Meningococcal ACYWX Polysaccharide Conjugate Vaccine

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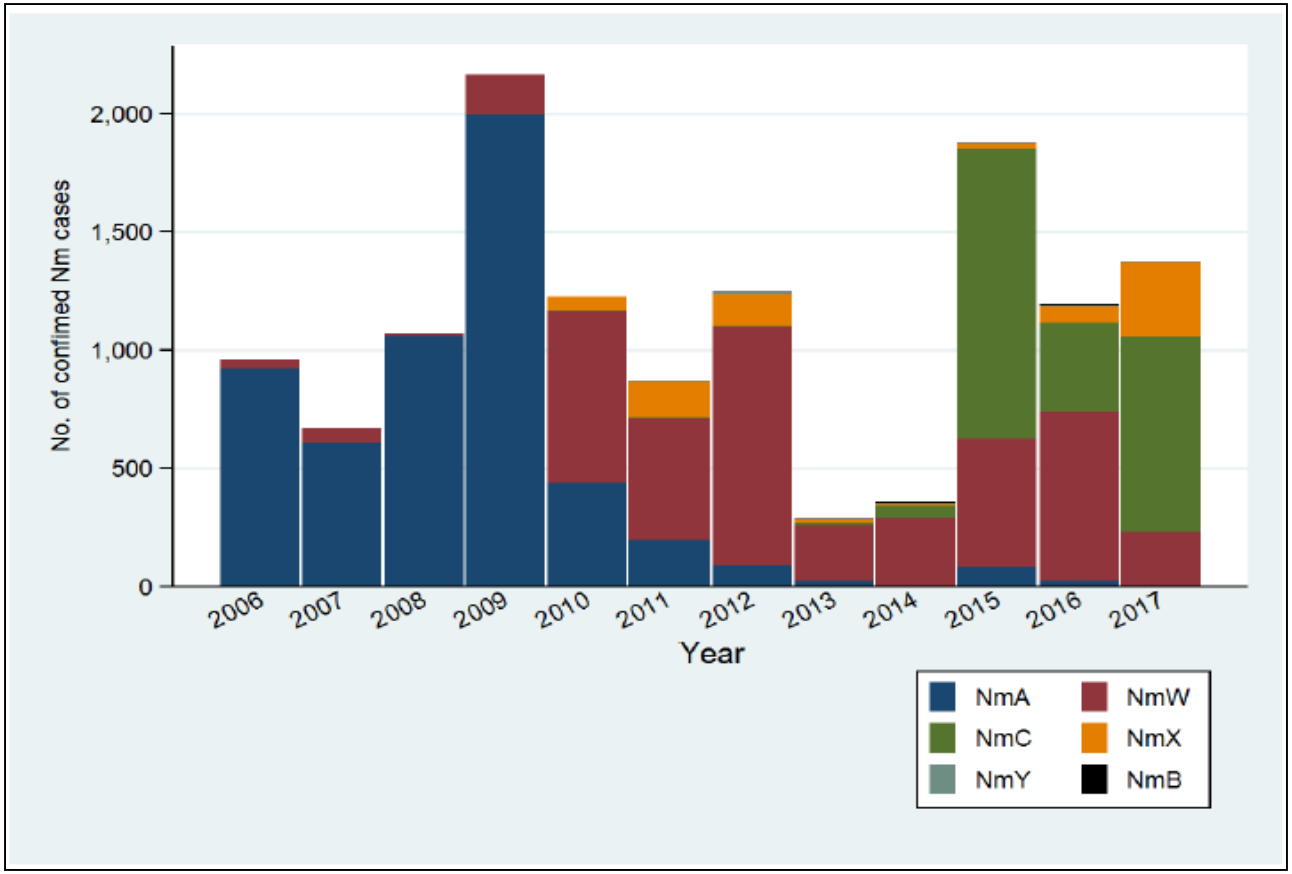


- Since 2010, ~350 million Africans have been immunized with **MenAfriVac** through a series of country and WHO sponsored preventive campaigns in 1-29 year olds.
- Post MenAfriVac launch, **No Men A cases observed** in vaccinated population.

Mustapha, M. M et al, 2018, Human vaccines & immunotherapeutics, 1-9.

Non-Group A strains cause epidemics

- **Serogroup W** was introduced into Africa through returning Hajis in 1999 and 2000. Major W epidemic in Burkina Faso in 2002- over 12,000 cases; incidence about 100/100,000.

- **Serogroup X** meningococci have caused focal epidemics in many Sub-Saharan countries, Niger reporting over 4000 cases in 2008.

- **Serogroup Y** *N. meningitidis* commonly recovered in carriage studies, has potential to cause meningitis outbreaks.

- **Serogroup** C, In 2015, the meningitis belt witnessed an outbreak of serogroup C in which 1201 lab confirmed cases were reported in Niger and Nigeria. Another, outbreaks of serogroup C occurred in Nigeria in the early part of 2017.

Product Development Rationale

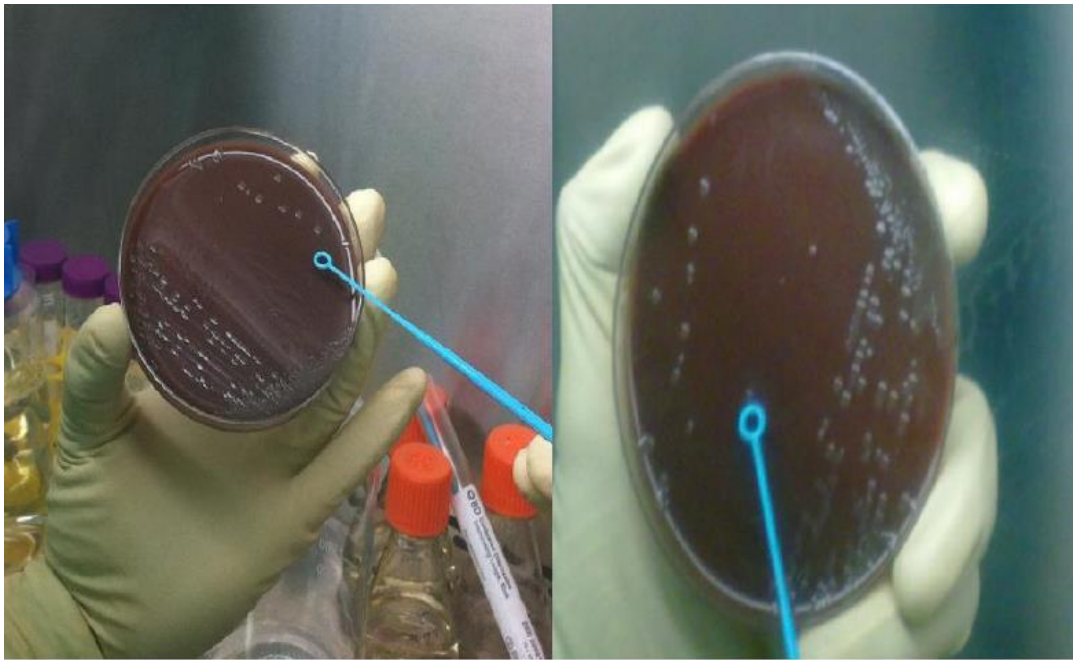
- Learning from the *MenAfriVac* experience, conjugate vaccines are the real long-term solution. Despite of having three ACYW conjugate vaccines, more cost effective and thermostable vaccines are required.

- WHO Position Paper, Nov 2011

  Vaccines available against serogroups A, C, W and Y. **"However, lack of a vaccine against group X meningococci is a cause for concern given the outbreaks caused by meningococci of this serogroup in the past few years."**

- This is one of the main drivers behind the SIIL's collaboration with PATH to develop a pentavalent meningococcal conjugate vaccine (ACYWX, lyophilized).

Cell Banking



- Strains : Serogroup A, C and W - USFDA (CBER).

- Six currently circulating strains of Serogroup Y and five strains of Serogroup X were screened. The **serogroup Y strain from CDC** and **X strain from CEBER** showed uniform growth kinetics and high yields and desired polysaccharide characteristics.

- A **two-stage colony selection** protocol was followed for isolating individual colonies. MSL & WSL seed lots were characterized.

Polysaccharide Manufacturing

- Manufacturing meningococcal serogroup A, C, W and Y polysaccharides (PS) are well established through **fed- batch processes**. **SIIL developed a fermentation medium which has resulted in acceptable yields of purified X PS.**

- PS purification protocols are largely based on **surfactant precipitation and extraction by alcohol.**

- Purified PS yields are typically above **500 mg/L** of harvest, Men A,C,Y and X PS are O-acetylated and X is N-acetylated.

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Process development and immunogenicity studies on a serogroup 'X' Meningococcal polysaccharide conjugate vaccine

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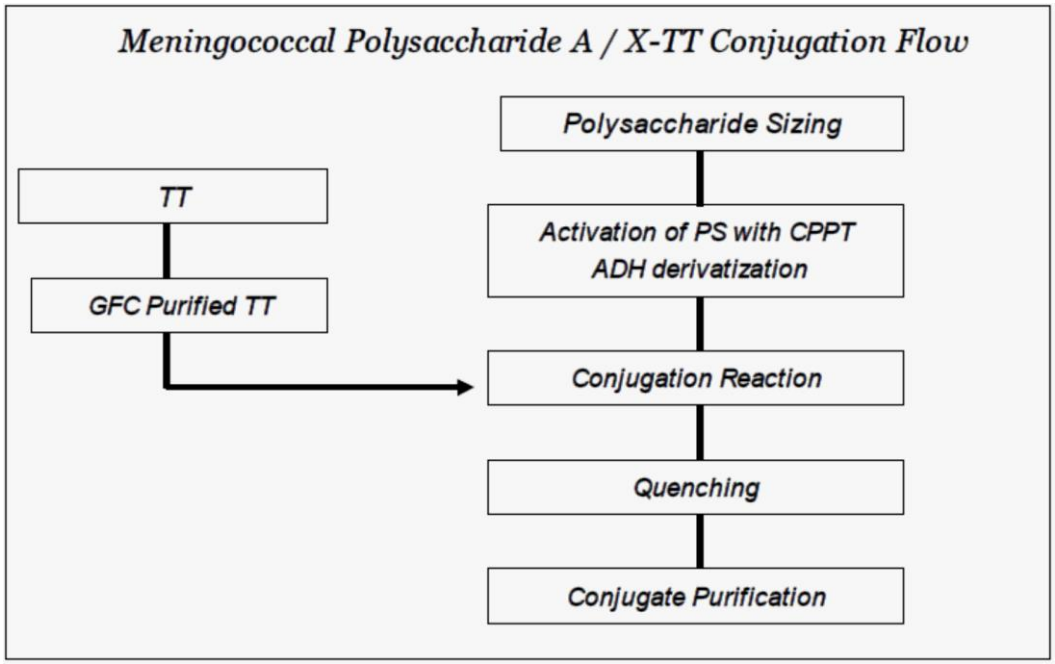
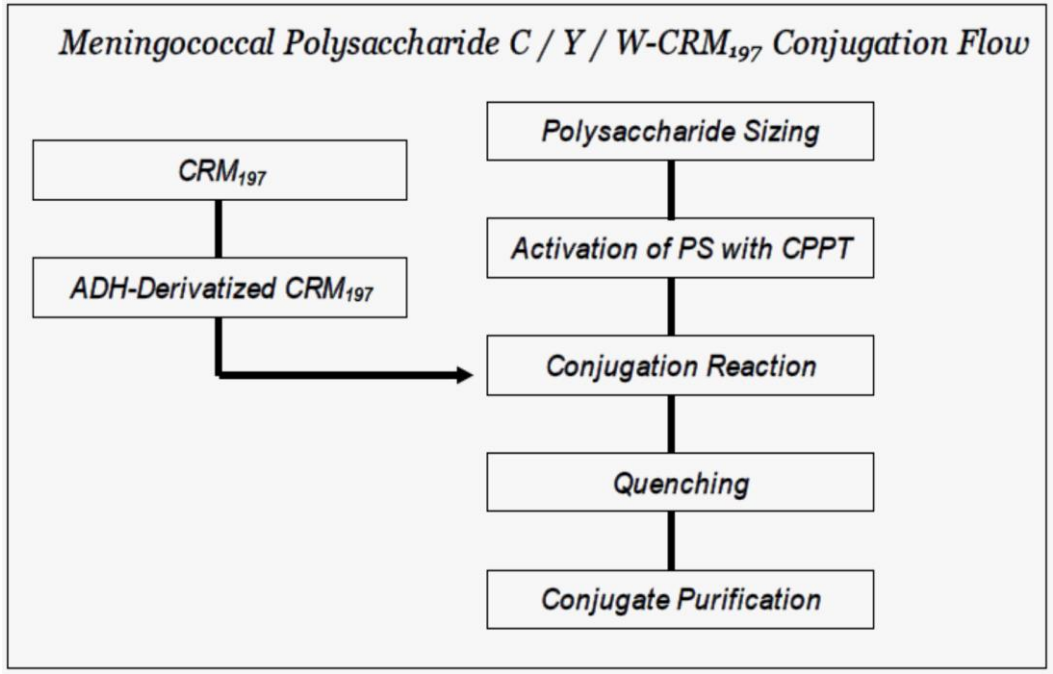
ABSTRACT

Meningococcal group X (MenX) is responsible for recent outbreaks of meningitis reported in sub-Saharan region of Africa. Although protective vaccines are available for meningitis, they are not effective against MenX. An efficacious, monovalent conjugate vaccine was designed against MenX and a fed-batch fermentation process was developed. The MenX polysaccharide (PS) was purified and yield estimated to be 15-fold higher than the reported elsewhere. Structure of MenX polysaccharide was confirmed by ^{1}H, ^{13}C NMR spectroscopy analysis. Molecular weight of PS was found to be 310 kDa using HPLC-SEC coupled to refractive index (RI) detector. The MenX–Tetanus toxoid (TT) monovalent conjugate proved to be highly immunogenic in mice, and the bactericidal titers of MenX–TT conjugate were 10-fold higher than native PS. Increasing the dose of MenX–TT conjugate from 0.5 μg to 1.0 μg induced an 8-fold higher antibody titer as well as serum bactericidal titer. The current work suggests that the MenX–TT conjugate is a candidate vaccine against meningitis caused by Meningococcal group X strains.





Conjugation Scheme - CPPT conjugation is similar to CDAP chemistry used in licensed meningococcal / pneumococcal vaccines

A,C,W, X PS – sized mechanically (100-150 kDa, Y PS: chemically (100-125 kDa)

Crude individual conjugates purified by ultrafiltration with yields of 20-35%, PS:PR ratio of 0.3-1.

The purified conjugates with stabilizer are stored below -20°C.

Formulation Development

- **Thermal analysis** of the five meningococcal PS confirmed **Men A PS is more susceptible** to thermal degradation, next is Men C, followed by W/Y and lastly X PS. The data also indicated that the **primary drying should be below -30°C.**

- Of the 18 different stabilizer mixtures, based on cake properties and reconstitution time, 7 mixtures were chosen for further studies with conjugates.

- **Men A-TT and Men C-CRM,** each separately at 125 µg / 0.5 ml fill volume, were lyophilized. Lyophilized vials were stored at **40°C for six months** and free PS levels were analyzed, as an indicator of conjugates stability.

- Pentavalent vaccines with **3 stabilizer combinations** (Sugar+Salt+Buffer) were evaluated immunologically. In turn, the lead formulation from the three stabilizers was **assessed in a rabbit immunogenicity study both with and without adjuvant.**

Formulation Development: Stabilizer Selection

Meningococcal A –TT Conjugate (Lyophilized)					
Unbound (Free) Polysaccharide (%) at different time intervals at 40°C					
Stabilizer Code	Initial	1 Month at 40°C	2 Months at 40°C	3 Months at 40°C	6 Months at 40°C
S3-L2	8.12	10.07	10.19	10.61	10.88
T3-L2	8.58	8.89	9.05	9.85	10.78
S3-SC50	4.92	5.00	5.60	7.13	9.01
T3-SC50	4.84	6.01	6.86	6.88	8.99
T2-SC50	5.60	6.66	7.29	7.89	9.02
S3-L2- SC50	7.52	6.25	7.81	8.15	8.95
T3-L2- SC50	6.62	6.65	8.08	9.56	10.30

Formulation Development: Stabilizer Selection

Meningococcal C – CRM Conjugate (Lyophilized)					
Unbound (Free) Polysaccharide (%) at different time interval at 40°C					
Stabilizer Code	Initial	1 Month at 40°C	2 Months at 40°C	3 Months at 40°C	6 Months at 40°C
S3-L2	2.13	2.58	3.59	3.04	3.14
T3-L2	1.96	2.16	2.57	3.33	3.47
S3-SC50	<0.96	< 0.98	< 1.06	< 1.09	1.74
T3-SC50	1.31	2.10	2.20	3.01	3.21
T2-SC50	1.64	2.19	2.72	3.06	3.05
S3-L2- SC50	1.54	2.37	2.5	3.31	3.74
T3-L2- SC50	1.48	2.75	3.23	3.79	3.99

Based on this data, wide use and cost-effectiveness S3SC50 stabilizer is finalized for NmCV-5

Immunogenicity Assessment

Three SIIPL developmental ACYWX (each at 5 µg) formulations with adjuvant and licensed product vaccines.

SIIPLF2: Men A (*MenAfriVac*) bulk , Men C-CRM, Men Y-CRM, Men W-CRM, Men X –TT

SIIPLF3: Men A TT(CPPT) Men C-CRM, Men W-CRM, Men Y-CRM, Men X-TT

SIIPLF4: Men A TT, (CPPT) Men C-TT, Men W-TT, Men Y-DT, Men X -TT

Animal Model	New Zealand White Rabbits
Age	2-3 months, 800-1200 gm body weight
Rabbits per formulations	Six rabbits (3 males, 3 females)
Formulations	Licensed Product, SIIPL- F2, SIIPL- F3, SIIPL-F4
Injection volume	0.5 ml / dose
Route of immunization	IM
Immunization Schedule	Day 0, 14,28
Bleeding Schedule	Day 0, 28, 35
Immune response	IgG & SBA

Immunogenicity Assessment for Conjugate Construct in Rabbit Model

Formulation	Men A Conjugate		Men C Conjugate		Men W Conjugate		Men Y Conjugate		Men X Conjugate	
-	Day 0	Day 35	Day 0	Day 35	Day 0	Day 35	Day 0	Day 35	Day 0	Day 35
IgG (GMT)										
Licensed product	50	44572	50	25600	50	29407	50	38802	NA	NA
SIIPL F2	50	72408	50	51200	50	60887	50	72408	50	60887
SIIPL F3	**50**	**58813**	**100**	**58813**	**50**	**89144**	**50**	**117627**	**200**	**51200**
SIIPL F4	50	33779	100	9701	50	25600	50	29407	50	51200
SBA (GMT)										
Licensed product	**2**	**446**	**2**	**223**	**2**	**223**	**4**	**256**	**NA**	**NA**
SIIPL F2	**2**	**724**	**2**	**215**	**2**	**256**	**2**	**152**	**16**	**1024**
SIIPL F3	**2**	**676**	**2**	**512**	**2**	**588**	**2**	**388**	**8**	**1024**
SIIPL F4	**2**	**338**	**2**	**64**	**2**	**147**	**2**	**111**	**2**	**676**

- Licensed product is a quadrivalent vaccine.
- **SIIPL F3 [Men A TT(CPPT) Men C-CRM, Men W-CRM, Men Y-CRM, Men X-TT] is finalized for further development .**

Immunogenicity Assessment for thermally challenged (40°C for 1 month) vaccine in Rabbit Model

Vaccine / Description	Men A		Men C		Men W		Men Y		Men X	
	Day0	Day35	Day0	Day35	Day0	Day 35	Day0	Day35	Day0	Day35
IgG (GMT)										
Licensed Product (Stored at 2-8 ^{0}C)	71	25600	159	18102	100	28735	50	22807	-	-
SIIPL – ACYWX (lyophilized) stored at 40^{0}C for 1 month	56	22807	50	40637	50	20319	50	25600	111	18102
SBA (GMT)										
Licensed Product (Stored at 2-8 ^{0}C)	**2**	**456**	**2**	**256**	**2**	**181**	**2**	**181**	**-**	**-**
SIIPL – ACYWX (lyophilized) stored at 40^{0}C/1month	**2**	**645**	**2**	**512**	**2**	**228**	**2**	**203**	**4**	**575**

- The formulation found immunogenic even after heating, indicating potential of a thermostable vaccine.

Product Profile

Composition	Qty / Dose
Men A PS -TT	5 µg
Men C PS-CRM	5 µg
Men Y PS -CRM	5 µg
Men W PS -CRM	5 µg
Men X PS-TT	5 µg
Stabilizer (for Freeze drying)	< 10% w/v

Diluent	Sodium chloride in WFI (0.9% w/v)
Preservative	No

NmCV-5 Presentations : Single Dose, 5 Dose.

Pre-Clinical Study (MPI Research Inc. MI USA)

- ✓ **Test Articles: Adjuvanted and Non-adjuvanted Vaccine Formulations with Saline control in New Zealand White Rabbits**
- ✓ **Doses: 4 IM Injections every other week for 7 weeks.**
- ✓ **Assessment: Mortality, Clinical observations, dermal scoring, body weight & temperature, food consumption; ophthalmoscopic examinations, clinical and anatomic pathology with immunogenicity evaluations (at NIBSC).**
- ✓ **Delayed toxicity Assessment: In recovery (6-week) group**

Study Design				
Group Number	Dose Level	Dose Volume (mL/rabbit)	Number of Animals	
			Male	Female
1	Saline Control	0.5	10[a]	10[a]
2	25 µg meningococcal (Non-Adjuvanted)	0.5	10[a]	10[a]
3	25 µg meningococcal (Adjuvanted)	0.5	10[a]	10[a]
[a]Four animals/sex/group were maintained for a 6-week recovery period.				

In summary, four bi-weekly intramuscular administrations of NmCV-5 vaccine with or without adjuvant was **well tolerated** and **raised immune response** against **all 5 serogroups**.

Separate Tox Study for Conjugation byproduct (4PP)

Test	Study Outcome
Oral single dose in rats	At 10 mg/kg dose, no impact on body weights or food consumption observed, hence NOAEL considered to be 10 mg/kg.
IM single dose in rats	0.2 mg dose well tolerated No-Observed-Adverse-Effect Level (NOAEL) is 0.2 mg/site.
In vitro mutagenicity, prokaryotic cells	4-PP was concluded to be negative in the Bacterial Reverse Mutation Assay.
In vitro mutagenicity, eukaryotic cells (mouse lymphoma)	4-PP was concluded to be negative in the L5178Y/TK+/- Mouse Lymphoma Assay, under the test conditions.
Skin sensitization Guinea Pig study	4-PP, was determined to be a non-sensitizer (0% Sensitization Index) following assessment of skin sensitization using the Maximization Method.

Thermostability Assessment: Final Lot used in Phase-1 Study

% Free Polysaccharide Data						
Serogroup	**Temperature**	**Men A**	**Men C**	**Men Y**	**Men W**	**Men X#**
Real Time	2-8°C 12 Months	< 4.8	4.0	7.8	< 3.7	13.3
Accelerated	25°C/RH 60±5%, 6 Months	< 4.9	3.9	8.7	< 3.7	13.8
Stress	40°C/ RH 75±5%. 4 weeks	< 4.9	4.0	7.2	< 3.7	11.9
	40°C / RH 75±5%. 16 weeks	< 5.0	4.0	9.1	< 3.7	12.8
	40°C / RH 75±5%. 24 weeks	< 4.9	3.7	8.7	< 3.7	15.6

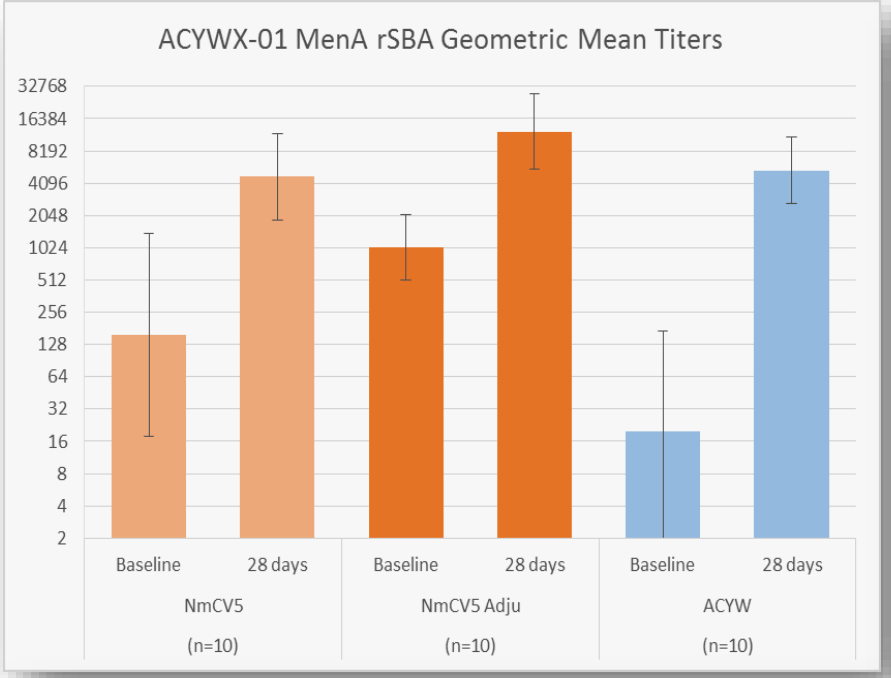
#: Initial Free PS is ~10.0%

Phase-1 Study: Safety Conclusions
(Center for Vaccine Development, Baltimore, USA)

60 subjects post day 28:

- No SAEs
- Solicited events were mild and moderate
- Unsolicited related events were few (n=4) and mild
- All AEs resolved without sequelae except for a mild hyperpigmentation on site of injection

The unblinded analysis concluded that both the vaccine formulations are safe and immunogenic.


ACYWX-01 MenA rSBA Geometric Mean Titers
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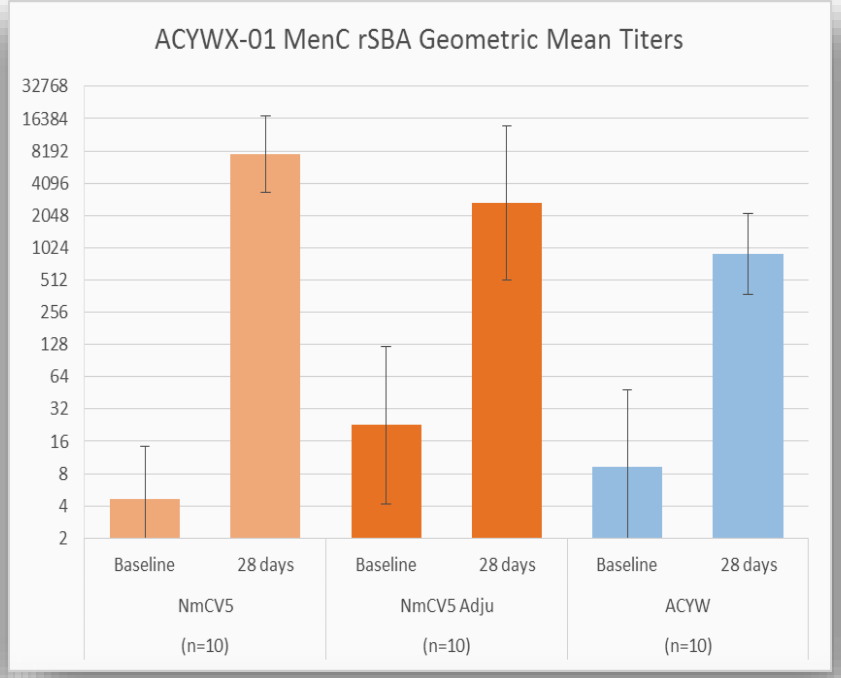
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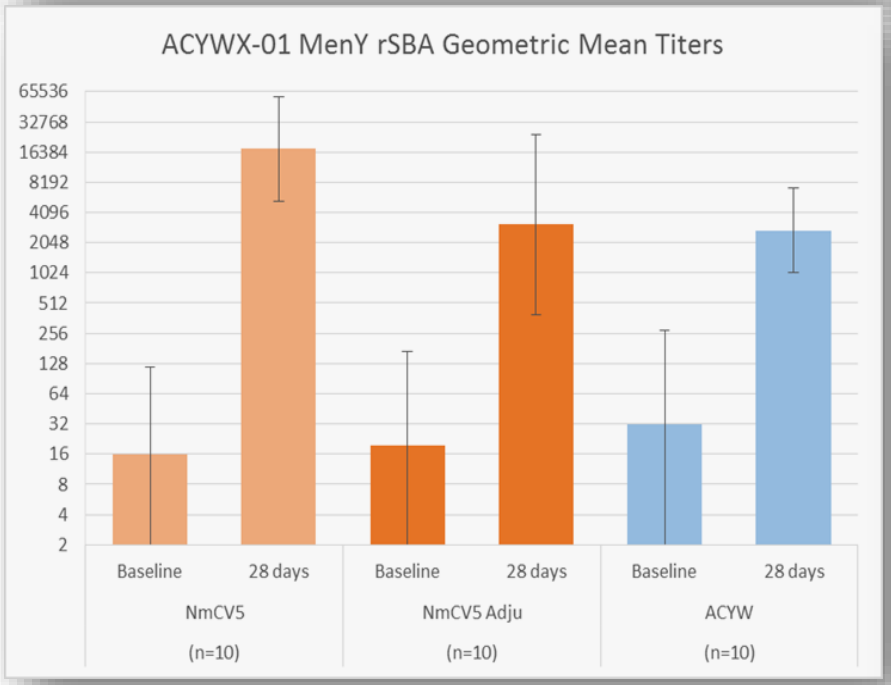
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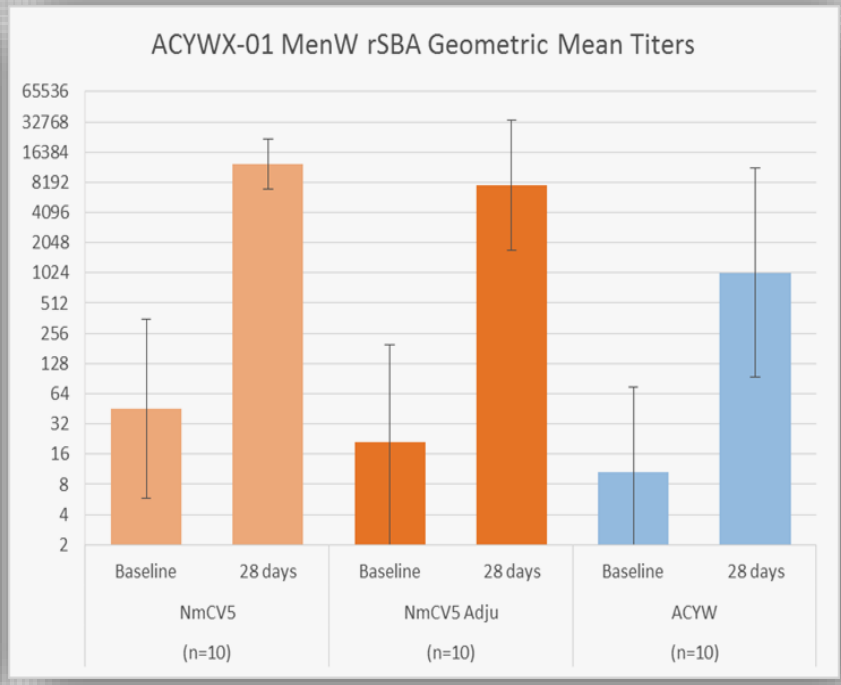
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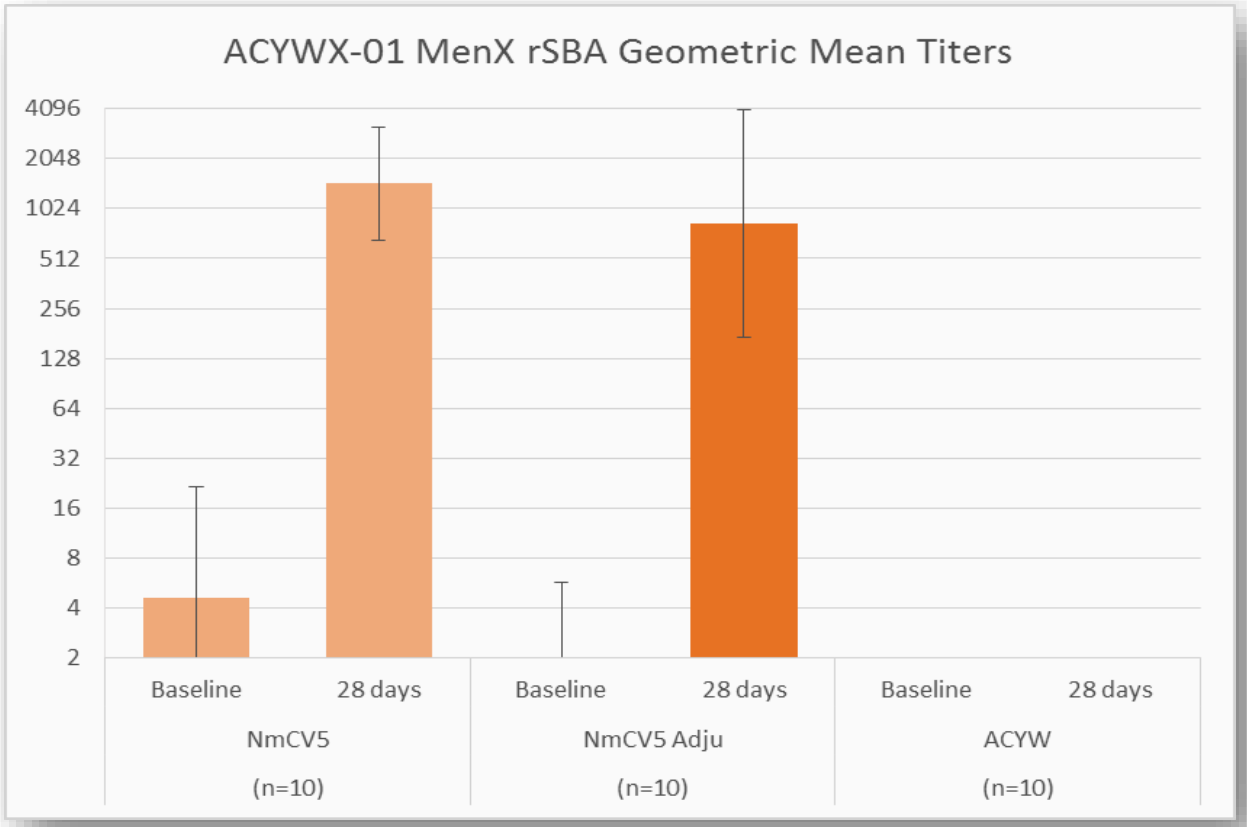


- NmCV-5 adjuvanted and non-adjuvanted formulations elicit responses to all five Men groups.
- Adjuvanted NmCV-5 seems to elicit a stronger response against Men A.
- NmCV-5 non-adjuvanted seems to elicit a stronger response against C, W, Y, X.
- Both formulations of NmCV-5 elicit similar or higher responses than LICENSED A, C, Y and W vaccine.

Phase-2 Study

(Center for Vaccine Development, Bamako, Mali)

- Phase-2 trial on Polymen vaccine (ACYWX conjugate, NmCV-5) in Mali.

- The study was done in 375 toddlers of 12 to 16 months.

- The objective of the study was to assess safety of the vaccine in toddlers as well as to select one of the two NmCV-5 formulations - with or without adjuvant.

- Safety point of view, there were few local and systemic reactions in all three groups. The incidence was small and similar in all three groups. No serious adverse event was reported till one month after the first dose.

- Serum samples were tested for serum bactericidal assay *antibody titres (rSBA) at the Public Health England, Manchester*.

Immunogenicity Results after the first dose of the vaccines

Long term seroprotection (titre > 1:128) at one month after the first dose

Sero Group	Non-adjuvanted NmCV-5 (N=147)	Adjuvanted NmCV-5 (N=148)	Comparator (N=74)
A	100%	100%	98.6%
C	98.6%	97.3%	54.1%
W	98.6%	98%	90.5%
X	100%	99.3%	20.3%
Y	97.3%	99.3%	87.8%

Geometric mean titres (GMT) at one month after the first dose

Sero Group	Non-adjuvanted NmCV-5 (N=147)	Adjuvanted NmCV-5 (N=148)	Comparator (N=74)
A	7704.9	7286.9	3872.1
C	1146.7	1058.1	70.9
W	6563.6	5476.0	1135.1
X	7596.7	7965.0	6.7
Y	2415.5	2951.1	629.2

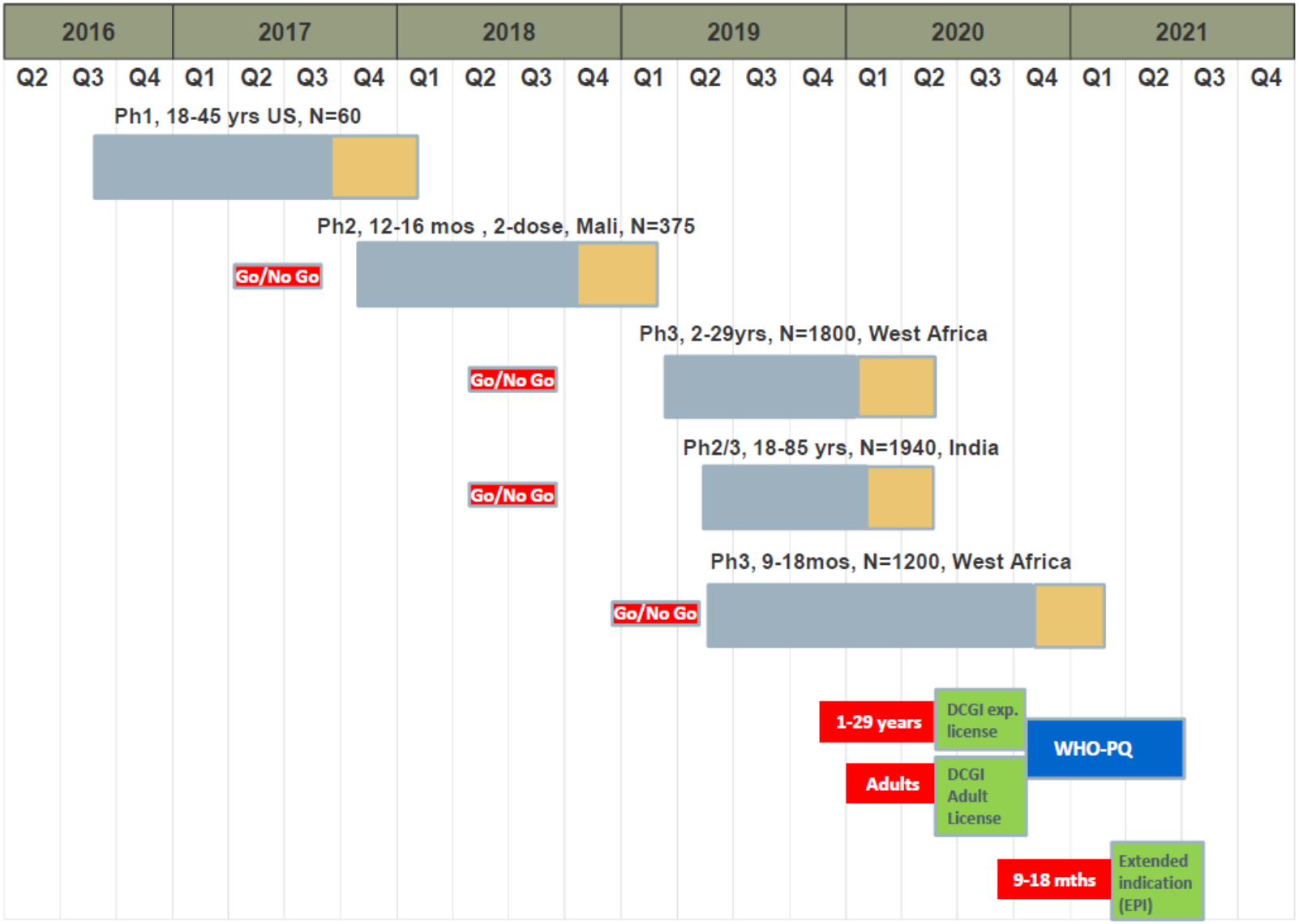
Findings from Phase-2 Study

- The immunogenicity results look much better with SIIPL vaccine as compared to the Comparator vaccine (especially in terms of GMTs and also for Serogroup C).

- There is practically no difference in the responses with the adjuvanted and unadjuvanted formulations. It looks like there is no need for an adjuvant in the vaccine.

- Unlike Comparator (ACYW) conjugate vaccine which recommends two doses for the toddler age group, it seems only one dose of SIIPL vaccine may be enough for this age group.

ACYWX Timelines



First Subject First Visit to Last Subject Last Visit

Analysis & Study Report

Regulatory Dossier for Licensure and Introduction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Thank You !!