Descifrando las vacunas del mañana:

Vacunología Estructural

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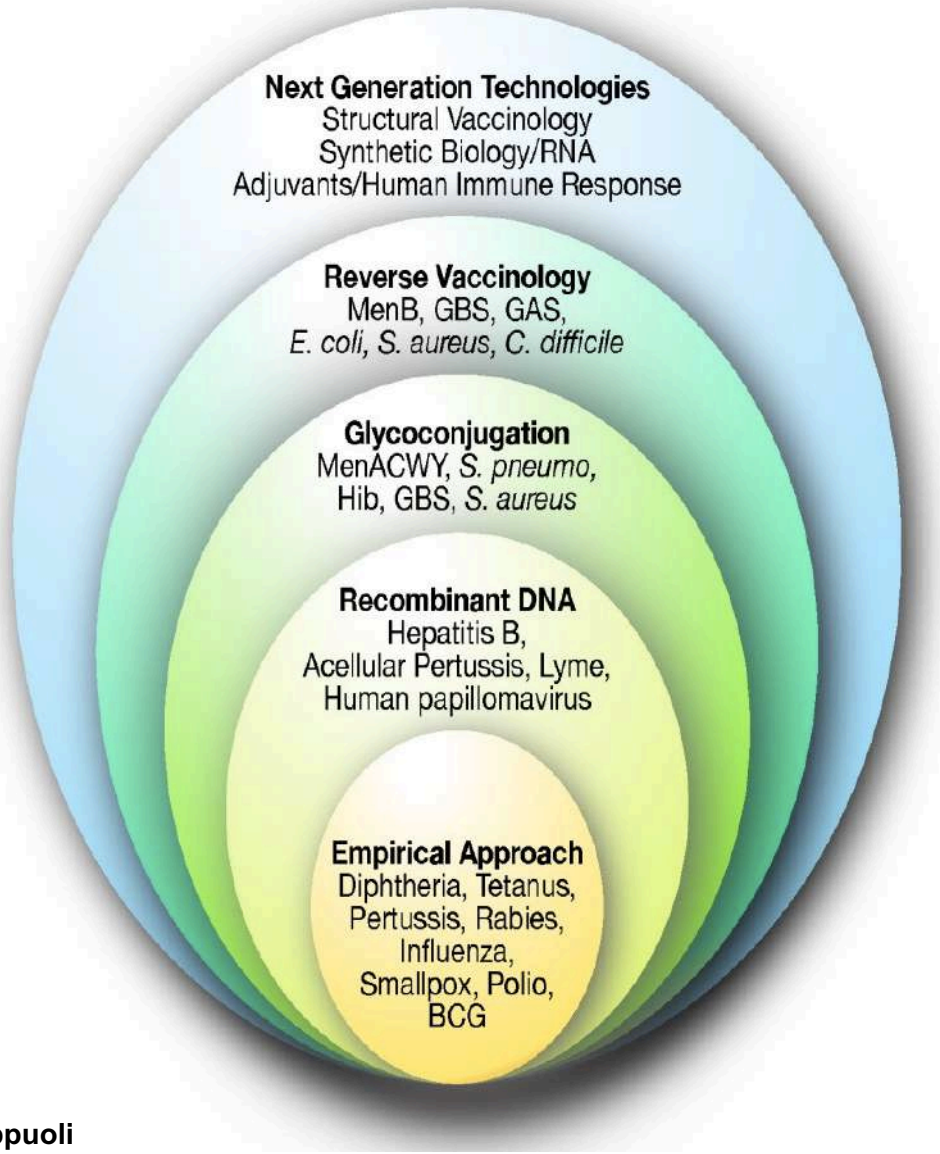
Genética, Vacunas, Infecciones y Pediatría (GENVIP)

Declaración de potenciales conflictos de interés

NO EXISTE NINGUN CONFLICTO DE INTERÉS EN RELACIÓN A ESTA PRESENTACIÓN

Durante los últimos 30 años, el desarrollo de nuevas tecnologías han hecho posibles vacunas imposible de desarrollar hasta la fecha.

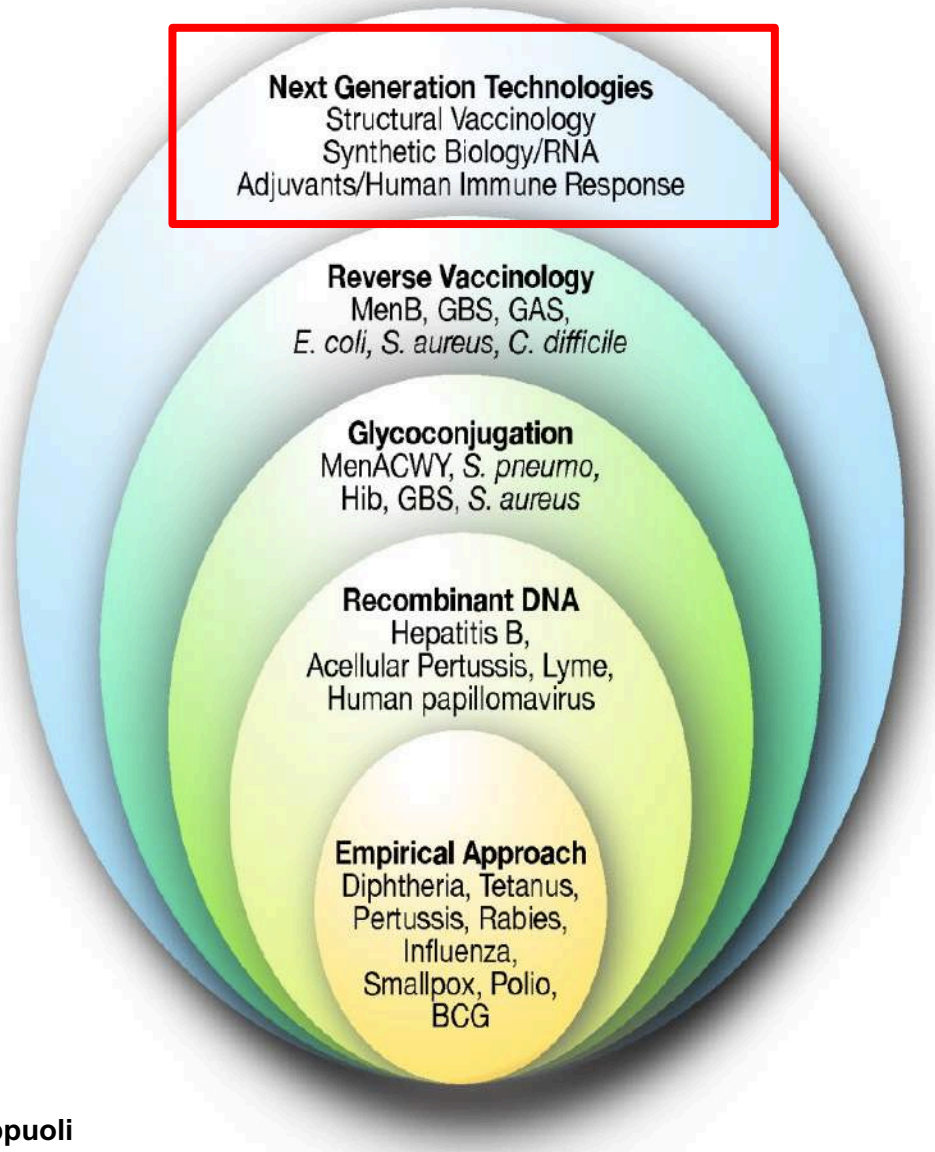


Rino Rappuoli
Conference on New Horizons for Vaccine Research and Innovation *Session on Innovation on Vaccine Design*

Structural Vaccinology

Structure-based antigen design

Adjuvants



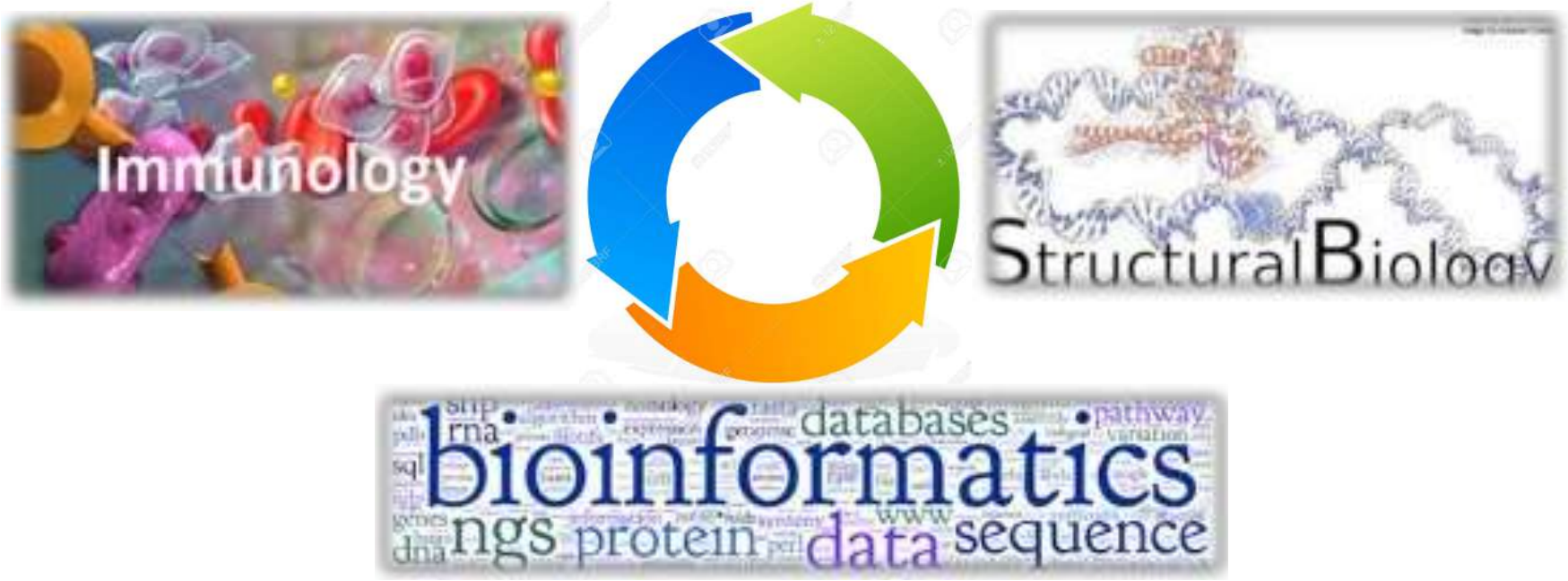
Synthetic biology

- Vectors
- Synthetic seeds
- Self Amplifying Messenger RNA **(SAM)**

Systems biology

Rino Rappuoli
Conference on New Horizons for Vaccine Research and Innovation *Session on Innovation on Vaccine Design*

VACUNOLOGÍA ESTRUCTURAL



Una respuesta inmune eficaz no requiere el reconocimiento de proteínas antigénicas enteras, simplemente el reconocimiento de EPITOPOS sencillos seleccionados es suficiente para inducir INMUNIDAD PROTECTORA

VACUNOLOGÍA ESTRUCTURAL

OBJETIVOS:

- Identificación de los EPITOPOS de células T/B considerados PROTECTORES en los antígenos
- Optimización de los antígenos en términos de estabilidad, presentación de epítopos, facilidad de producción y seguridad

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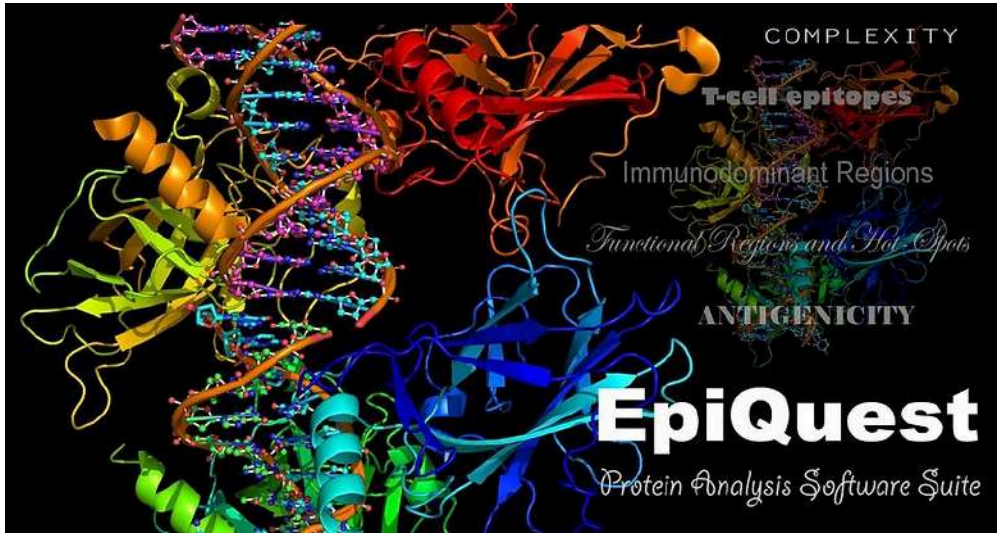
¿Como identificamos los EPITOPOS?



EpiQuest Programs at a glance:

EpiQuest-B
Prediction of linear continuous B-epitopes in primary amino acid sequence of a protein. Prediction of epitope structure and **immunogenicity**.

EpiQuest-T
Prediction of areas containing **strong** T-epitope peptides in primary aminoacid sequence of a protein

EpiQuest-A
Determination of accessibility/surface exposure of domains in a protein molecule.

EpiQuest-C
Evaluation of complexity (uniqueness) of primary sequence of a protein molecule. Allows to select areas relatively unique for given type of proteins

Match
Design of complementary peptides to a given amino acid sequence.

InCharge
Sequence analysis, sequence composition, prediction of isoelectric point for protein molecules on the basis of their linear sequence.

Predicting Immunogenicity of B-epitopes

Start	End	Length	Sequence	ELISA
25	33	9	VHTWTEQYK	Positive-High
29	37	9	DSGCVVSWK	Positive-High
31	39	9	GCVVSWKNK	Positive-High
33	41	9	VVSWKNKEL	Positive-High
89	97	9	TRLENLMWK	Positive-High
133	141	9	RPQPTELRY	Positive-High
135	143	9	QPTELRYSW	Positive-High
137	145	9	TELRYSWKT	Positive-High

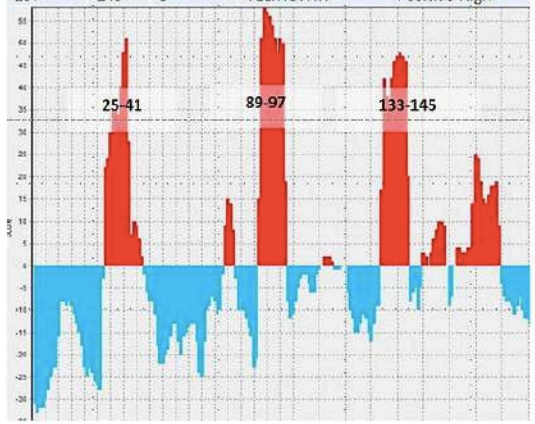


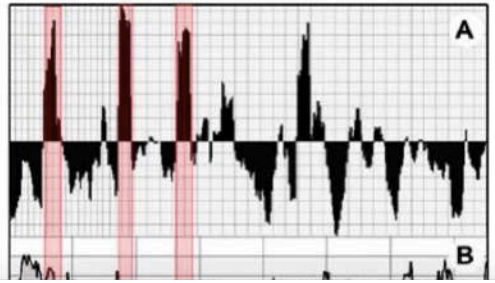
Exhibit 1. ***Predicted and actual immunodominant epitopes of NS1 protein [Dengue virus].*** *To illustrate what EpiQuest-B can predict, the NS1 is one of the best examples, as its immunodominant epitopes were investigated with high precision and detail. In total, there are 8 immunodominant epitopes covering 3 areas in the N-terminal part of the molecule. As can be seen, all 3 areas were detected by EpiQuest-B with high precision, as 3 main peaks in the antigenicity profile of the molecule.*

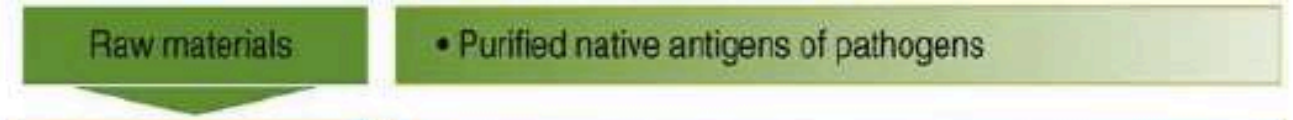
EpiQuest™-B

In predicting B-epitopes, whether for the needs of antibody production or finding the areas, inducing the humoral response (for diagnostics), the main question is: **whether this sequence is capable to elicit an immune response**? Our research (Litvinov et al, in preparation) showed that the structure of peptide sequence defines the probability of a strong humoral response to it.

Based on our ideas of peptide-peptide interactions, a new algorithm employed in EpiQuest-B evaluates the potential immunogenicity of the peptide sequence. The software calculates the **Antigenicity Index** for a given sequence (AGI).

The higher the AGI, the more likely is the strong humoral response to it. This is something NONE of the existing epitope prediction software is capable of, while EpiQuest-B does it with a high statistical significance (see below).





Etapas de la Vacunología Estructural

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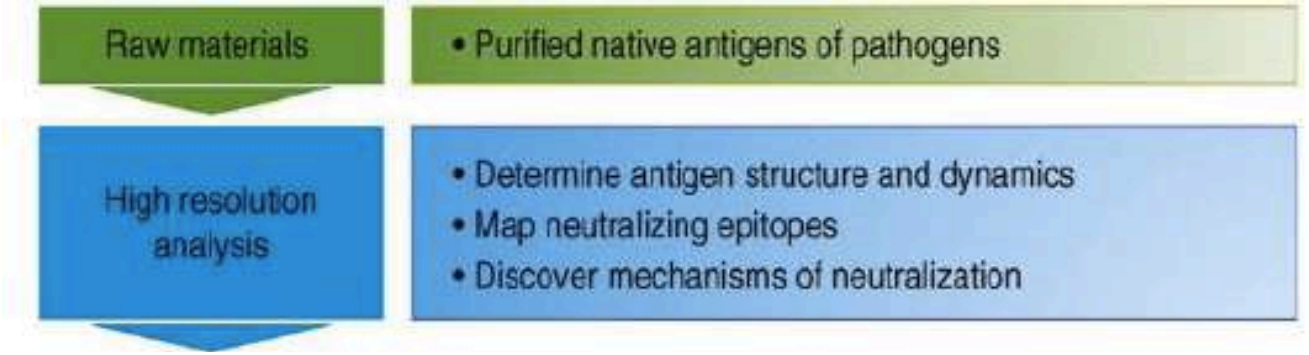
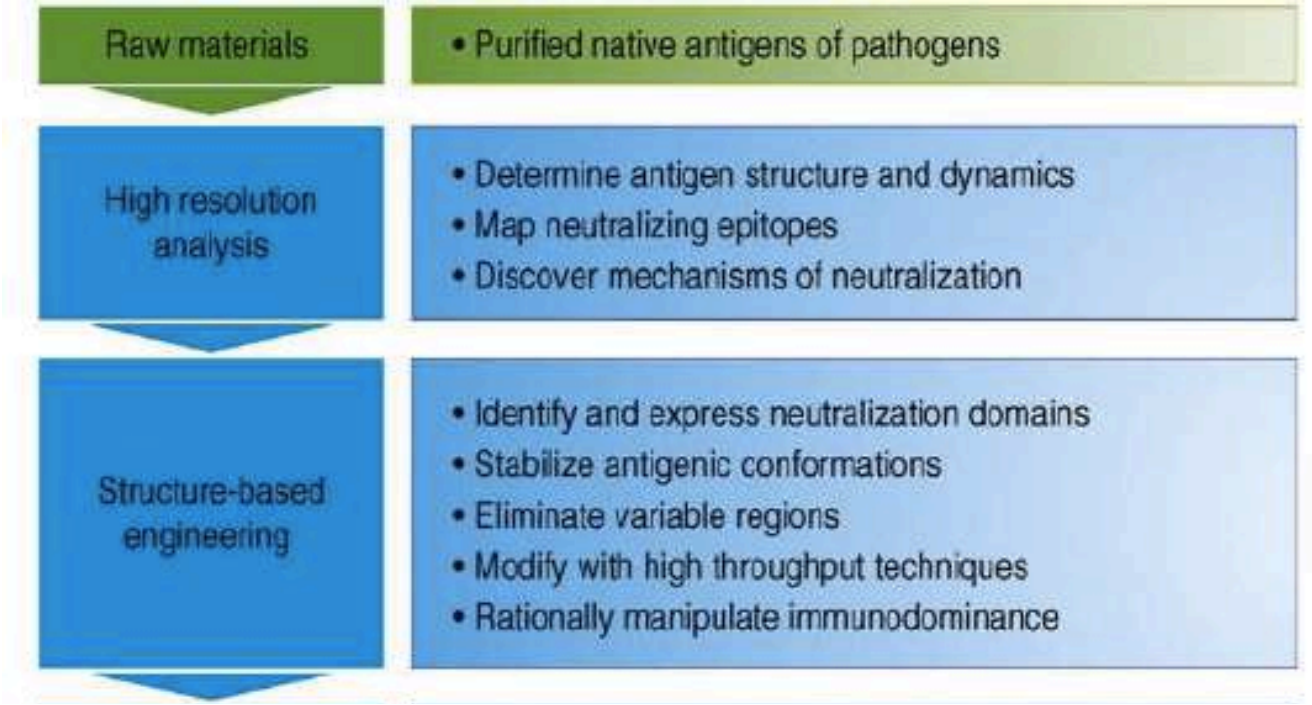
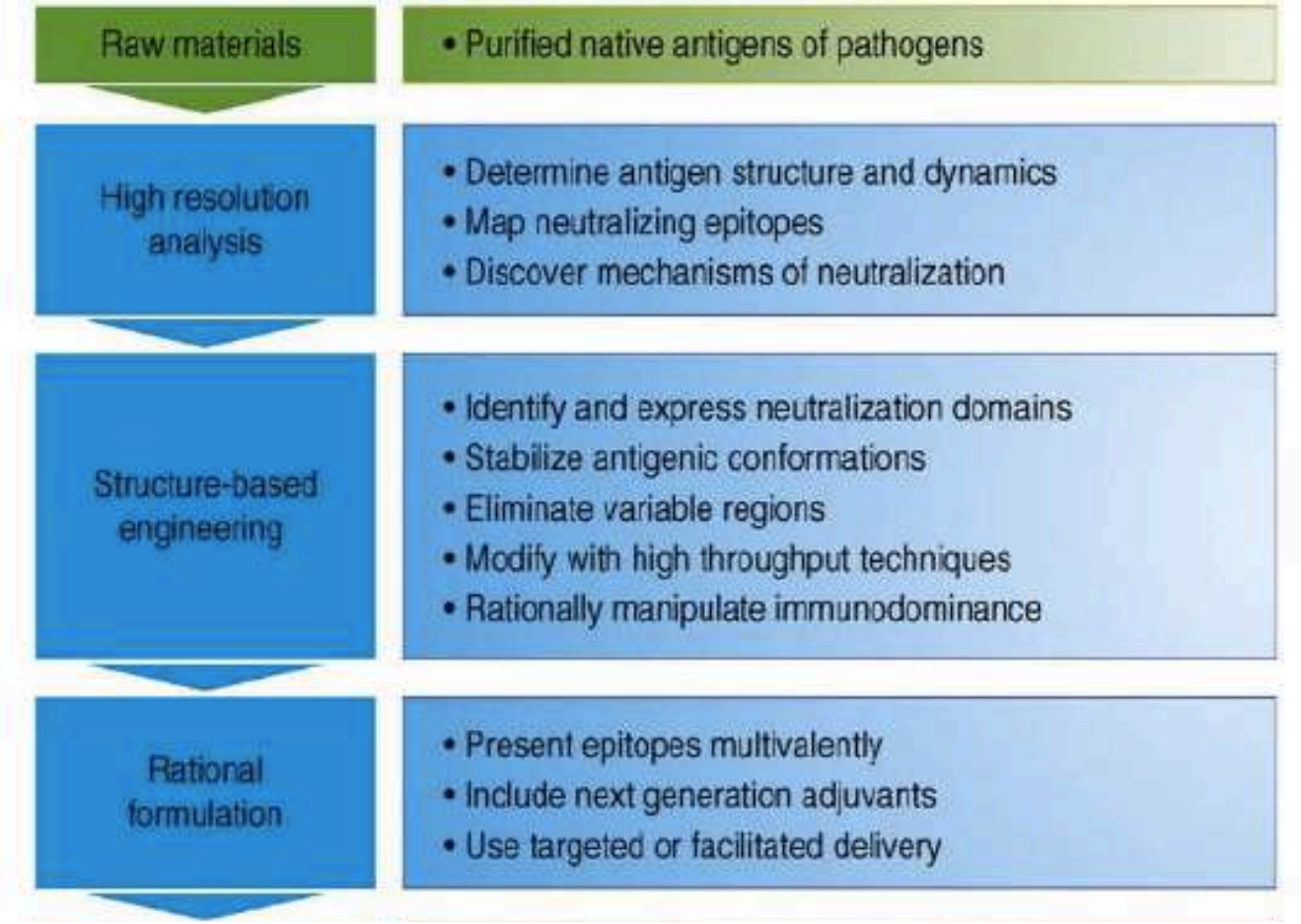


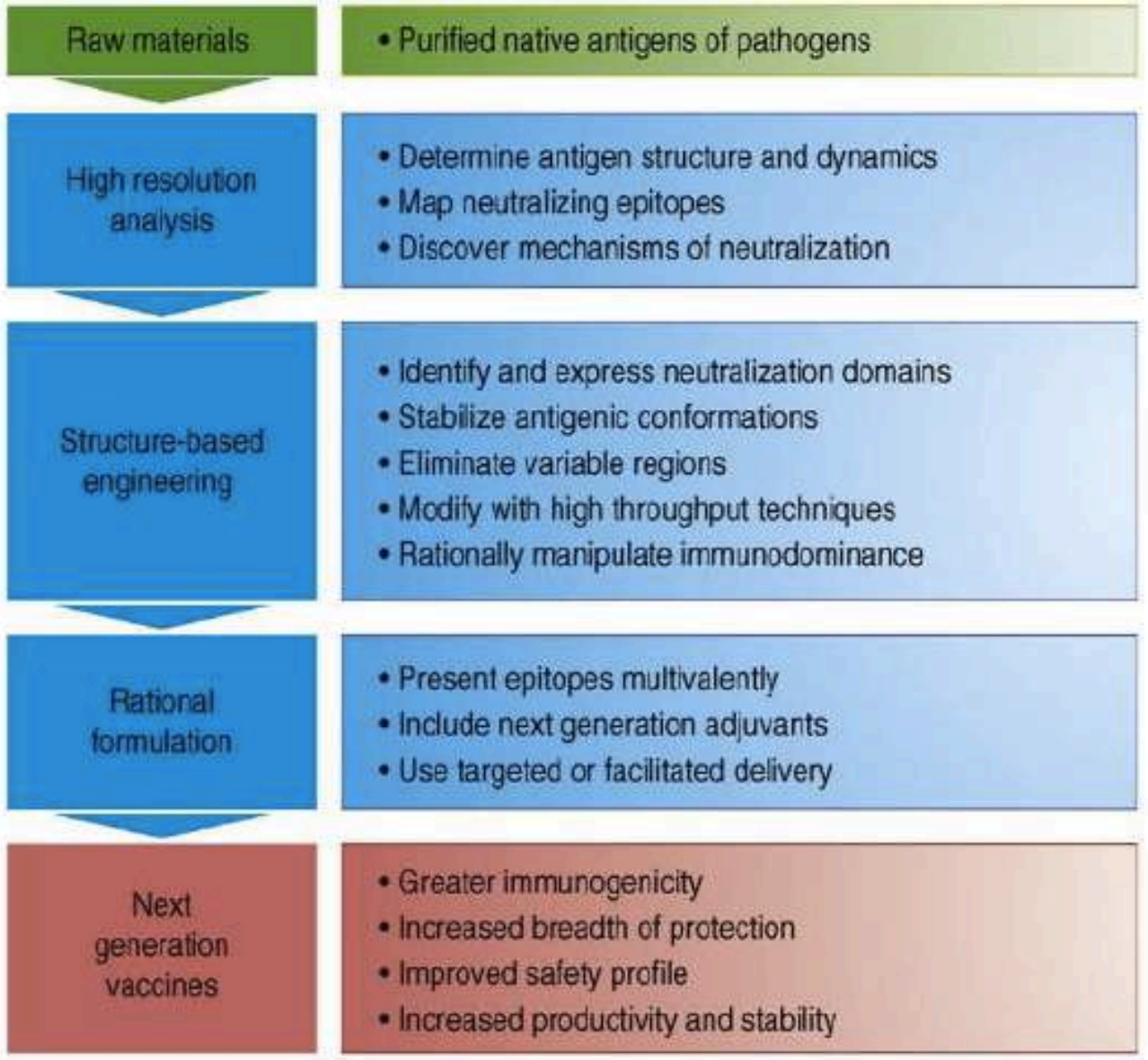
Etapas de la Vacunología Estructural



Etapas de la Vacunología Estructural



Etapas de la Vacunología Estructural



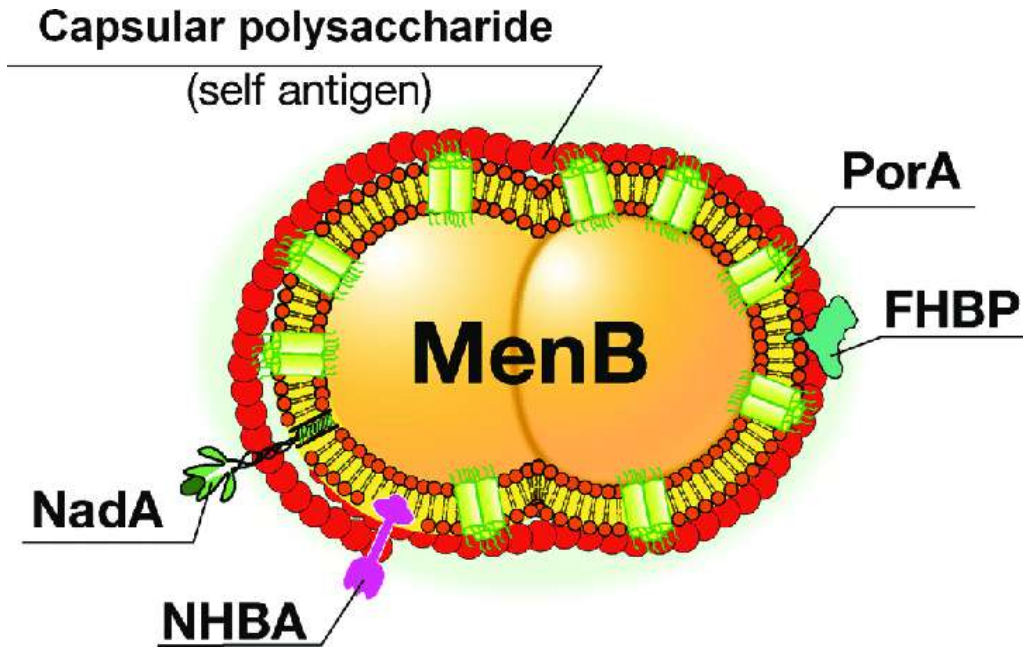
Gourlay L et al 2017. Trends in Biotechnology

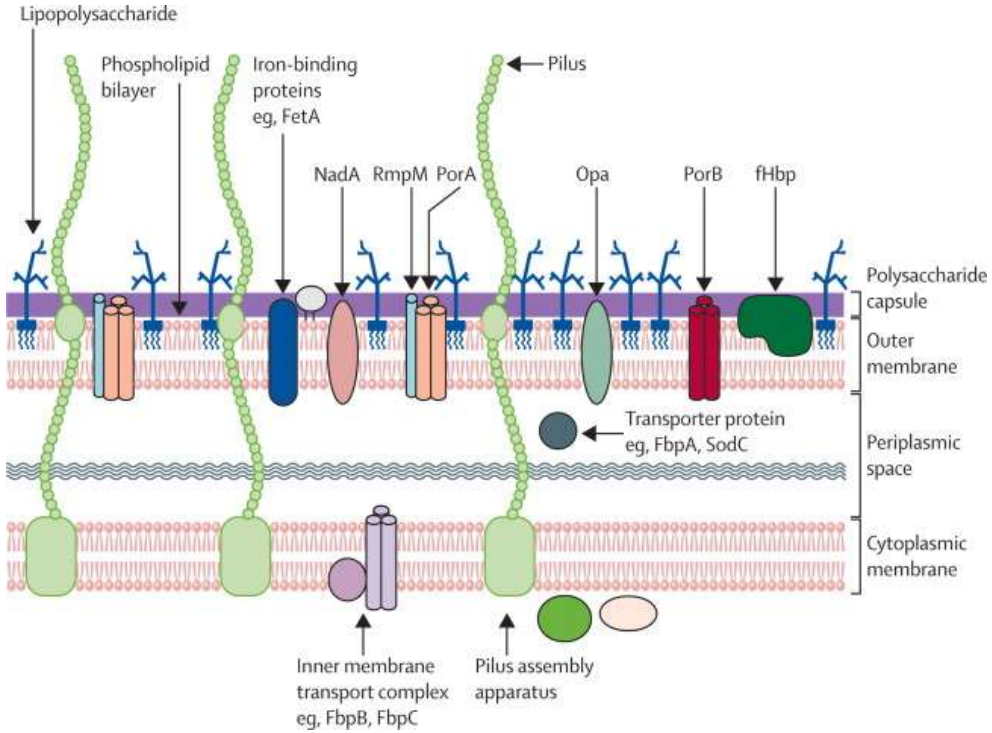
Diferentes estrategias en el diseño de Vacunas Estructurales

1. Broad-coverage immunogens
2. Epitope-focused immunogens
3. Germline-targeting immunogens

Broad-coverage immunogens

Vacunas que requieren conferir protección frente a varias cepas de un patógeno





Saradangani M & Pollard A. The Lancet Infectious Diseases 2010

Reverse Vaccinology

Agenomic approach to vaccine discovery

600 potential vaccine candidates identified

350 proteins successfully expressed in E.coli

91 novel surface-exposed proteins identified

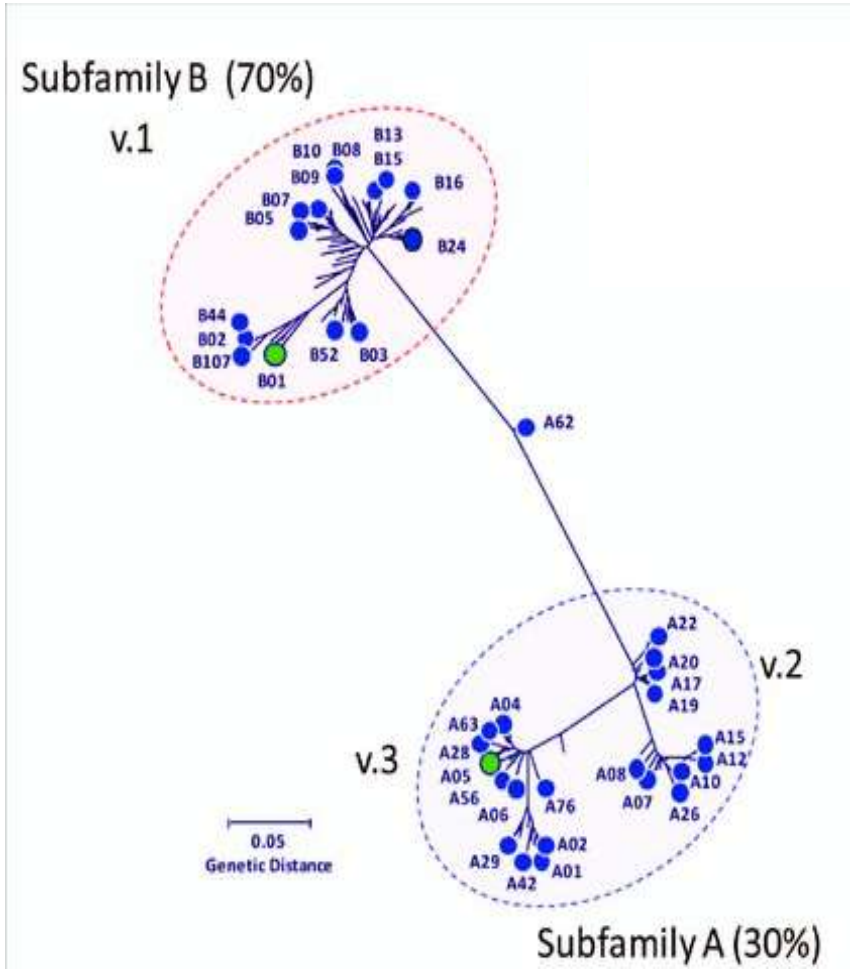
28 novel proteins have bactericidal activity

VACCINE CANDIDATES

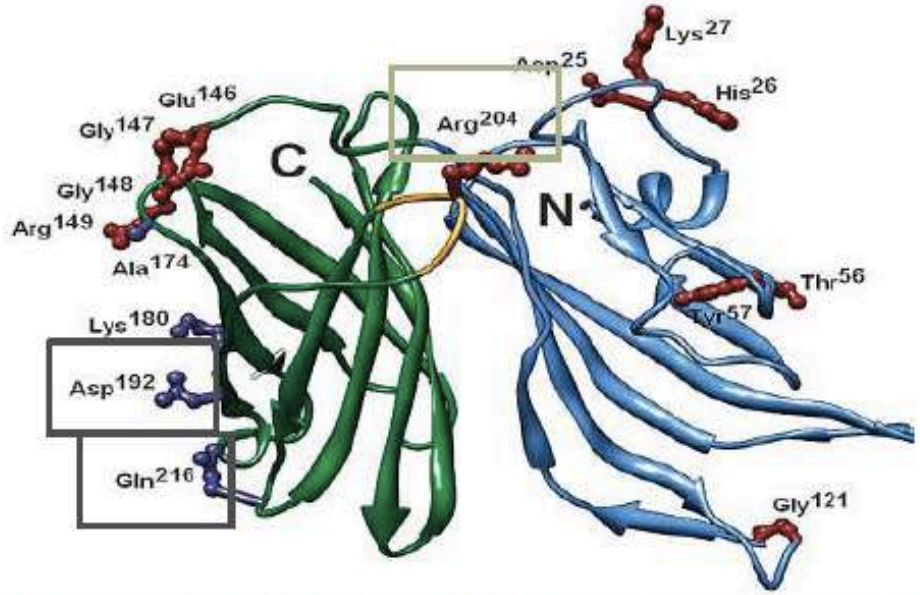
Express recombinant proteins

In silico vaccine candidates

Factor H Binding Protein (FHBP)



The protective epitopes of variant 1 and of variants 2 and 3 map in nonoverlapping regions located mostly in the amino- and carboxy-terminal regions of fHbp, respectively.



amino acids important for recognition by the antibodies against variant 1 colored red

amino acids important for recognition of variants 2 and 3 colored purple

Structural vaccinology starts to deliver

Philip R. Dormitzer, Guido Grandi and Rino Rappuoli

NATURE REVIEWS | MICROBIOLOGY
VOLUME 10 | DECEMBER 2012 | 807

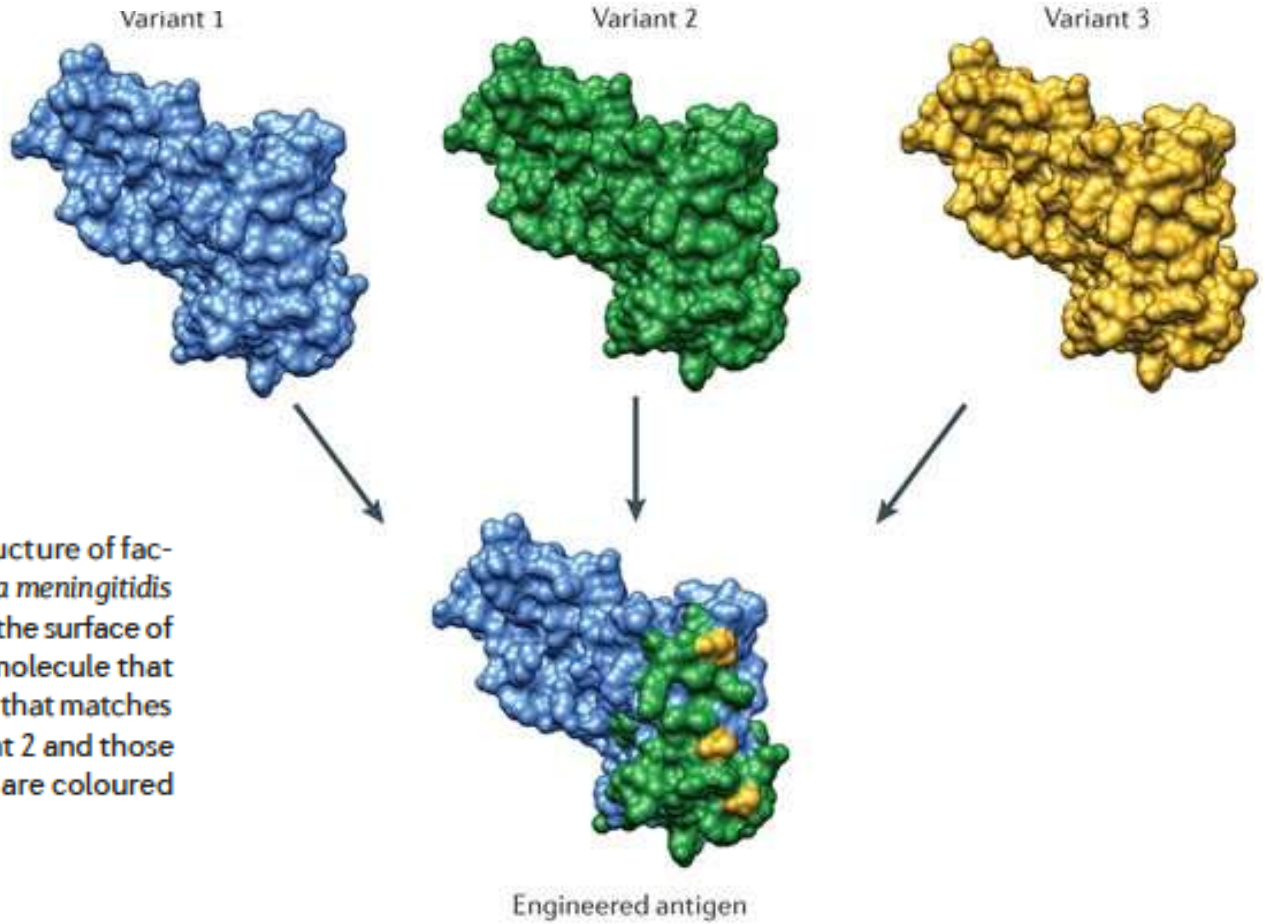


Figure 1 | **Rational design of a cross-protective factor H-binding protein.** The structure of factor H-binding protein variants 1, 2 and 3 from the serogroup B meningococcus (*Neisseria meningitidis* serogroup B), and the engineered antigen for the vaccine. A cluster of amino acids from the surface of variants 2 and 3 was engineered into the variant 1 structure to generate a chimeric molecule that elicits immunity against all three variants. In the antigen, the patch of the protein surface that matches variant 2 is coloured green (this patch includes both residues that are specific to variant 2 and those that are shared with variants 1 and 3), whereas residues that are specific to variant 3 are coloured yellow, and residues that are specific for variant 1 are coloured blue.

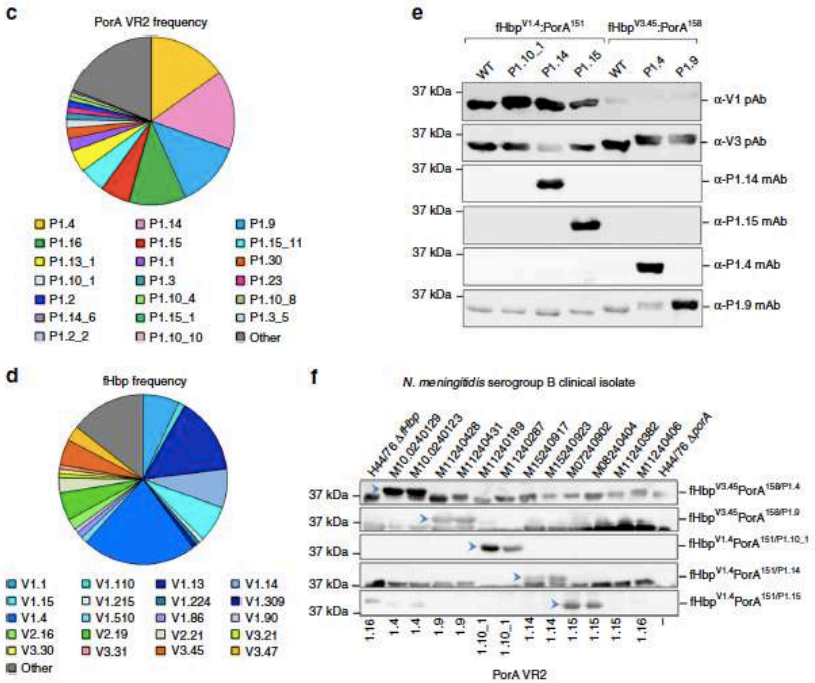
nature COMMUNICATIONS

ARTICLE

DOI: 10.1038/s41467-018-03146-7 OPEN

Structure-based design of chimeric antigens for multivalent protein vaccines

S. Hollingshead[1], I. Jongerius[1,2], R.M. Exley[1], S. Johnson[1], S.M. Lea[1] & C.M. Tang[1]



Frequency of PorA VR2 (**c**) and fHbp peptides (**d**) in *N. meningitidis* serogroup B strains ($n = 243$) isolated in 2016 in the UK.

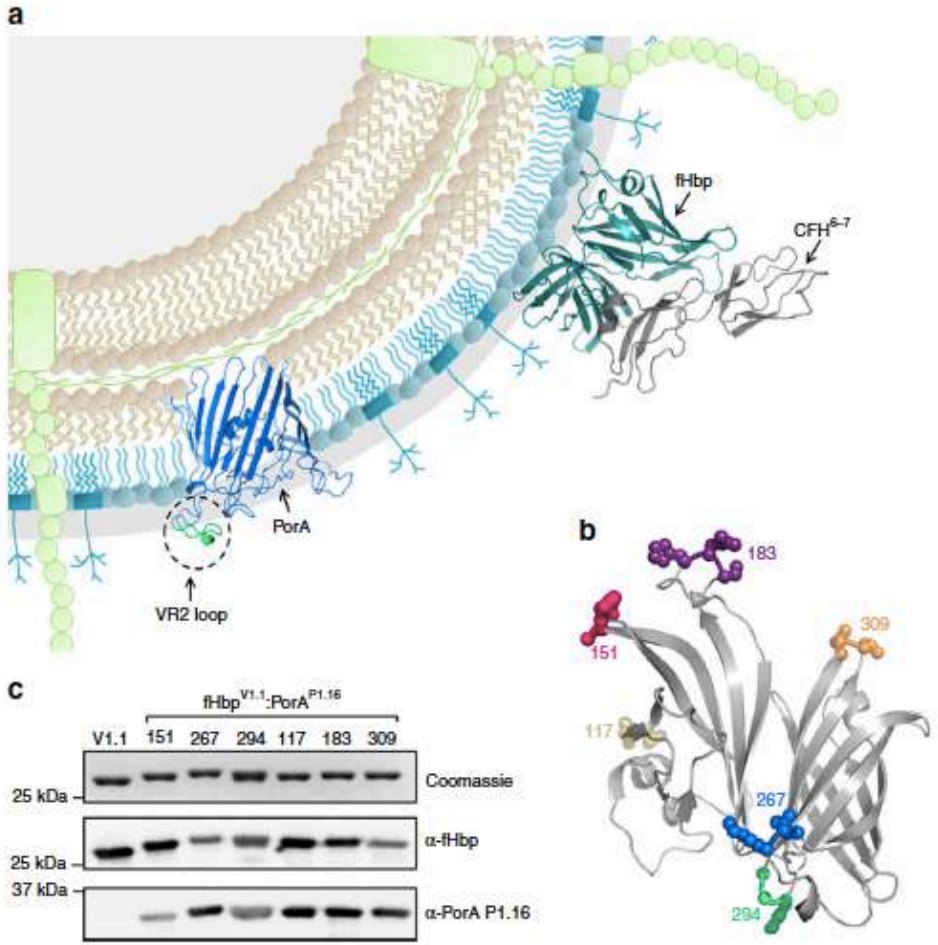


Fig. 1 Structure-based design of ChAs. **a** Schematic of meningococcal cell surface, depicting the key surface-exposed antigens, fHbp and PorA. **b** Location of fHbp residues replaced with VR2 P1.16. **c** Analysis of recombinant ChAs by SDS-PAGE and western blot. Immunoblots are probed with α-V1.1 fHbp pAb and α-PorA P1.16 mAb. Complete gel and western blots are shown in Supplementary Figure 5

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fHbp y PorA son antigénicamente variables

Mediante VE se generan Ag quiméricos formados por las formas mas prevalentes de fHbp/PorA circulantes

fHbp (V1.4, V2.19, V3.45) **PorA** (VR2 P1.4, P1.9, P1.14) cobertura de secuencia exacta frente al 57% de las cepas circulantes serogrupo B en UK 2016

fHbp and PorA are both antigenically variable. To improve vaccine coverage, we used the comprehensive epidemiology data available for UK meningococcal isolates[41] to generate ChAs composed of the most prevalent fHbp and PorA antigens. This maximises vaccine coverage as ChA composition mirrors the prevalent fHbp and PorA antigens circulating within a given geographical area. For example, a vaccine composed of the three most common fHbp peptides from each variant group (fHbp peptides V1.4, V2.19 and V3.45) with a single PorA VR2 insertion (PorA VR2 P1.4, P1.9 and P1.14) would give exact sequence coverage against 57% of serogroup B strains circulating in the UK in 2016; this compares favourably with currently licensed meningococcal serogroup B vaccines Bexsero (36%) and Trumenba® (4.8%)[33,34,41].

In summary, using structure-based design, we generated ChAs that retain epitopes of fHbp and PorA and generate immune responses against both antigens, demonstrating that a soluble antigen can be exploited as a scaffold to display epitopes from an integral membrane protein. Our work provides proof-in-principle for bacterial vaccine design employing structure-led protein engineering previously used in viral proteins to graft functional motifs onto unrelated protein scaffolds[52–55], or β-hairpin peptide mimetics[56,57] to develop novel conformationally restricted antigens.

Epitope-focused immunogens

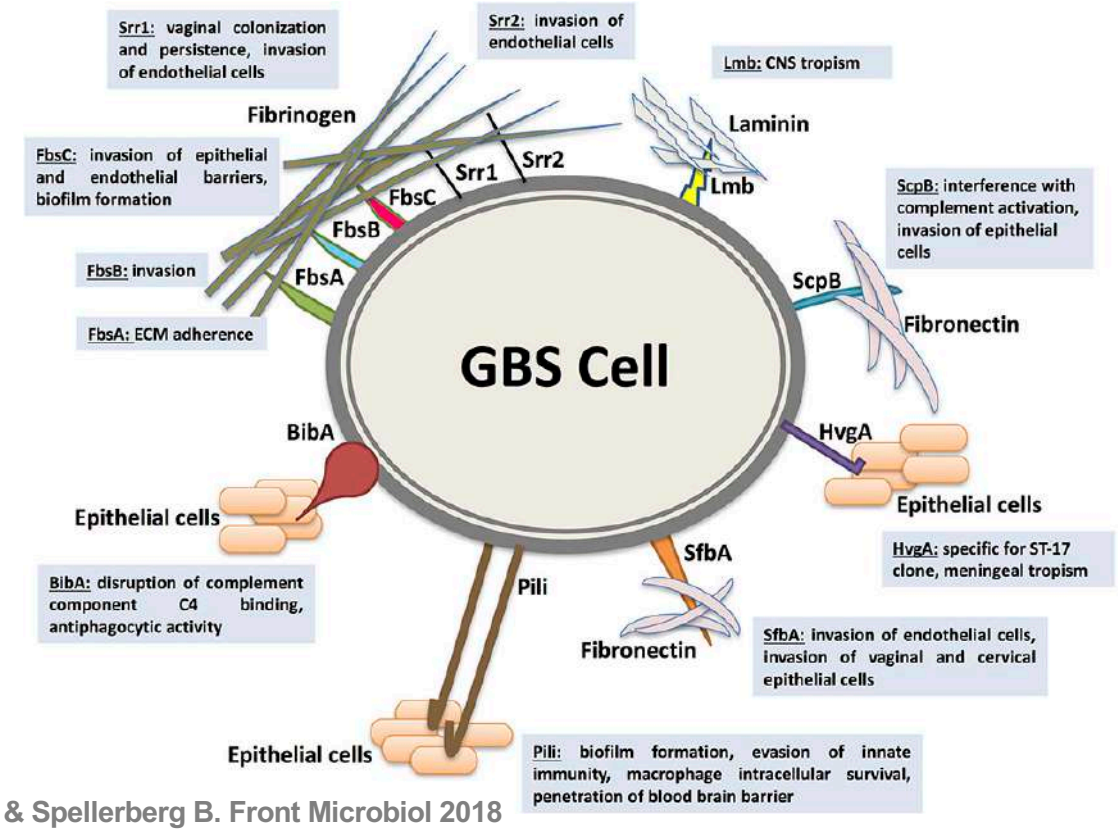
Minimización del Antígeno: centrar la respuesta inmune en **epítopos protectores** evitando otros epítopos no-deseables

<u>Estreptococo Grupo B (GBS)</u>

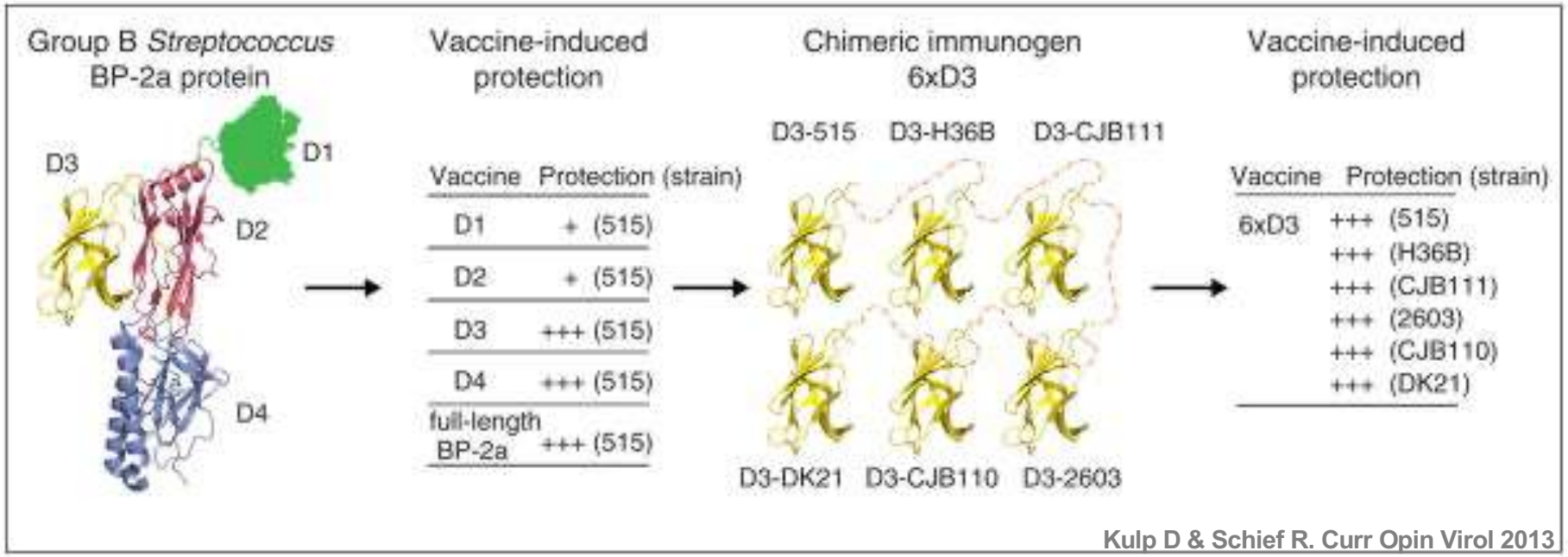
10 Serotipos diferentes

Todos los serotipos expresan Pili (Factor de virulencia/Antígeno Protector)

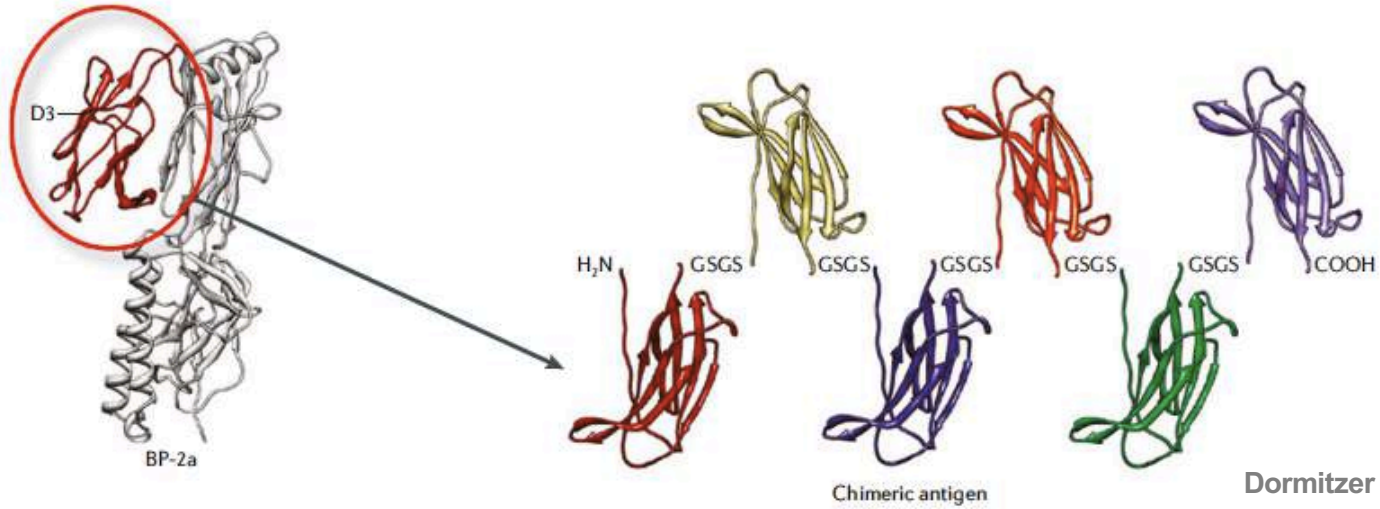
BP-2a: seis variantes antigénicas



Shabayek S & Spellerberg B. Front Microbiol 2018



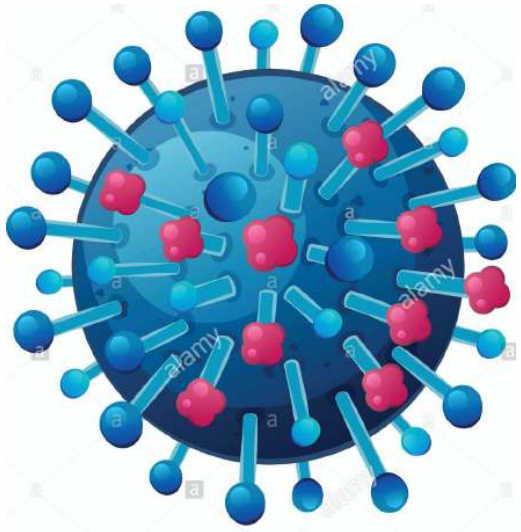
Kulp D & Schief R. Curr Opin Virol 2013



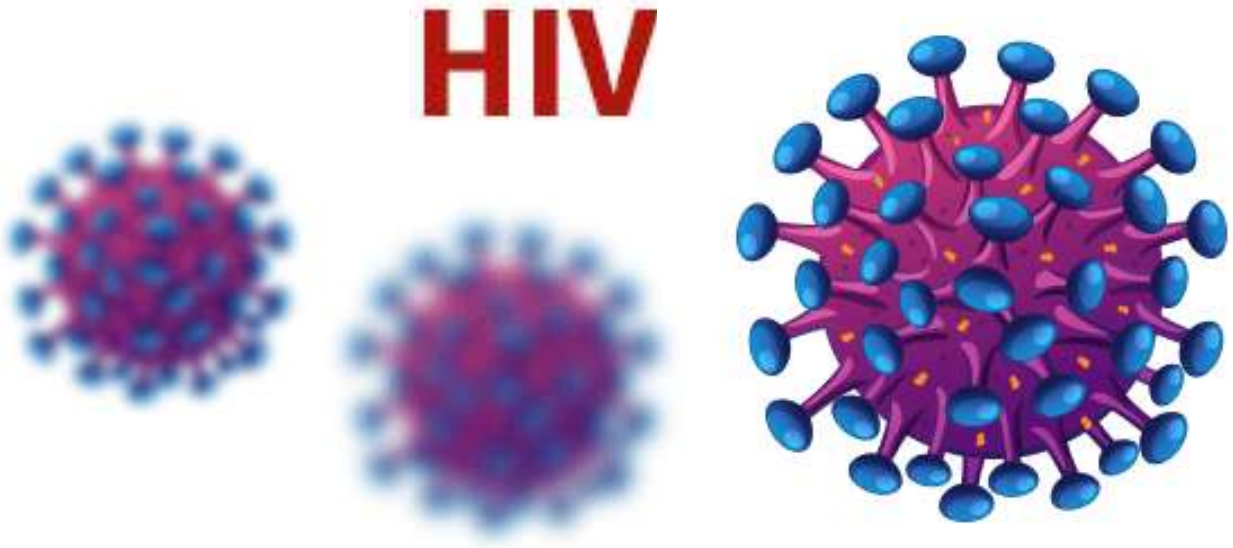
Dormitzer PR et al. Nat Reviews Microbiol 2012

Germline-targeting immunogens

Virus como el **VIH**, **Influenza** o **Hepatitis-C** suponen un reto para el diseño vacunal debido a que sus proteínas de superficie son altamente variables y están recubiertas por glicanos

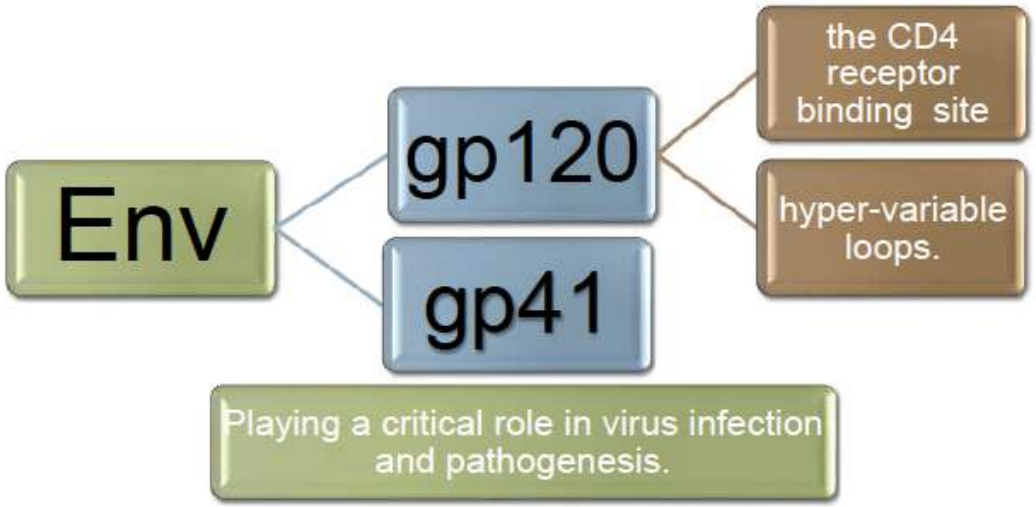


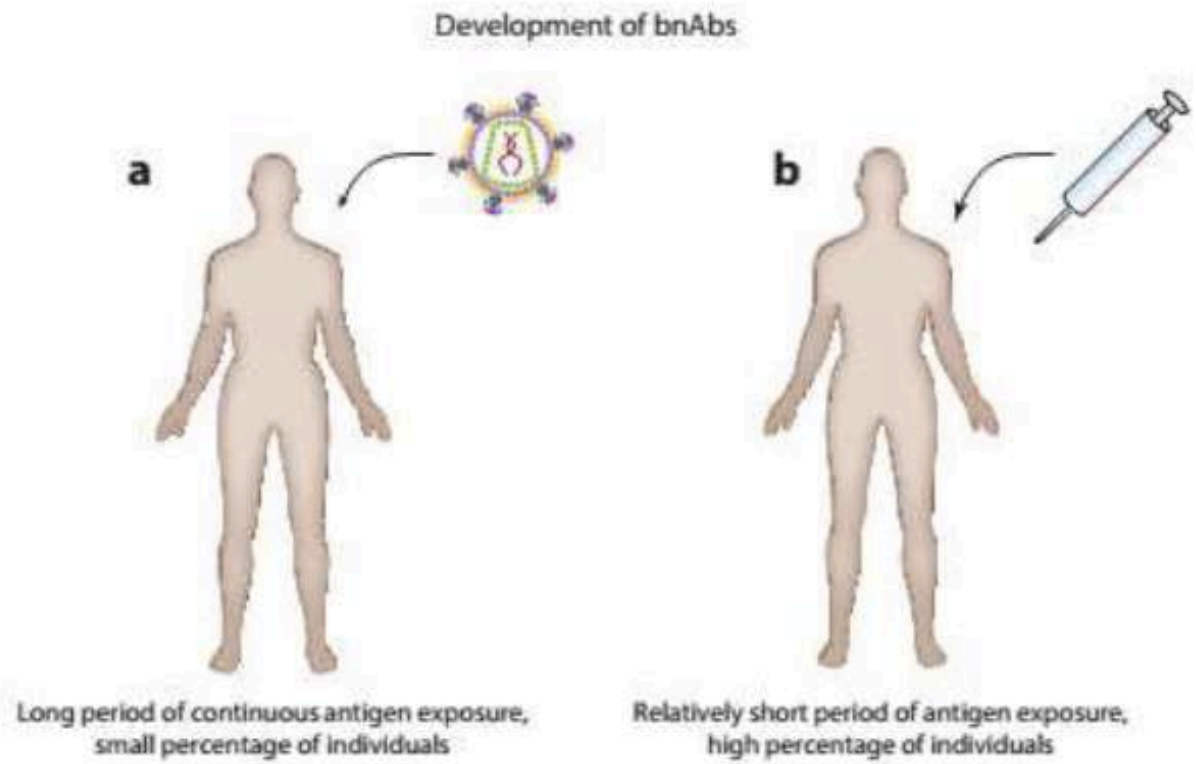
Influenza



El descubrimiento de la existencia de **Anticuerpos Neutralizantes de amplia cobertura** (broadly neutralizing antibodies or **bNAbs**) dio lugar a una nueva estrategia de diseño de inmunógenos vacunales

Broadly neutralizing antibodies (bnAbs), i.e., antibodies capable of neutralizing the majority of strains of a given highly antigenically variable pathogen, have attracted considerable attention





These bNAbs are highly mutated from germline, and have been produced by HIV-infected individuals only after two to three years of infection.

Hence it is expected that elicitation of similar bNAbs by vaccination will be very difficult and may require a lengthy and complex immunization regimen.

Rational HIV Immunogen Design to Target Specific Germline B Cell Receptors

Joseph Jardine,[1,2,3,4]* Jean-Philippe Julien,[2,3,5]* Sergey Menis,[1,2,3,4]* Takayuki Ota,[1] Oleksandr Kalyuzhniy,[1,2,3,4] Andrew McGuire,[6] Devin Sok,[1,2,3] Po-Ssu Huang,[4] Skye MacPherson,[1,2,3,4] Meaghan Jones,[1,2,4] Travis Nieusma,[2,3,5] John Mathison,[1] David Baker,[4] Andrew B. Ward,[2,3,5] Dennis R. Burton,[1,2,3,7] Leonidas Stamatatos,[6,8] David Nemazee,[1] Ian A. Wilson,[2,3,5,9] William R. Schief[1,2,3,4]†

www.sciencemag.org SCIENCE VOL 340 10 MAY 2013

Desarrollo de un inmunógeno del VIH a partir de los **bNAbs** dirigido a estimular la población de células B germinales

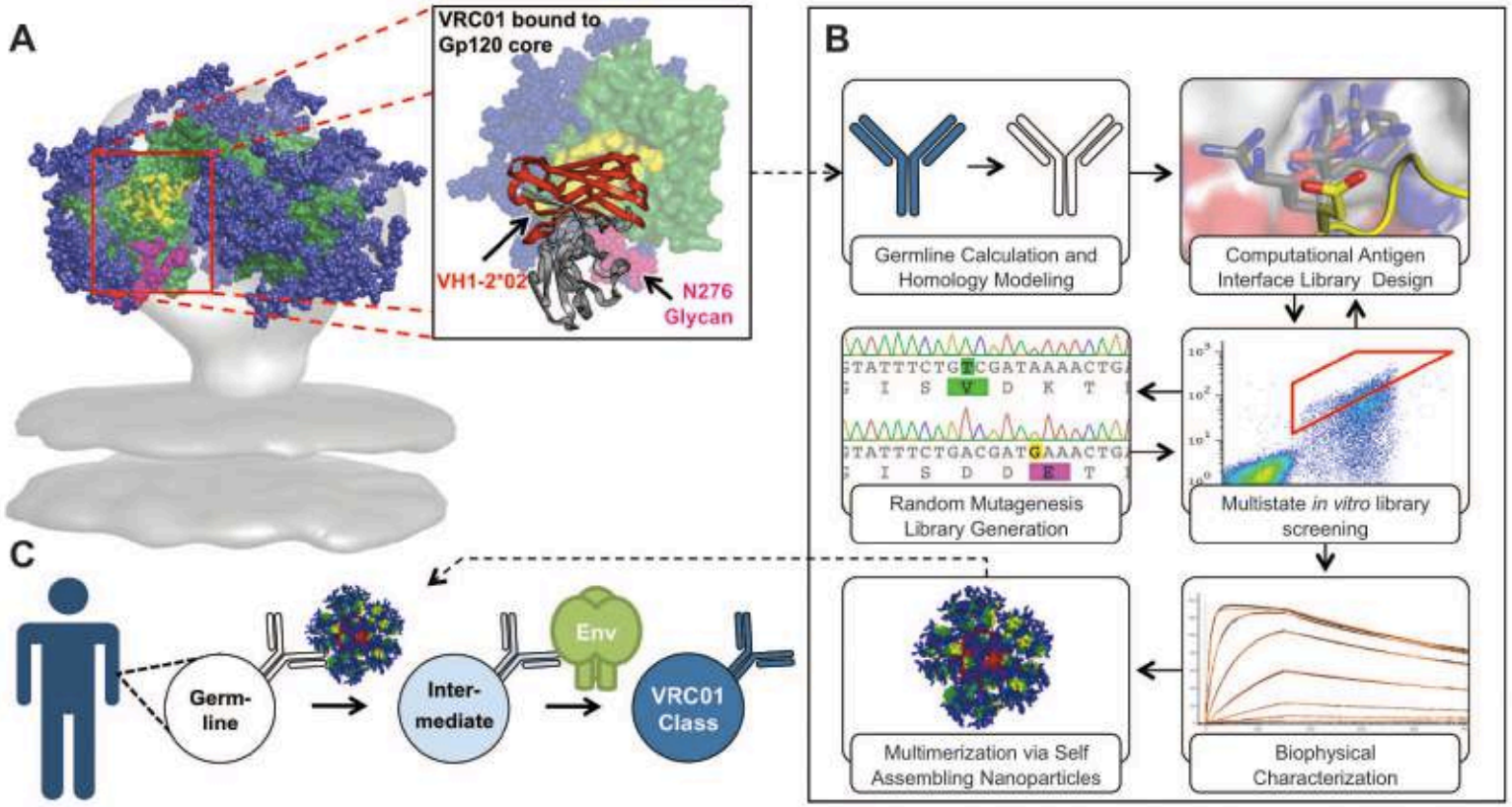


Fig. 1. Development of a germline (GL)–targeted HIV immunogen. (A) VRC01-class bNAbs bind to gp120 primarily through paratope residues encoded by VH 1-2*02. gp120 is colored green, with the CD4 binding site highlighted in yellow. Glycans are represented as blue spheres with the critical N276 highlighted in magenta. VRC01 is shown as a secondary structure rendering and colored gray, with the VH1-2*02 region highlighted in red. **(B)** Steps in the engineering of a modified gp120-based nanoparticle capable of activating GL VRC01-class B cells. **(C)** This nanoparticle can be used in an HIV-1 vaccine GL-prime-boost strategy to bridge this initial recognition gap and initiate clonal expansion and start somatic hypermutation of VRC01-class bNAbs precursors.

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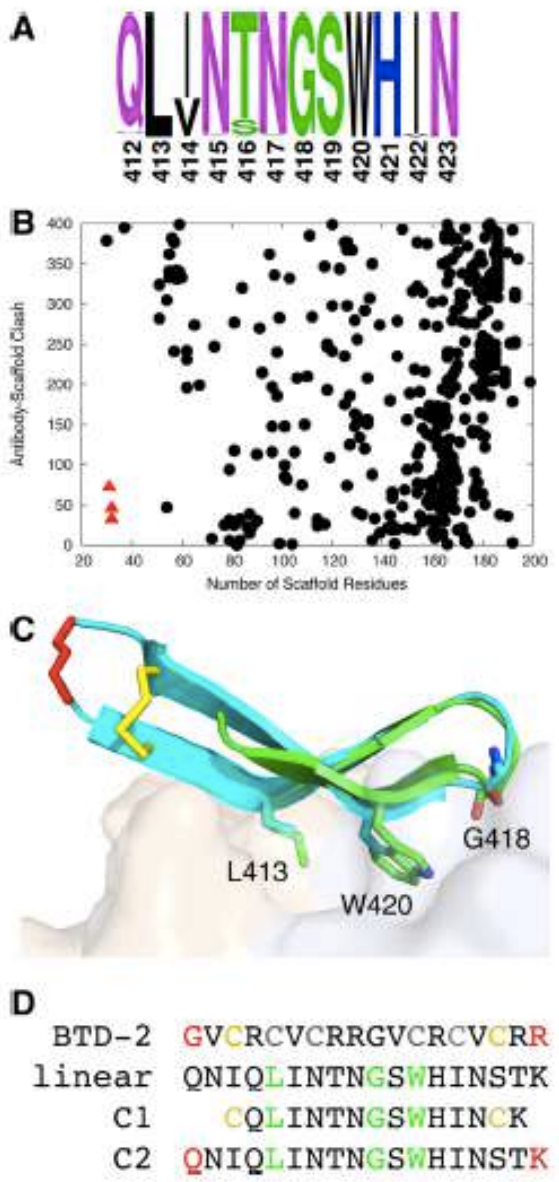
AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology®

Structure-Based Design of Hepatitis C Virus Vaccines That Elicit Neutralizing Antibody Responses to a Conserved Epitope

October 2017 Volume 91 Issue 20 e01032-17

Brian G. Pierce,[a,b] Elisabeth N. Boucher,[c*] Kurt H. Piepenbrink,[d*] Monir Ejemel,[c] Chelsea A. Rapp,[d] William D. Thomas, Jr.,[c*] Eric J. Sundberg,[d,e] Zhiping Weng,[a] Yang Wang[c]

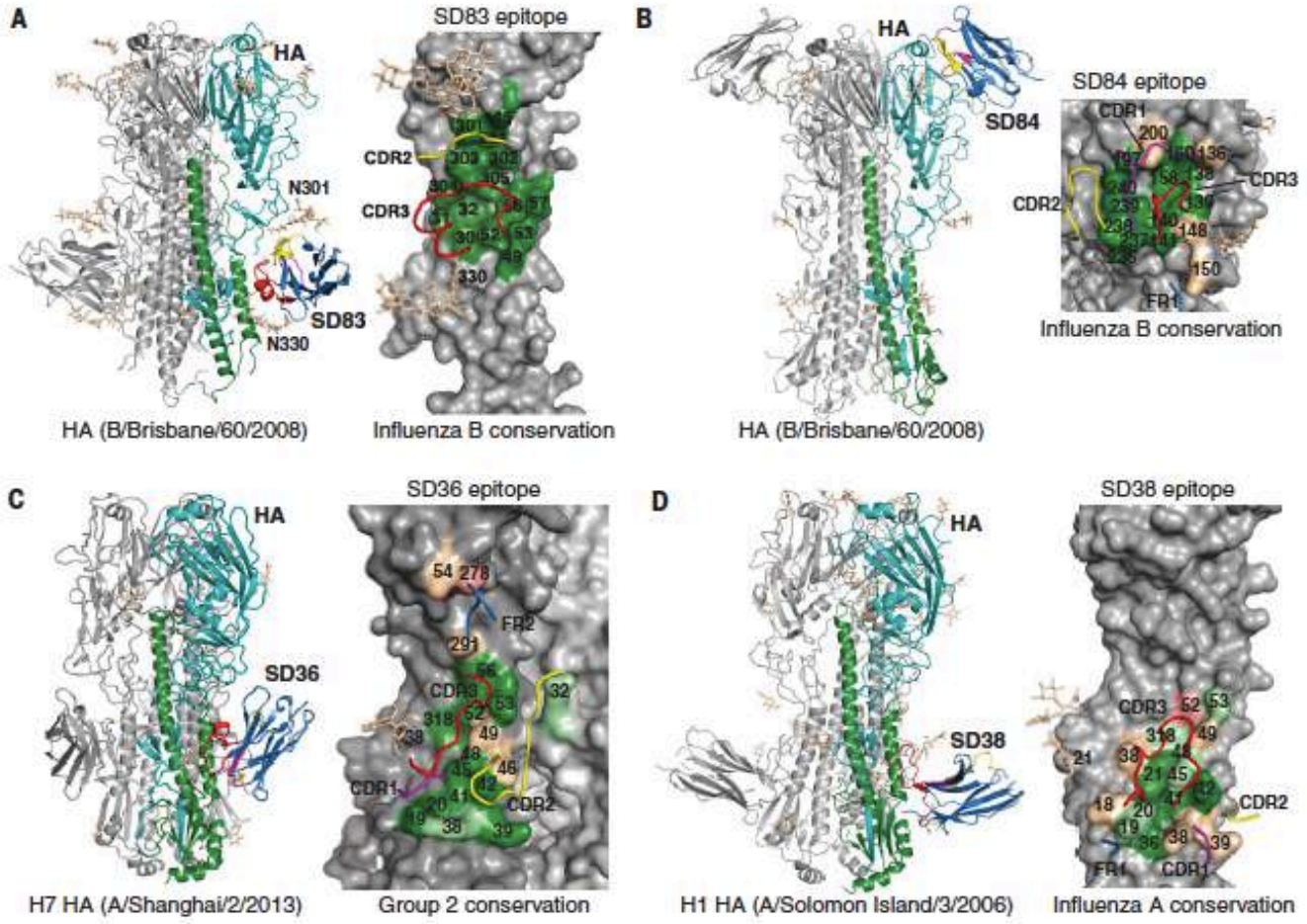
Diseño de inmunógenos vacunales frente al VHC, basándose en la estructura de un epítopo conservado diana de anticuerpos neutralizantes de amplia cobertura



ANTI-FLU THERAPY *Science* 362, 595–602 (2018) 2 November 2018

Universal protection against influenza infection by a multidomain antibody to influenza hemagglutinin

Nick S. Laursen[1*], Robert H. E. Friesen[2]†, Xueyong Zhu[1], Mandy Jongeneelen[3], Sven Blokland[3], Jan Vermond[4], Alida van Eijgen[4], Chan Tang[3], Harry van Diepen[4], Galina Obmolova[2], Marijn van der Neut Kolfschoten[3], David Zuijdgeest[3], Roel Straetemans[5], Ryan M. B. Hoffman[1], Travis Nieusma[1], Jesper Pallesen[1], Hannah L. Turner[1], Steffen M. Bernard[1], Andrew B. Ward[1], Jinquan Luo[2], Leo L. M. Poon[6], Anna P. Tretiakova[7]‡, James M. Wilson[7], Maria P. Limberis[7], Ronald Vogels[3], Boerries Brandenburg[3], Joost A. Kolkman[8]§, Ian A. Wilson[1,9]§



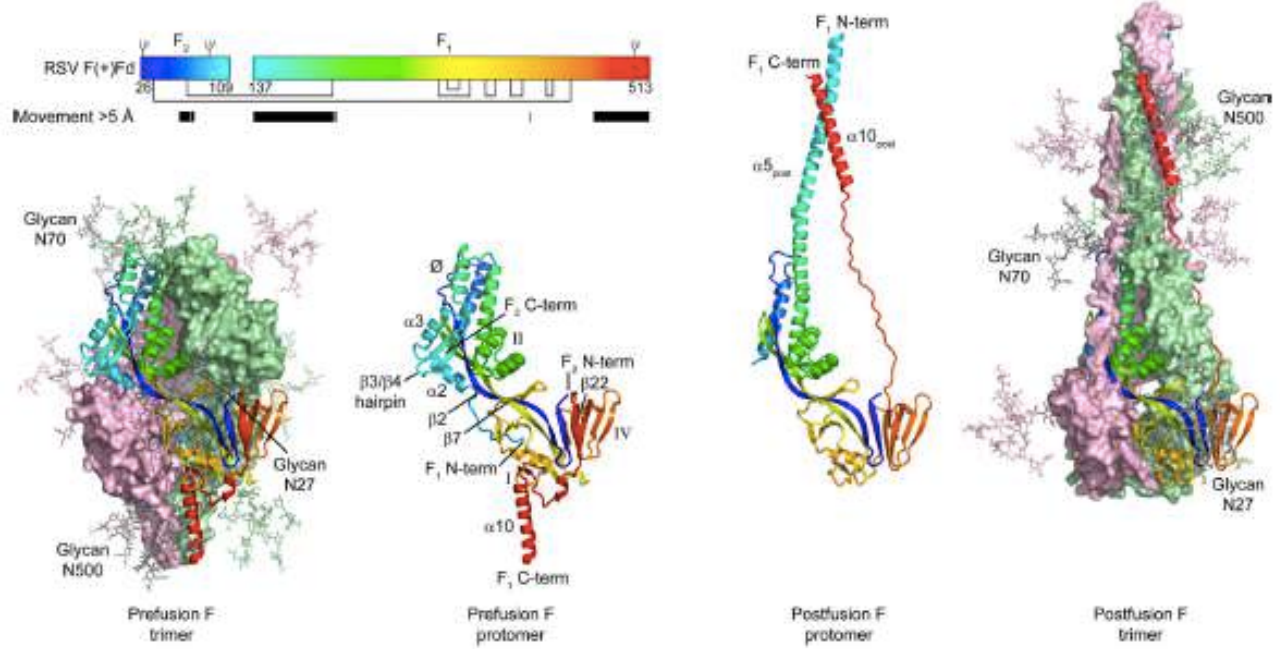
Diseño de Anticuerpo multi-dominio cuya diana son múltiples epítopos del tallo de la Hemaglutinina de virus Influenza A y B

Science. 2013 May 31; 340(6136): 1113–1117. doi:10.1126/science.1234914.

Structure of RSV Fusion Glycoprotein Trimer Bound to a Prefusion-Specific Neutralizing Antibody

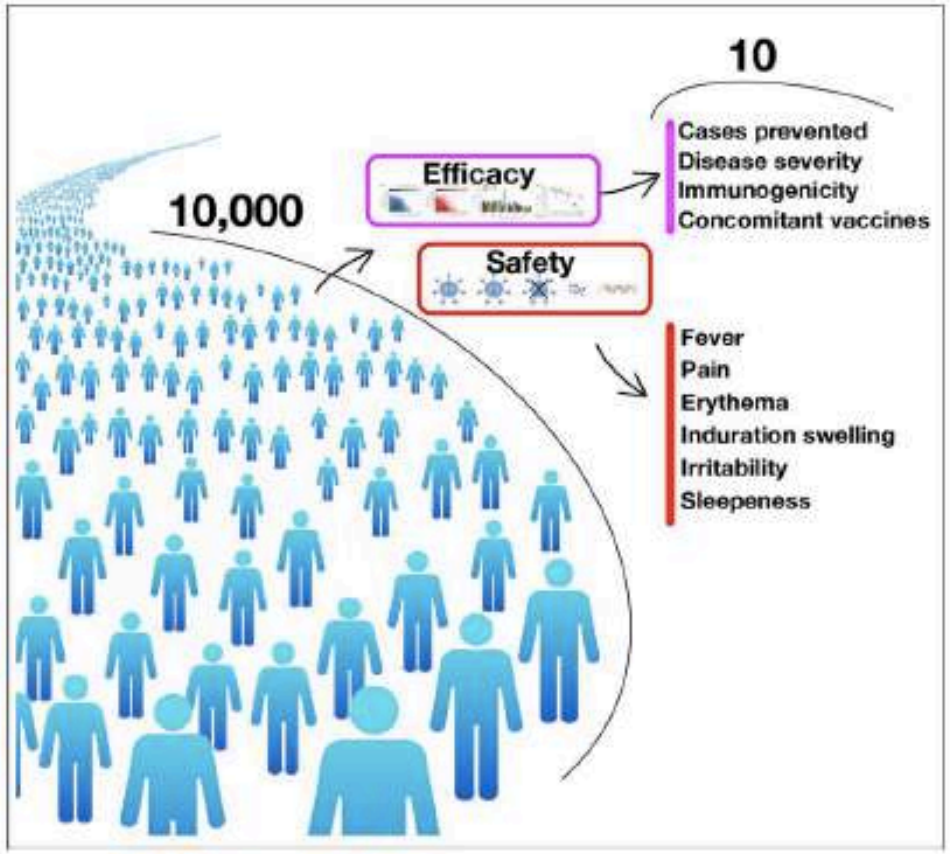
Jason S. McLellan[1,*], **Man Chen**[1], **Sherman Leung**[1], **Kevin W. Graepel**[1], **Xiulian Du**[1], **Yongping Yang**[1], **Tongqing Zhou**[1], **Ulrich Baxa**[2], **Etsuko Yasuda**[3], **Tim Beaumont**[3], **Azad Kumar**[1], **Kayvon Modjarrad**[1], **Zizheng Zheng**[4], **Min Zhao**[4], **Ningshao Xia**[4], **Peter D. Kwong**[1,*], and **Barney S. Graham**[1]

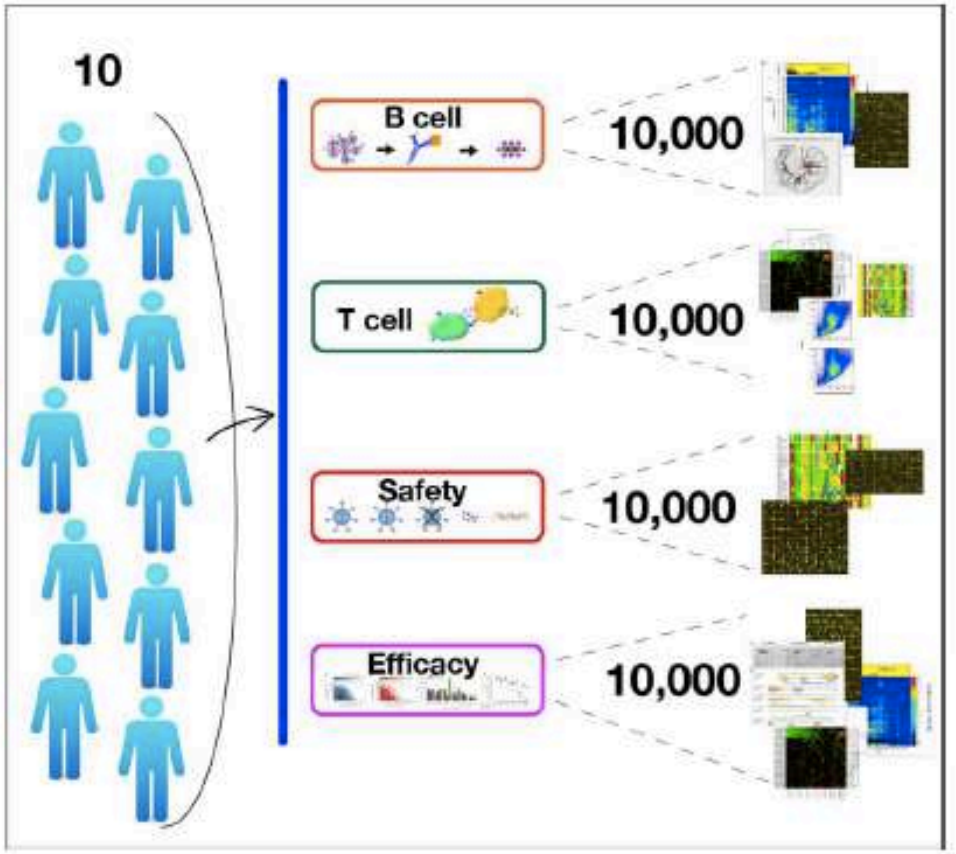
Identificaron Ac dirigidos a la proteína de pre-fusión F del VRS, que permiten el diseño de antígenos vacunales mejorados frente al VRS.



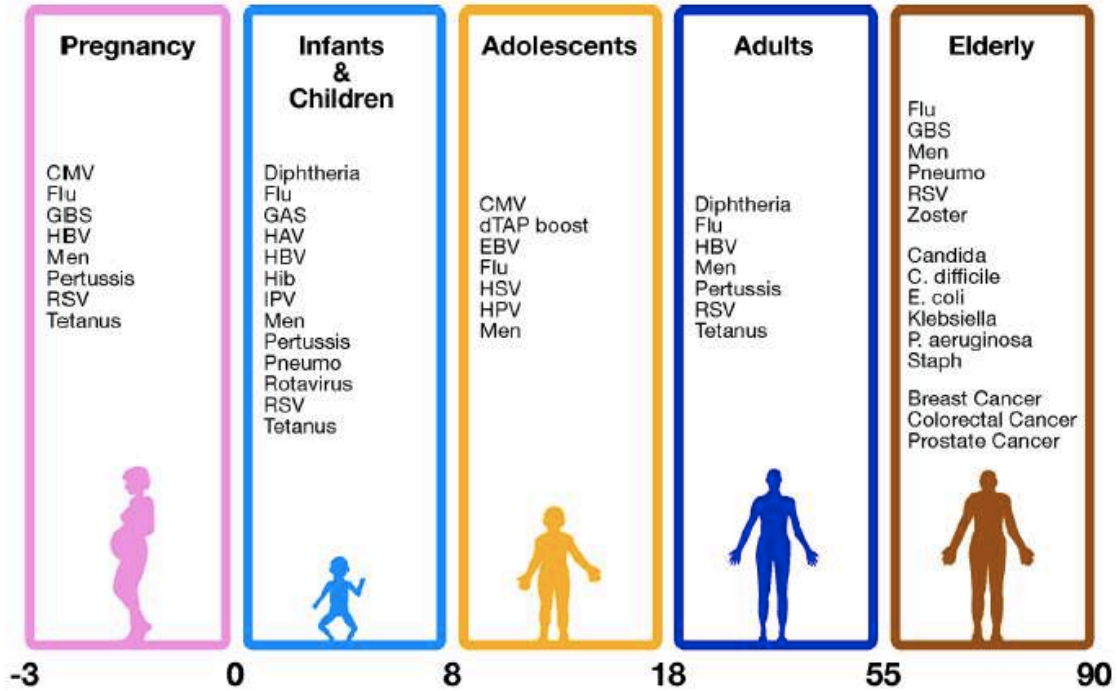
Systems biology

From 10,000 people with 10 data each
to
10 people with 10,000 data each

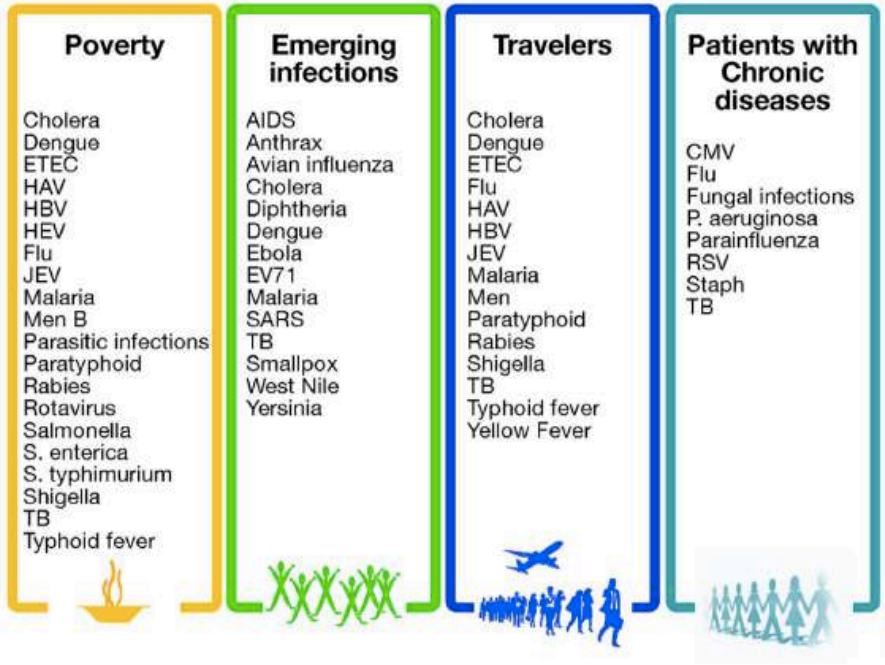




Vaccines for every age



Vaccines for today's society



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Nature Reviews Immunology | November 2011; doi:10.1038/nri3085

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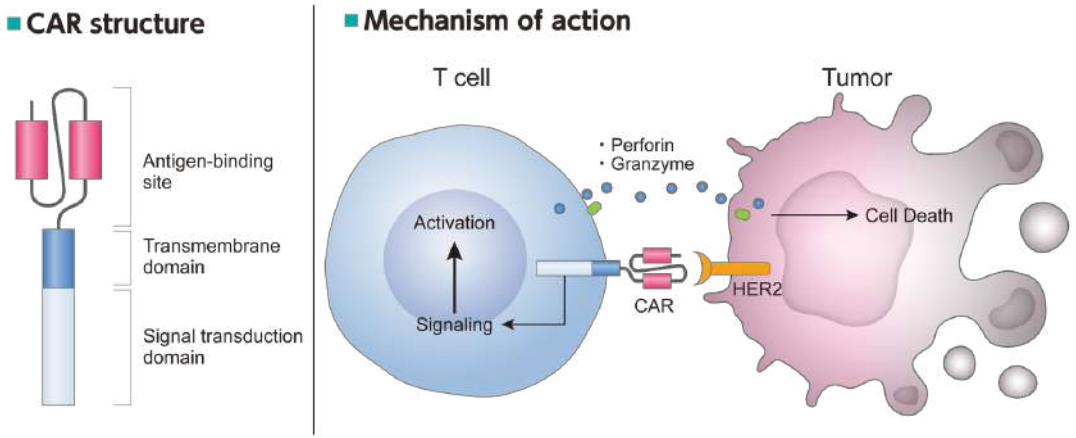
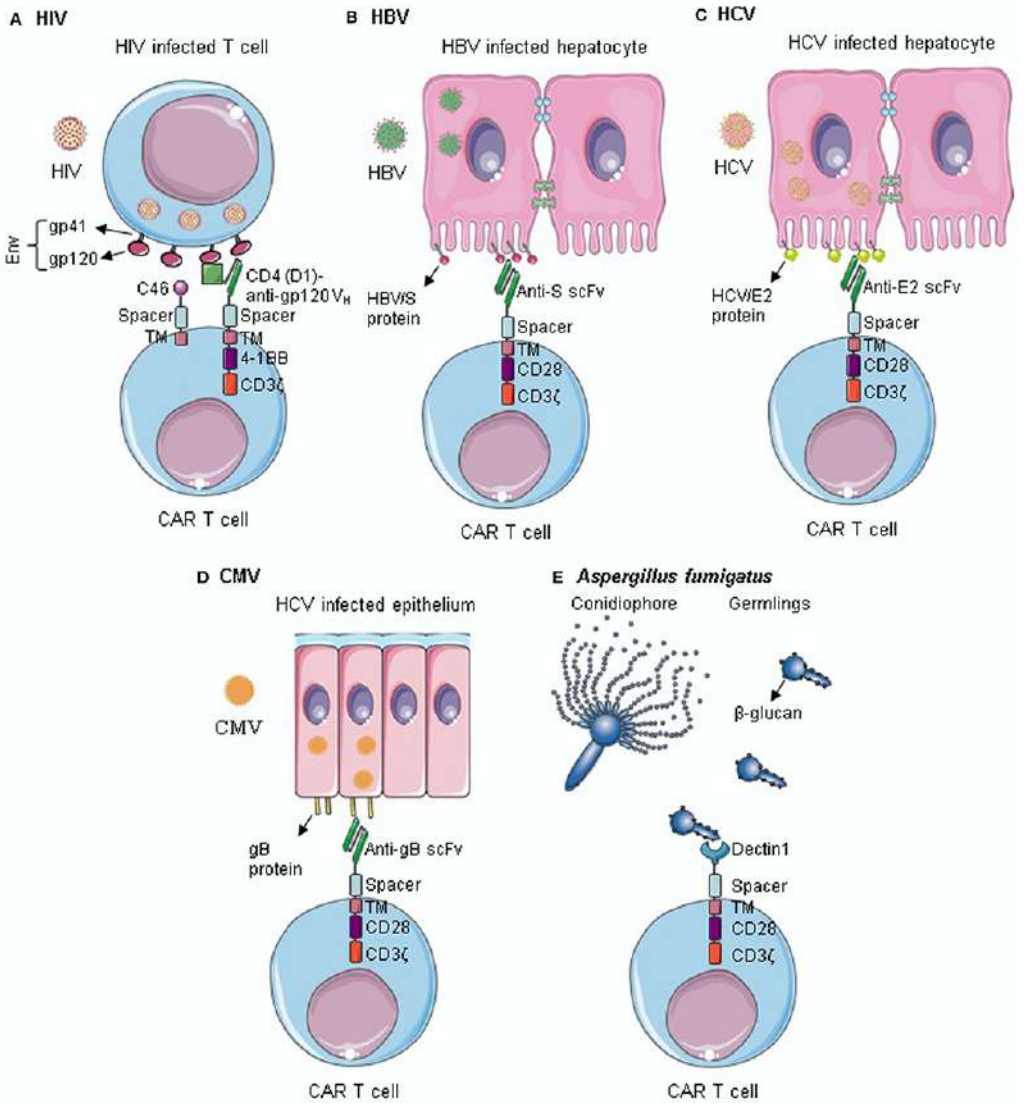
CAR T Cells Beyond Cancer: Hope for Immunomodulatory Therapy of Infectious Diseases

MINI REVIEW
published: 21 November 2019
doi: 10.3389/fimmu.2019.02711

*Michelle Seif, Hermann Einsele and Jürgen Löffler**

CHIMERIC ANTIGEN RECEPTOR (CAR) – T CELLS





Flattening the COVID-19 Curve With Natural Killer Cell Based Immunotherapies

REVIEW
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Marisa Market[1,2†], Leonard Angka[1,2†], Andre B. Martel[1,2,3], Donald Bastin[4], Oladunni Olanubi[1,2], Gayashan Tennakoon[1], Dominique M. Boucher[2], Juliana Ng[1], Michele Ardolino[1,2,5†] and Rebecca C. Auer[1,2,3*†]*

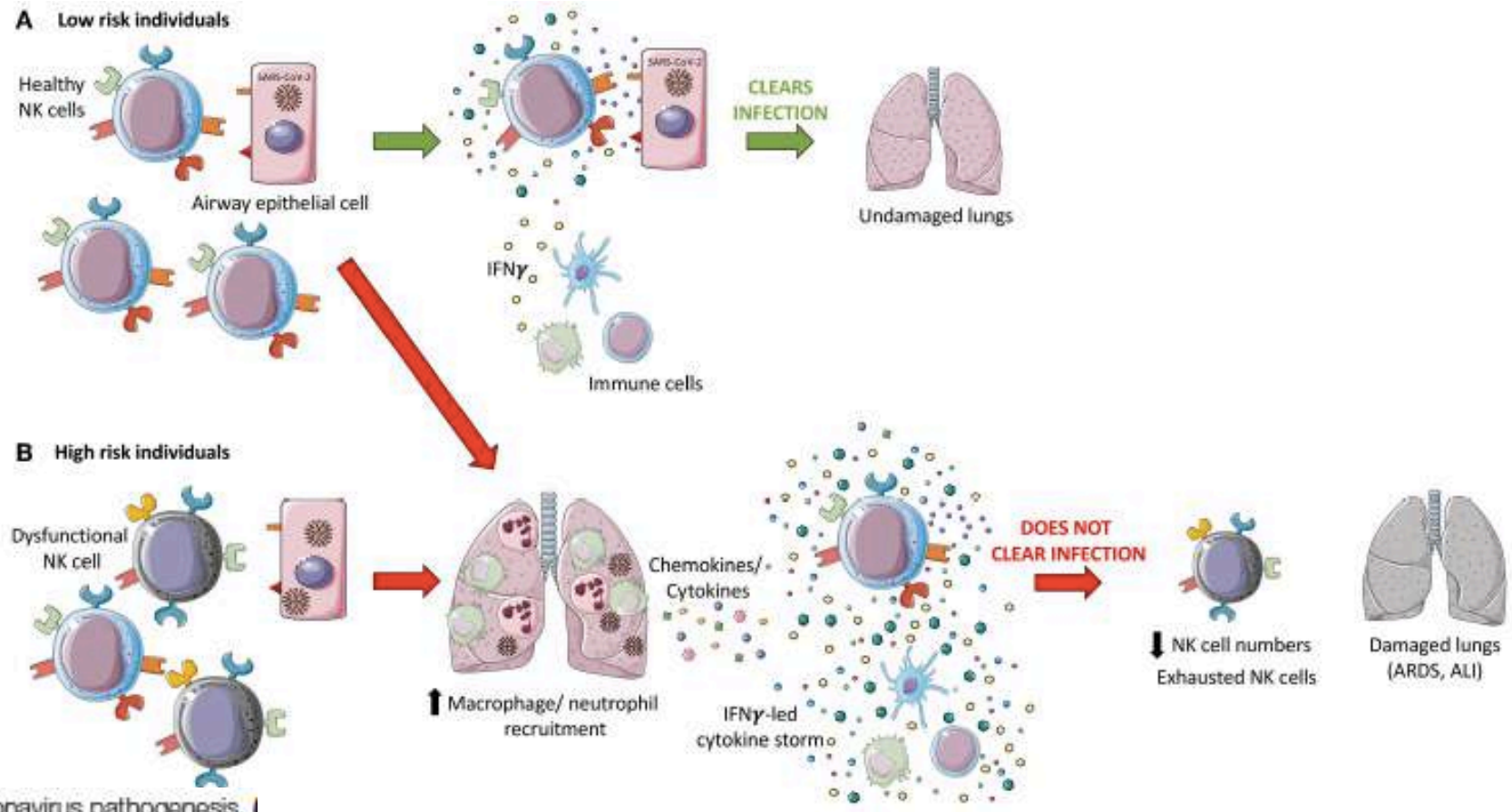

FIGURE 1 | Hypothesized dual role of NK cells during coronavirus pathogenesis. (

CONCLUSIONES

- La combinación de la **Biología Estructural** y la **Vacunología Reversa** ha permitido evolucionar hacia el desarrollo de la **VACUNOLOGÍA ESTRUCTURAL**

- Principal **Ventaja**: uso de la información obtenida a nivel atómico se emplea para el diseño racional de antígenos, reduciendo de forma considerable la posibilidad de fracaso en los ensayos clínicos, pudiendo centrar los esfuerzos en los candidatos óptimos y reduciendo los tiempos de desarrollo

- La VE tendrá un importante papel en el desarrollo de las futuras vacunas, sobre todo en aquellas hasta la fecha han fracasado

- Estas Vacunas Estructurales con reducida complejidad y amplia eficacia pueden beneficiar a un numero mayor de individuos de la población