
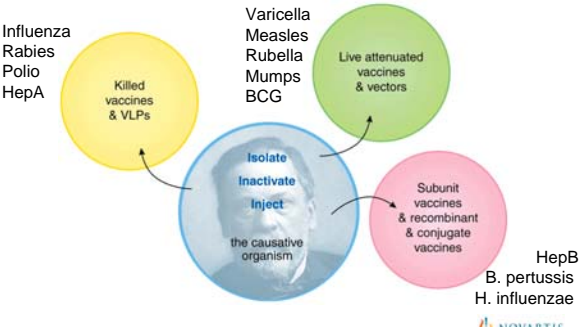


Pathogen Genomics: Approaches and Applications

Vega Masignani
 Genomics for Animal Health: Outlook for the Future
 Paris, October 12th-15th 2009




Standard Vaccinology: the Pasteur principles to develop vaccines

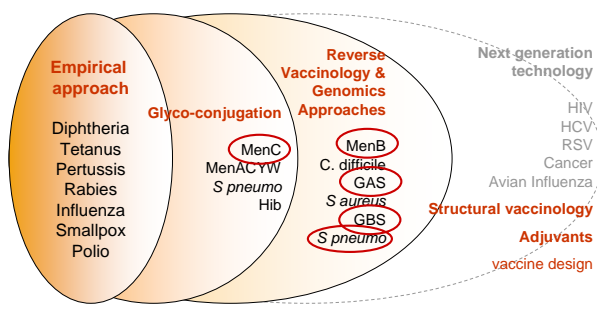


Influenza, Rabies, Polio, HepA (Killed vaccines & VLPs)
 Varicella, Measles, Rubella, Mumps, BCG (Live attenuated vaccines & vectors)
 HepB, B. pertussis, H. influenzae (Subunit vaccines & recombinant & conjugate vaccines)

Isolate, Inactivate, Inject, the causative organism



New technologies during the past 30 years expanded the pool of human vaccine targets



Empirical approach: Diphtheria, Tetanus, Pertussis, Rabies, Influenza, Smallpox, Polio


Glyco-conjugation: MenC, MenAC, YW, S pneumo, Hib

Reverse Vaccinology & Genomics Approaches: MenB, C. difficile, GAS, S aureus, GBS, S pneumo

Structural vaccinology: HIV, HCV, RSV, Cancer, Avian Influenza


Adjuvants vaccine design

Next-generation technology

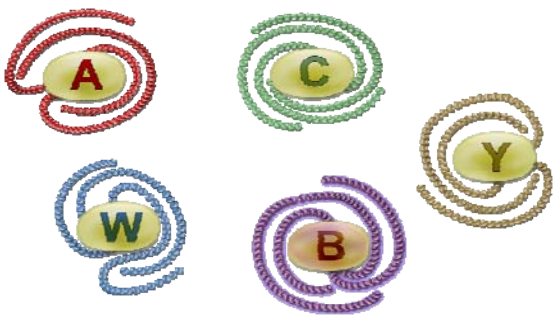



Neisseria meningitidis: a dreadful pathogen

- *N. meningitidis* is a strictly human pathogen and the leading cause of bacterial sepsis and meningitis in children and young adults
- When the patients survive, they are often left with important disabilities which range from neurological disorders to limb amputation
- *N. meningitidis* can cause outbreaks, but it's also endemic in certain regions, such as Africa (the "meningitis belt")
- Of the 13 serotypes associated with *N. meningitidis* only five cause disease (A, B, C, Y, W)

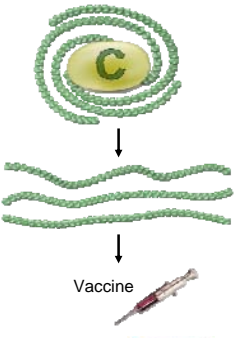



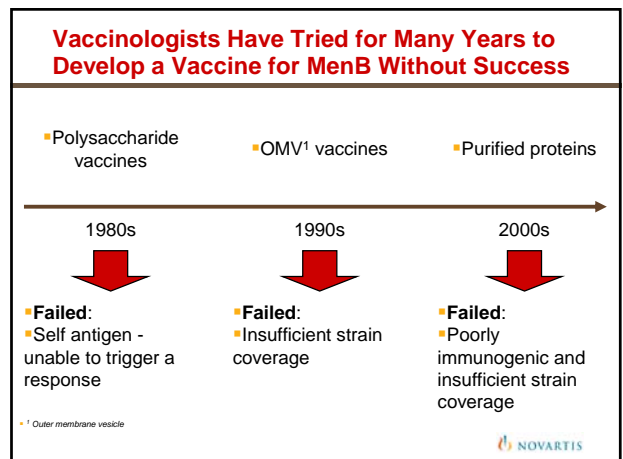
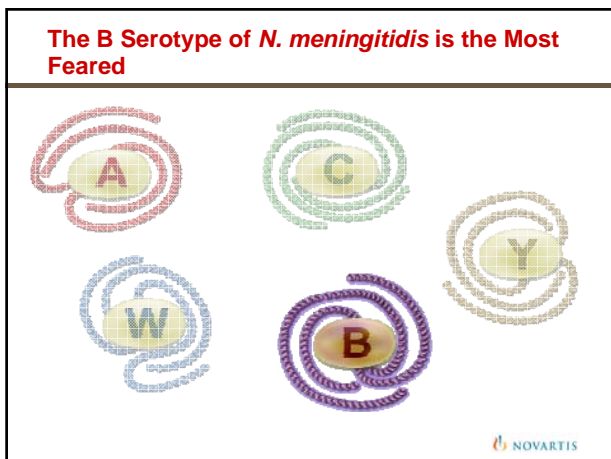
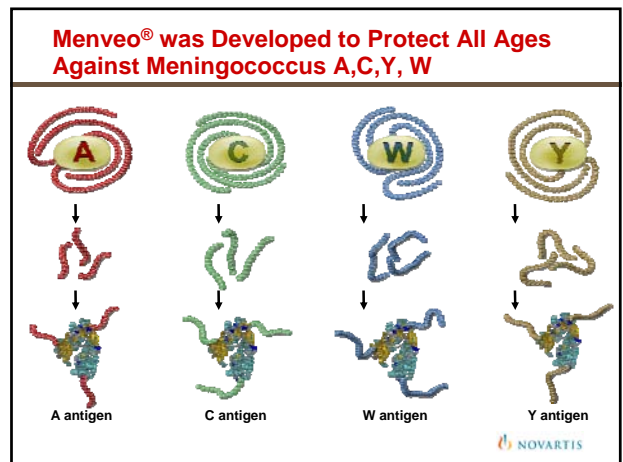
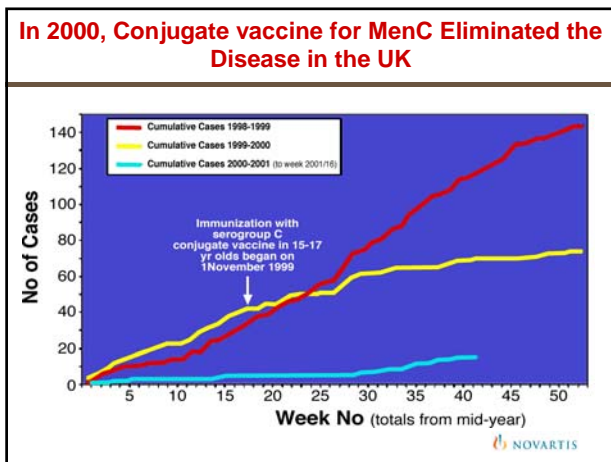
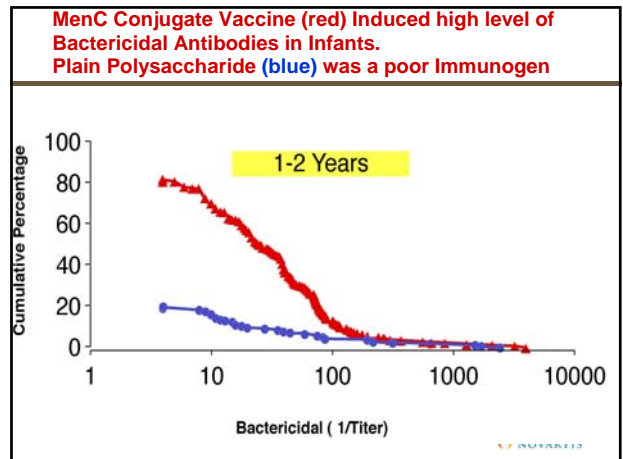
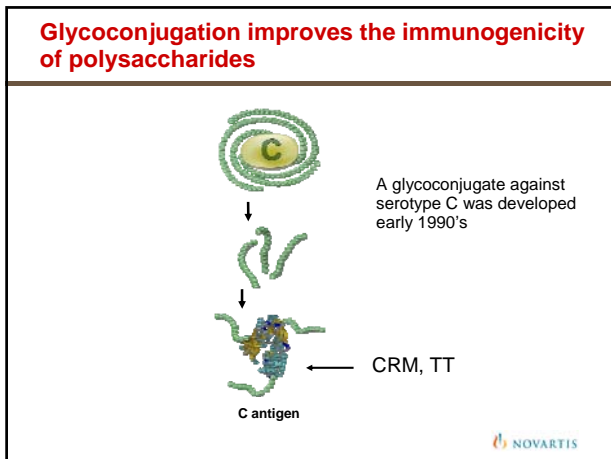
***N. meningitidis* has many serotypes – Each with a distinct coat polysaccharide**

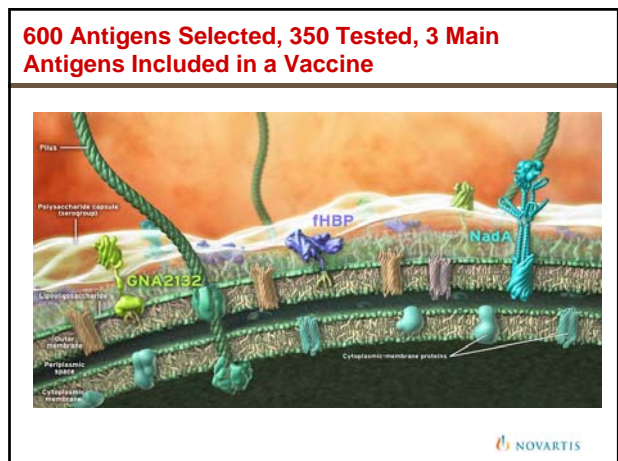
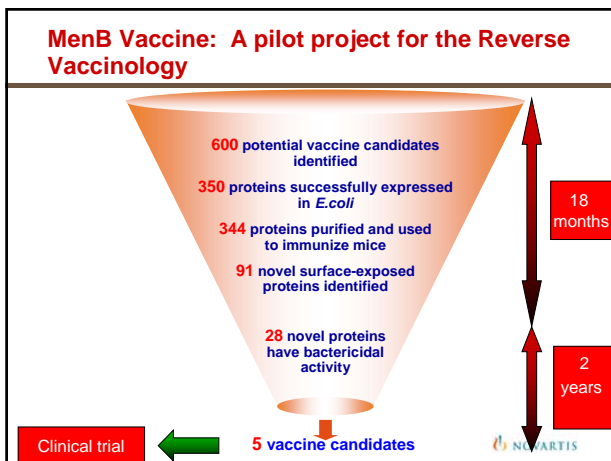
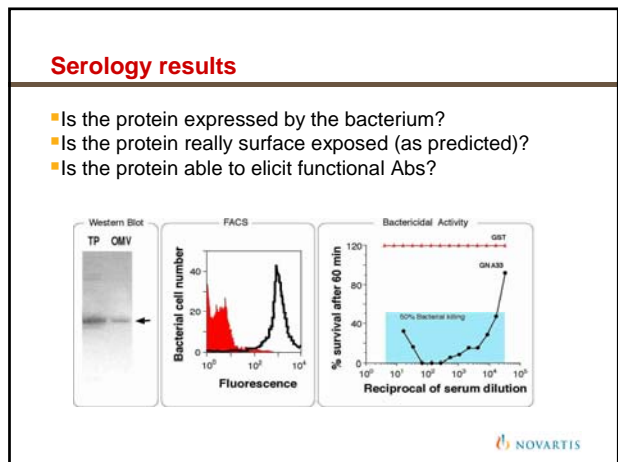
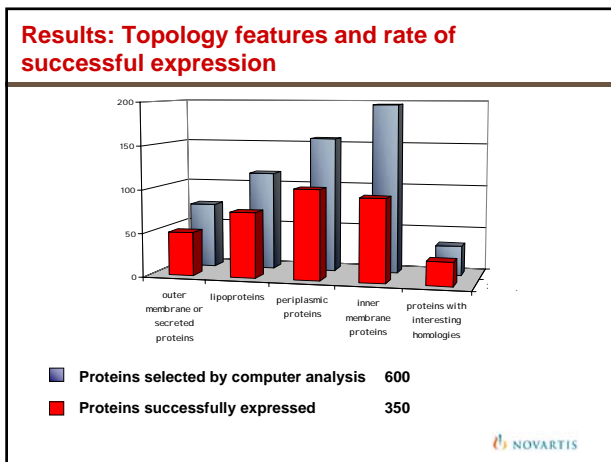
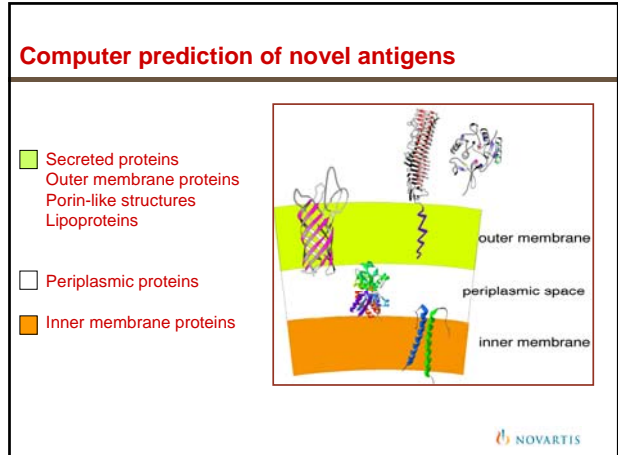
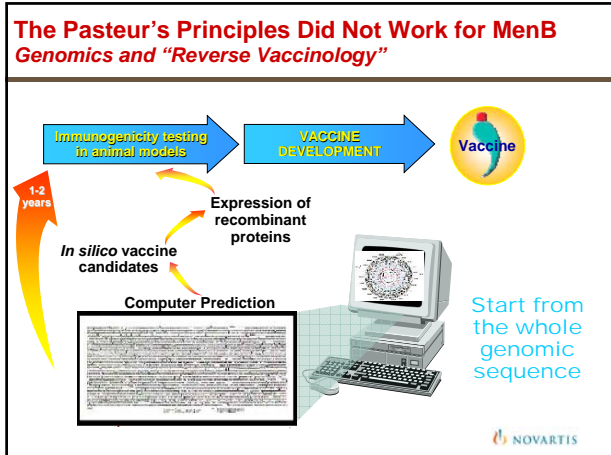



Polysaccharides are not Good Vaccine Antigens

- Polysaccharides trigger a limited immune response
 - B cell response only
 - Lacks potency, durability and memory (boost)
- Infants do not respond to polysaccharide vaccines

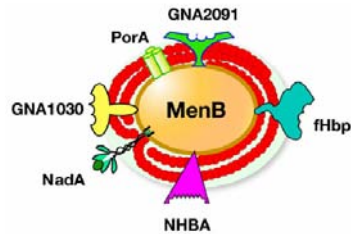




**New surface proteins were discovered
 5 were used to make a vaccine**

Vaccine composition

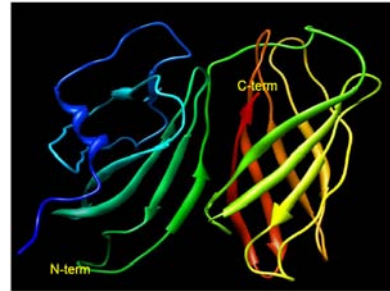
- NadA
- GNA2091-fHBP
- NHBA-GNA1030



The MenB vaccine is in Phase III clinical trials



**Reverse Vaccinology explains Biology
 One of the MenB antigens binds Factor H**

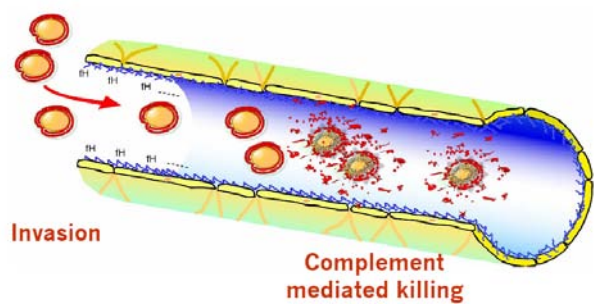


The complement system

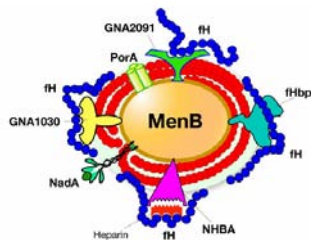
- Complement is the first barrier that protects humans from invasion of pathogens
- Several bacteria interfere with complement factors to escape phagocytosis
- Factor H is a negative regulator of the complement cascade and protects self tissues from complement's attack
- Surface proteins able to strongly bind FH protect the bacterium from complement attack and can survive in the blood



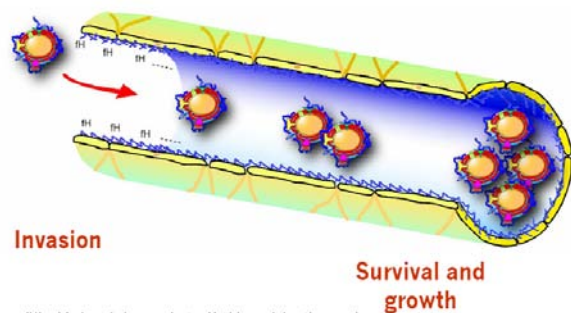
Bacteria are recognized as "non self" by the alternative pathway of complement, C3 binds and complement kills them



**Binding Factor H is a survival mechanism for MenB
 4 out of the 5 vaccine components bind FH**



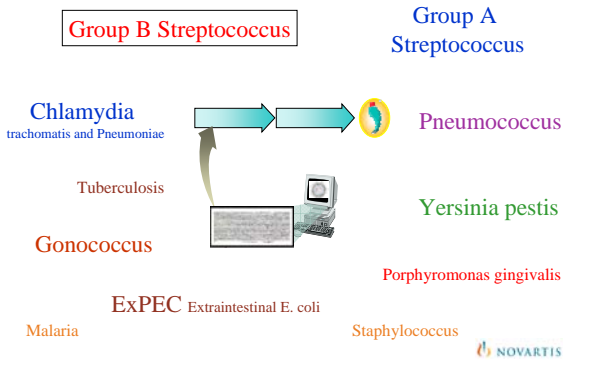
***N. meningitidis* coated by FH is not recognized as "non-self" by C3, survives and multiplies in human blood**



fHbp binds only human factor H, this explains the species specificity of meningococcus and why we never had an animal model for this bacterium

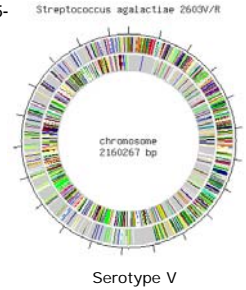


Reverse vaccinology has become a routine discovery approach



A step further: the Group B Streptococcus

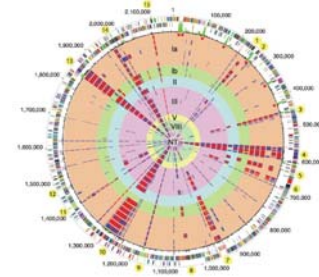
- GBS generally behaves as a commensal organism that colonizes the genital tract of 25-40% of healthy women
- GBS is the major Gram+ cause of sepsis and meningitis in infants
- Eight serotypes, of which 5 are associated with disease
- Increasingly important cause of systemic infection in the elderly
 - Bacteremia
 - Skin or soft tissue infection
 - Pneumonia
 - Urosepsis
 - Endocarditis



Summary of the Reverse Vaccinology Approach on GBS

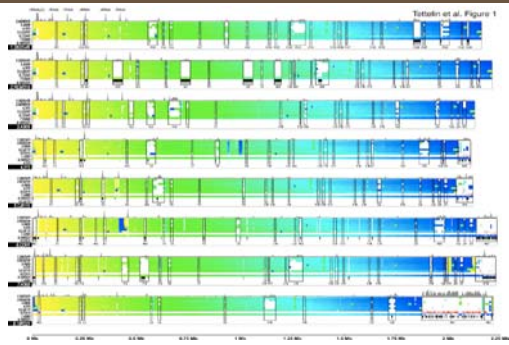
- 473 antigens selected *in silico*
- 357 antigens successfully expressed (75.5%)
- 62 antigens showed protection in mouse model and/or high surface expression
- To assess the presence/absence/conservation of the genes, a DNA chip of the complete genome was hybridized with total DNA from 20 different GBS isolates belonging to 6 serotypes (CGH)

Is one genome enough for exhaustive antigen identification?

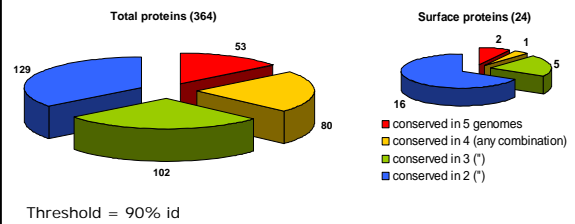


- CGH against multiple isolates shows that approximately 18% of the reference genome is absent from at least one strain!
- How many surface proteins have been lost during our first antigen selection?

Seven additional GBS genomes were sequenced



Reverse Vaccinology applied to the GBS pan-genome identified new potential candidates




Universal protection of a protein-based GBS vaccine

Identification of a Universal Group B *Streptococcus* Vaccine by Multiple Genome Screen

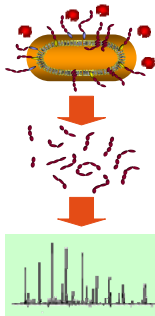
Domenico Malone, ^{1*} Immaculada Margarit, ^{1*} Cira D. Rinaudo, ¹ Vega Masignani, ¹ Marirosa Mora, ¹ Maria Scarselli, ¹ Hervé Tettelin, ² Cecilia Brettoni, ¹ Emilia T. Iacchini, ¹ Roberto Rosini, ¹ Nunzio D'Agostino, ¹ Lisa Morin, ¹ Scilla Buccato, ¹ Massimo Marian, ¹ Giuliano Galli, ¹ Renzo Nogarotto, ¹ Vincenzo Nardi Dal, ¹ Filippo Vegni, ¹ Claire Fraser, ¹ Giuseppe Mancuso, ¹ Giuseppe Teti, ¹ Lawrence C. Madoff, ² Lawrence C. Padgett, ² Rino Rappelli, ¹ Dennis L. Kasper, ² John L. Telford, ¹ Guido Grandi ¹

Group B *Streptococcus* (GBS) is a multiserotype bacterial pathogen representing a major cause of life-threatening infections in newborns. To develop a broadly protective vaccine, we analyzed the genome sequences of eight GBS isolates and cloned and tested 312 surface proteins as vaccines. Four proteins elicited protection in mice, and their combination proved highly protective against a large panel of strains, including all circulating serotypes. Protection also correlated with antigen accessibility on the bacterial surface and with the induction of opsonophagocytic antibodies. Multigenome analysis and screening described here represent a powerful strategy for identifying potential vaccine candidates against highly variable pathogens.

The protective vaccine was formulated using 4 antigens, none of which is shared by all strains




Surface digestion of live bacterial cells: The Group A streptococcus example



Digestion with proteases

Peptide recovery

Protein identification by MS



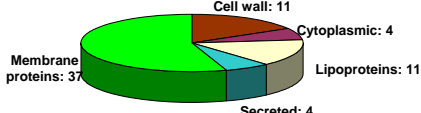

72 surface-exposed proteins identified by Proteomic approach in GAS

ARTICLES

nature biotechnology

Characterization and identification of vaccine candidate proteins through analysis of the group A *Streptococcus* surface proteome



Mariuel J Rodriguez-Ortega, Nathalie Sorais, Giuliano Rossi, Sabrina Liberatori, Sabrina Cappi, Marirosa Mora, Maria Scarselli, Francesco Doms, Gerardo Ferrari, Ignazio Garagnoli, Tiziana Magli, Anita Neumann, Alessia Covei, John L. Telford & Guido Grandi



S. pneumoniae disease burden

- *S. pneumoniae* is a gram-positive encapsulated diplococcus, that expresses more than 90 different types of capsular polysaccharides.
- It causes approximately 25% of the vaccine preventable disease on a global scale (more than 150 million cases in children < 5yrs).
- Worldwide mortality is 1.6 million people, including more than 800,000 children under age five, annually (95% in developing countries).

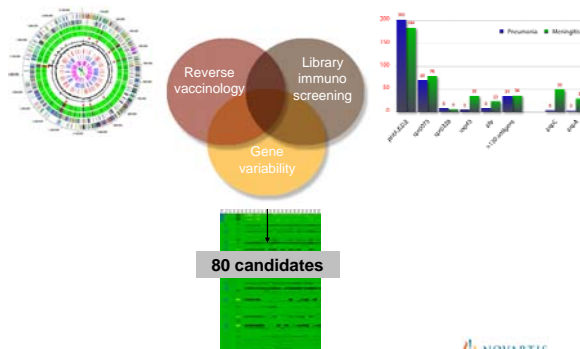

Pneumonia Disease Burden

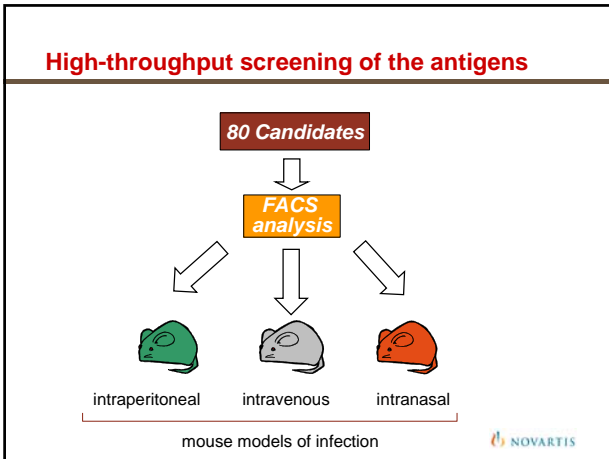



Conjugate vaccines cannot cover the >90 serotypes and overtime “non vaccine types” emerge

A combined approach for vaccine candidates selection

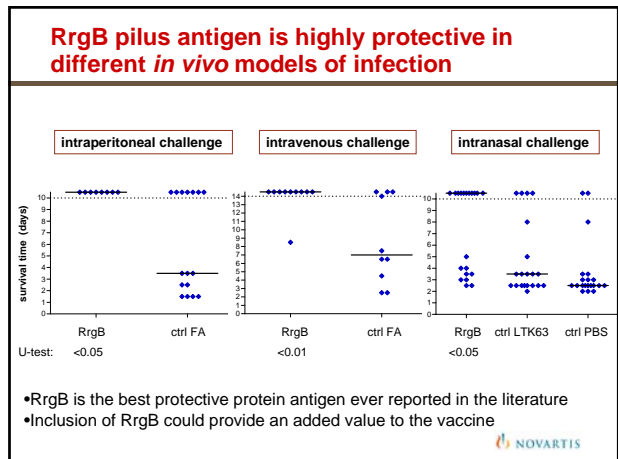
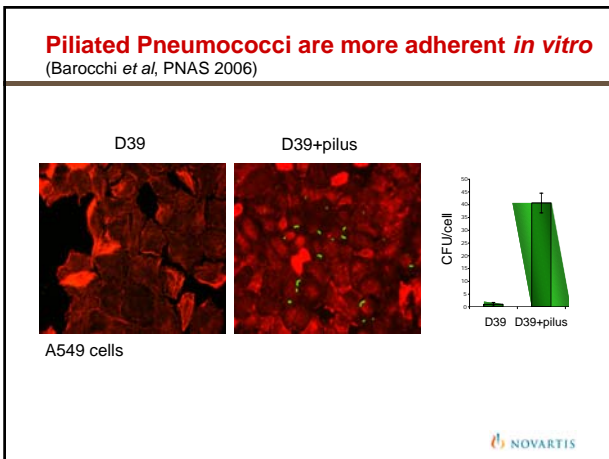
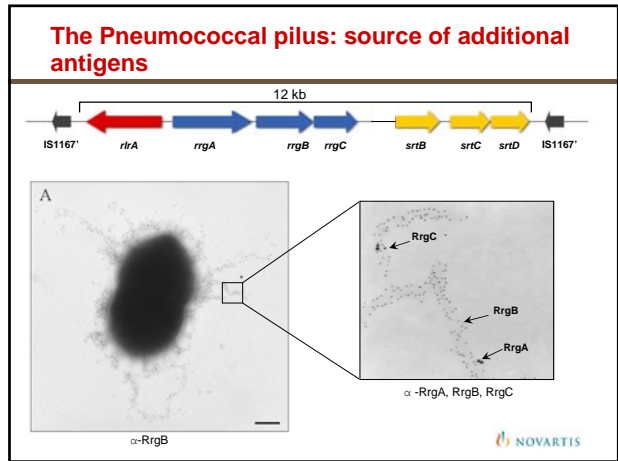
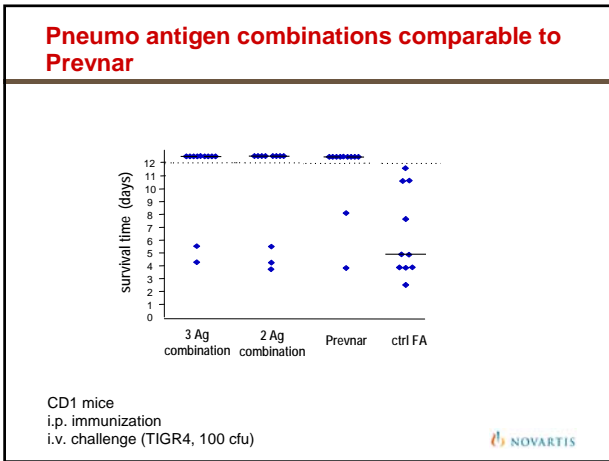





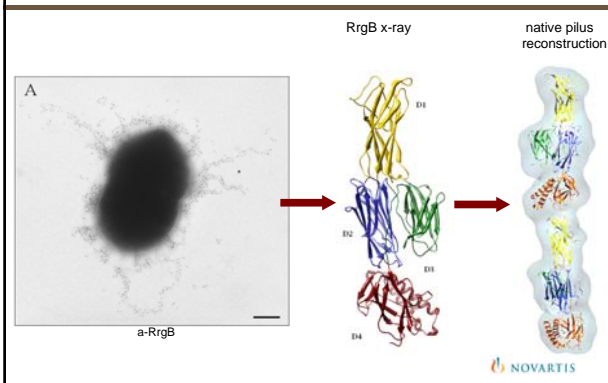
Conserved antigens display cross-protection

Ag Id	Conservation	FACS	Protection against serotypes			
			4	2	3	35B
Ag1	>97%	+++	+	++	-	-
Ag2	>99%	+/- (secreted)	+	+	++	++
Ag3	>99%	+/- (secreted)	++	++	-	-
Ag4	>96%	+++	++	++	++	-
Ag5	>99%	++	++	-	+	+
Ag6	>99%	+	++	++	n.d.	-
Ag7	>99%	+	++	-	n.d.	++
Ag8	>99%	++	++	+	n.d.	n.d.
Ag9	>99%	+/- (secreted)	+/-	+/-	-	n.d.

Development of antigen combinations



Understanding the molecular architecture of the pilus



Conclusions and Future Perspectives

- Genome-based approaches have proved successful for the identification of novel candidates for the development of vaccines against important human pathogens
- Following these approaches new vaccines are in clinical trials or in late stage of preclinical development
- Other human pathogens are currently being addressed by genomics (E. coli, C. difficile, Pseudomonas, etc)
- What about animal pathogens?
- Bacteria like Staph aureus, S. suis, S. equis, S. uberis, S. dysgalactiae could be easily approached by using similar strategies!

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Acknowledgements

- MenC and Menveo
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 - Mariagrazia Pizza & the MenB Team
- GBS
 - John Telford, Guido Grandi & the GBS Team
- GAS
 - Imma Margarit & the GAS Team
- Pneumococcus
 - Antonello Covacci, Vega Masignani, Michèle Barocchi & the Pneumo Team

Rino Rappuoli

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