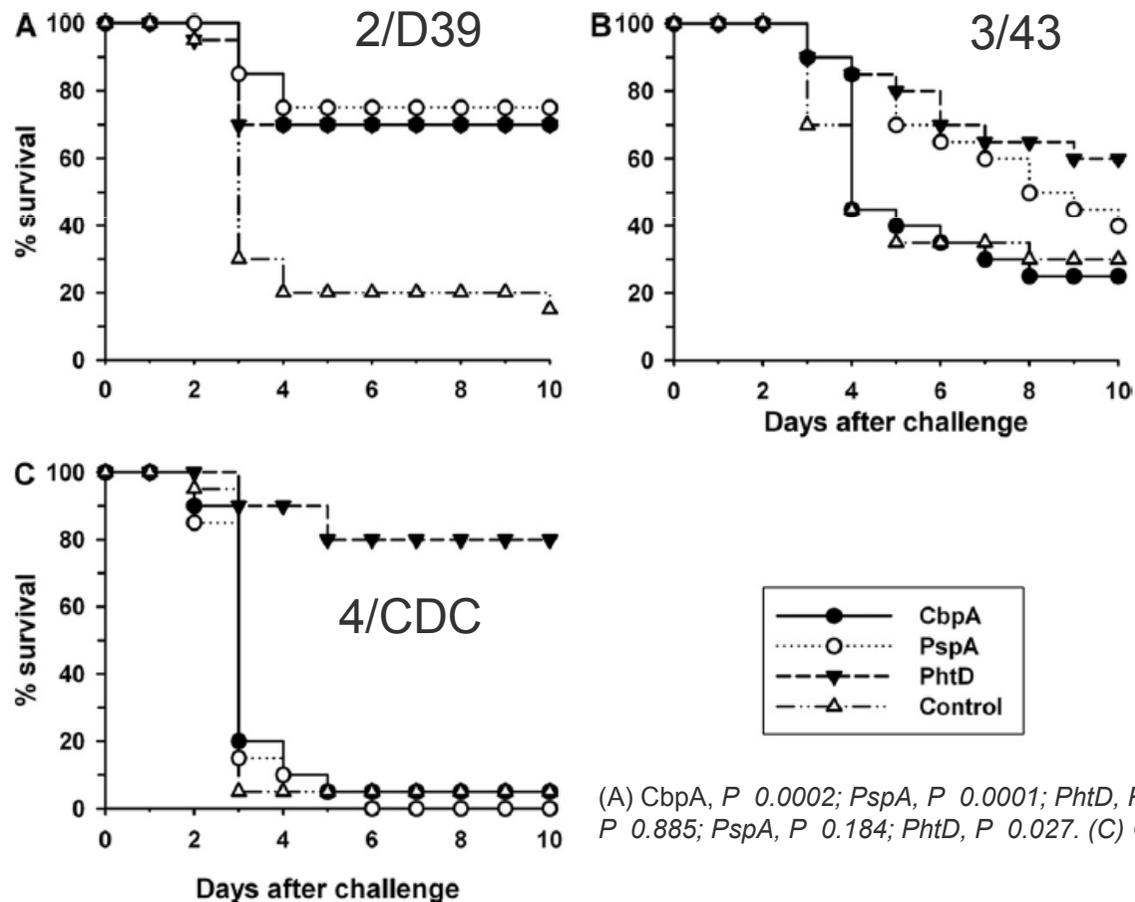


Figure courtesy Tim Mitchell

PhtD protects against Spn in a mouse lethal challenge model

Mouse survival upon lethal *S. pneumoniae* intranasal challenge

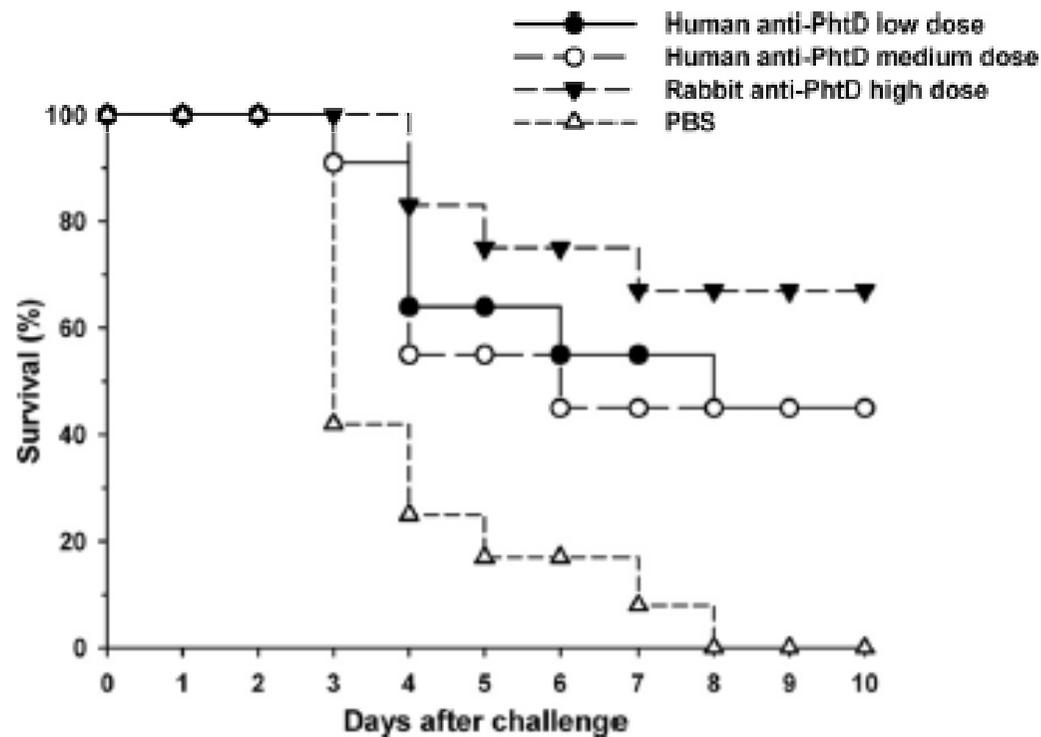
– N=20/group; mice immunized with proteins adjuvanted in AS02V



(A) CbpA, P 0.0002; PspA, P 0.0001; PhtD, P 0.0009. (B) CbpA, P 0.885; PspA, P 0.184; PhtD, P 0.027. (C) CbpA, P 0.825; PspA, P 0.538; PhtD, P 0.0001

Human anti-PhtD antibodies protect against Spn in a mouse lethal challenge model

- Mouse survival upon lethal *S. pneumoniae* (3/43) intranasal challenge
 - Naturally occurring human anti-PhtD antibodies (20 μ g, low dose; 60 μ g, high dose) transferred into mice ($n=20/group$)



Compared with PBS: human anti-PhtD low dose, $P = 0.0054$;
human anti-PhtD high dose, $P = 0.0029$; rabbit anti PhtD, $P = 0.0001$.

dPly and PhtD together: Monkey pneumonia model

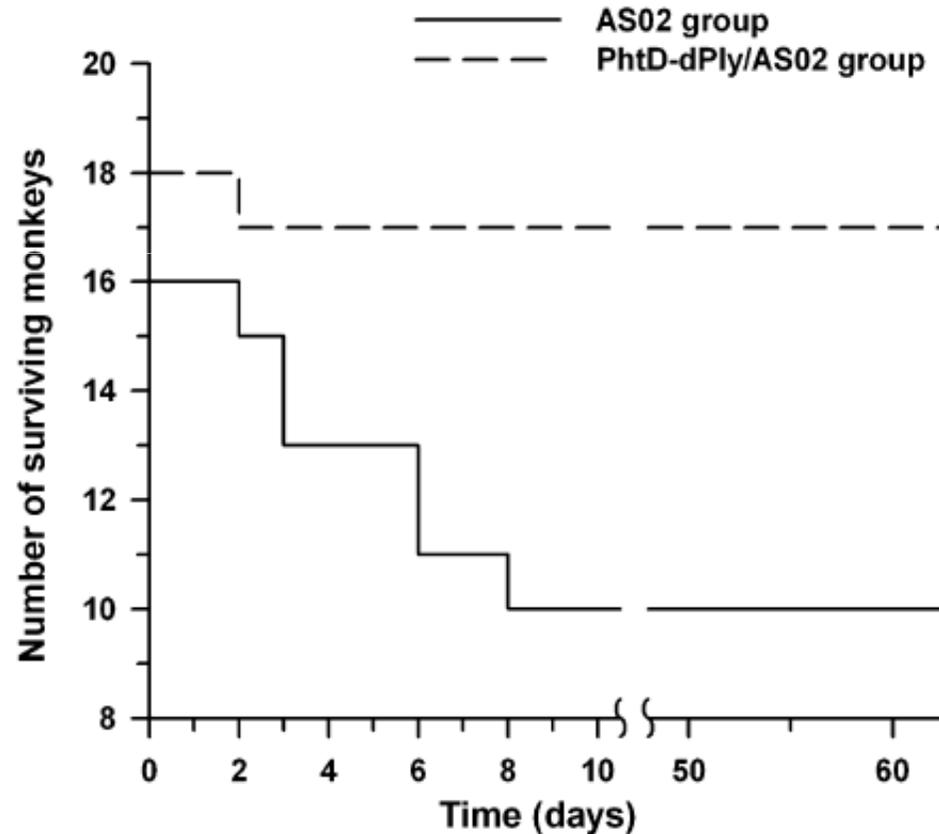
- Adult macaques
- Intra-bronchial inoculum with ST 19F
 - BAL with:
 - positive culture (> 2 weeks)
 - Elevated neutrophils, cytokines
 - Sign of respiratory distress
 - positive X-ray



X-ray plate showing cellular infiltrate in caudal right lung lobe of animal CE45 (arrow). Radiograph obtained 4 days post-inoculation.

Protection against pneumonia in monkeys

Survival of AS02- and PhtD–dPly/AS02-vaccinated monkeys after challenge with *S. pneumoniae*



Clinical evaluation of formulations comprising dPly and/or PhtD

- Goal: assess various formulations, antigen doses and adjuvantation for safety, reactogenicity and immunogenicity
 - Phase I and II studies in adults - completed
 - Phase II studies in toddlers & infants— some completed and others ongoing
- *S. pneumoniae* immunization carriage (SPICAR)* phase II study with Ply & PhtD combined with PHiD-CV conjugates started Feb2011, N= 1320
 - Stage 1: safety, tolerability and immunogenicity in 2-4 year old children
 - Stage 2: tolerability, immunogenicity, and impact on nasopharyngeal carriage in Gambian infants 8-10 weeks of age

*collaboration with MRC, The Gambia, LSHTM, PATH

Conclusions

- Will the “dream” become a reality?
- We’re still in the early days but preclinical data with pneumococcal proteins look promising
- Likely that large scale trials will be needed to license protein-based vaccines
 - Could surrogate endpoints be used to accelerate licensure pathway?
- Stay tuned...