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Human monoclonal antibodies for discovery, therapy, and vaccine acceleration

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Screening of single B cells from convalescent or vaccinated people allows the discovery of novel targets for infectious diseases and rapid production of engineered human monoclonal antibodies (mAbs) that can prevent or control infections by passive immunization. Here we propose that the development of human mAbs can also significantly accelerate vaccine development by anticipating some of the key biological and regulatory questions.

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Passive and active immunization

Since the discovery of 'the serum' against diphtheria by von Behring in 1890 [1], passive protection via serum therapy and active protection via vaccines have been used in parallel to fight infectious diseases. In the early days, development of both solutions went hand in hand, often by the same manufacturers. Indeed, our GSK vaccine sites in Siena (Italy) and in Marburg (Germany) were independently started in 1904 to produce sera against anthrax and diphtheria respectively, and most of the institutes born in this period used to be named 'serum and vaccine institutes'. During the second part of the last century, the use of serum therapy declined over time due to tolerability issues and the triumphal advent of antibiotics. Today passive immunization is limited mainly to post exposure prophylaxis for infections such as rabies, tetanus or hepatitis B [2].

Vaccination, to the contrary, has become the primary approach for infectious disease prevention and eradication. Vaccines – providing broad and long-lasting protection – represents one of the greatest success stories of healthcare. Over the decades they prevented millions of deaths and debilitating sequelae of infectious diseases, and they keep on doing so [3•]. However, vaccines – like any other healthcare intervention – have some important limitations, as the slow onset of action (weeks to months), the suboptimal effect in some populations – such as neonates, elderly or immunocompromised – or even the risk of disease enhancement in some infections such as Dengue or RSV [4,5].

Constant innovation in the field of vaccination has partially filled these gaps. Adjuvanted vaccines help to boost immune responses in the elderly [6,7], and maternal immunization has the potential to prevent diseases in neonates [8,9]. However, the single most notable limitation of vaccines, which is unlikely to be overcome in the next decades, is the time required by a vaccine to stimulate the immune system to build an effective response within hours.

In this review we will show how the recent advent of highly potent, fully human mAbs may change again the picture of infectious disease prevention and therapy. We believe that mAbs will not only represent an important weaponry against infectious diseases, but they will also be a critical accelerator of vaccine development.

From serum therapy to modern antigen-specific mAbs

Since the first human monoclonal against SARS was cloned from a convalescent person [10•], a multitude of methods have been developed to identify, clone, engineer, and produce human mAbs against infectious diseases [11]. Advancement in genome sequencing and other breakthroughs have allowed the interrogation of immune responses at the single cell level and identification of extremely rare antigen-specific memory B cells [11,12]. The ability to produce the corresponding mAbs or Fabs (antigen-binding antibody fragments) has allowed to discover a plethora of highly functional and fully human molecules capable to neutralize the pathogen of interest [10•,11]. In the HIV field, human mAbs allowed the identification of vulnerability sites in the HIV envelope [13,14], the production of therapeutic antibodies that compete with the best drugs in controlling viremia as well as the development of germline targeting (GT) vaccinology to tailor the antibody responses to specific sites for which our immune system is blind [15–20]. In addition, a variety of engineering steps allow functional

improvements of mAbs transforming them into attractive commercial products. The wealth of recent progress in antibody technology and their potential applications for novel therapies is reviewed elsewhere [21–23].

Human mAbs for vaccine antigen identification and optimization

In addition of being excellent tools for prevention and therapy of infectious diseases, mAbs have been instrumental in several instances to identify new vaccine antigens and to define the nature and conformation of protective epitopes enabling vaccine design and development. This approach to vaccinology has been named 'reverse vaccinology 2.0' [24••].

Successful examples of human mAbs playing a pivotal role in vaccine development are the discovery of the pentamer-complex of cytomegalovirus (CMV) and the Pre-Fusion (Pre-F) conformation of the fusion (F) protein of respiratory syncytial virus (RSV). In the case of CMV, in the mid-1990s a recombinant form of the fusion protein gB, known to be the most promising antigen at that time, was combined with a potent adjuvant and tested in humans [25–28]. This vaccine showed only moderate efficacy and development was put on hold. The game changed when mAbs were isolated from people previously exposed to CMV. The most potent antibodies in neutralizing CMV infection of epithelial, endothelial, and myeloid cells, were not recognizing gB but a complex antigen made by five proteins (pentamer) [29]. Vaccination with pentamer-complex induced neutralizing antibodies that are orders of magnitude more potent than those induced by gB. This highly promising vaccine candidate produced recombinantly in CHO is currently being developed for human trials [30].

In the case of RSV, the first vaccine trial in infants, performed in 1966 with a formalin inactivated virus (FI-RSV), resulted in a catastrophic failure with disease enhancement and two infant deaths (15). The search for new vaccines continued without success for the next fifty years. Only the isolation and licensure of Palivizumab, a humanized mAb that prevents RSV disease in high risk infants, identified the fusion (F) protein of RSV as a good vaccine target. Since Palivizumab binds both the pre-fusion and post-fusion form of RSV-F, the initial efforts to develop a vaccine were focused on the use of post-F that was easily produced in a recombinant form as soluble antigen or displayed onto virus-like particles (VLPs) [31]. However, the vast majority of potently and fully human mAbs isolated from convalescent patients were shown to recognize exclusively the Pre-F making this conformation a better target for the development of a RSV vaccine [32]. Unfortunately, the Pre-F protein was very unstable and impossible to produce in its natural form as a vaccine antigen or for crystal structure studies. Again, the problem was solved by the Fab fragment of a particularly potent antibody (D25) that recognizes an epitope on the apex of the antigen, blocking it in its Pre-F state [32,33]. D25 allowed to grow crystals of the antigen–antibody complex and to determine the molecular structure of Pre-F. This information allowed a structure-based design of a stabilized RSV pre-F protein by introducing disulfide bridges and other stabilizing mutations [34•]. This molecule named DS-Cav1 has been shown to induce in humans neutralizing antibodies that are 10–15 times more potent than those of previous vaccines and is the leading candidate for most new RSV vaccines [35]. Several candidates based on DS-CAV1 are currently in Ph1 clinical development (by Janssen (NCT03334695); GSK (NCT03674177); or by NIH/NIAID/VRC (NCT03049488)) [36].

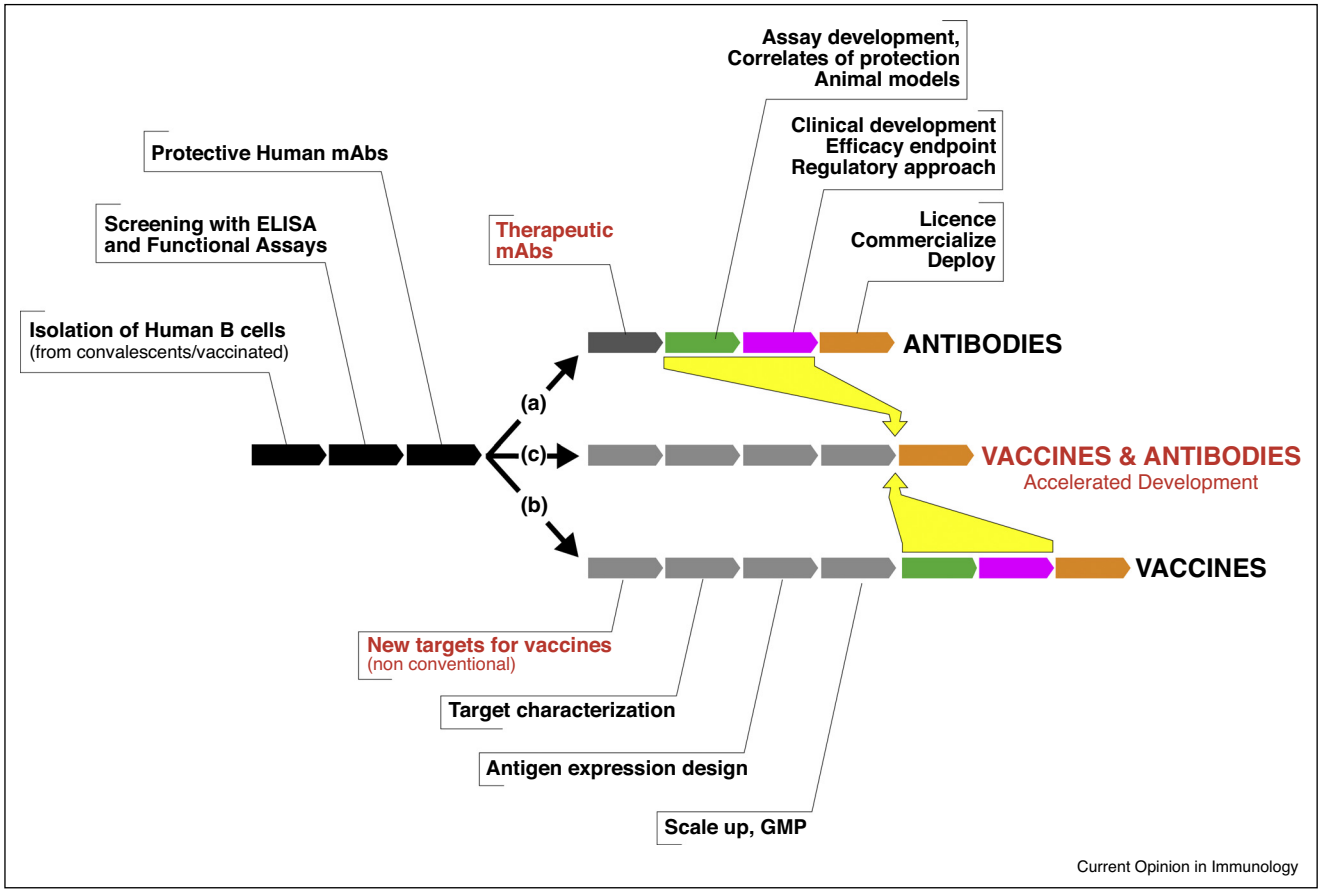
Human mAbs to control infectious diseases

D25 has also been the starting point to engineer its derivative MEDI8897, a potent mAb designed to compete with vaccination in the prevention of RSV. After CDR mutagenesis to enhance binding affinity, reversion of heavy chain germline residues to minimize the risk to elicit anti-drug antibodies (ADA) and half-life-extension by introducing Fc domain modifications, MEDI8897 was shown to persist up to three-times longer than Palivizumab in serum and to be up to 150-fold more potent [37,38]. These characteristics confer the extraordinary advantage to cover new-borns over one entire RSV season with a single intramuscular injection, while Palivizumab requires several monthly injections. A pivotal Ph2b study recently showed that MEDI8897 is efficacious in reducing medically attended RSV-confirmed lower respiratory tract infections in pre-term infants (NCT02878330). However, during late clinical development MEDI8897 (co-developed by MedImmune and Sanofi Pasteur) will be assessed in healthy late- and full-term infants (Ph2/3: NCT03959488 and Ph3: NCT03979313), in the offer preventive protection for all neonates independently of risk predisposition. This would be the first time that a mAb would be given to such a broad target population and highlights their potential to compete soon with vaccines in the space of neonatal infections.

Human mAbs as accelerators of vaccine development

So far, we have shown that the isolation of protective human mAbs can be a key step to identify new vaccines and therapeutic agents for infectious diseases. In this section we will show that the development and licensure of mAbs can also considerably accelerate vaccine development. The process of developing antibodies and vaccines are summarized in Figure 1a and b respectively. Briefly, following the isolation of protective human mAbs by screening single human B cells from naturally infected people (black arrows in Figure 1), development and licensure of an antibody requires engineering and optimization steps as described for MEDI8897. The molecule can then be produced and purified using well established technologies and start preclinical and clinical

Figure 1



Steps in the discovery and development of human mAbs and vaccines showing that mAb development **(a)** is faster than vaccine development **(b)**. However, the development of antibodies can accelerate vaccine development **(c)** by addressing some of the key biological and regulatory questions summarized in the green and violet boxes.

development. Key steps in this process are the identification and optimization of functional *in vitro* and *in vivo* assays and the validation of correlates of protection from infection and/or disease. In addition, epidemiological studies need to make sure that the functional antibody is broadly cross-reactive. After these initial steps, consultations with regulatory agencies can be initiated and decisions about clinical development plans, safety and efficacy endpoints, population where the clinical trials will be performed, and trial size will be taken. All these processes are illustrated as the green and violet boxes in path A in Figure 1. Following positive outcome of Ph3 clinical studies the antibody can finally be licensed.

While antibody development can take several years, it is much shorter than vaccine development. As shown in path B in Figure 1, the first step of vaccine development is the identification of the target recognized by protective antibodies. The new target needs then to be characterized, expressed and engineered to become a high-quality antigen. Vaccine-specific production and purification technologies need to be developed before manufacturing under good manufacturing practices. At this point, vaccine developers need to establish correlates of protection, *in vitro* and *in vivo* assays for safety and potency, show passive protection, perform epidemiological studies and consult with regulatory agencies on Ph2 and Ph3 clinical studies and endpoints. These activities usually take several years.

Vaccine development could be significantly shorter (path C in Figure 1) if a mAb targeting the same vaccine antigen had been already developed. In fact, vaccine development could leverage most of the assays, correlates, methods and regulatory approaches put in place during mAb-development and could move quickly to Ph3.

In conclusion, mAb development not only provides faster access to disease cure or prevention than any vaccine, but it can also accelerate vaccine development by anticipating some of the key biological and regulatory questions.

MAb/Vaccine co-development offers diverse treatments for different target populations

It is not difficult to imagine how mAbs and vaccines can complement each other. For example, 'superbugs' such as *Staphylococcus aureus* or *Pseudomonas aeruginosa* are multi-drug resistant pathogens and increasing concerns of nosocomial infections [39,40••,41]. Vaccines, if proven safe and effective, would be extremely useful for predictable events such as elective surgery or patients with chronic conditions expected to be hospitalized frequently. However, in acute conditions (e.g. emergency surgery and/or mechanical ventilation) there would not be enough time to vaccinate, and underlying conditions may reduce vaccine efficacy (immunocompromised or immunosuppressed subjects). These situations would likely be better addressed by passive administration of mAbs that could be used therapeutically in emergency scenarios such as Ebola outbreaks. Instead vaccines should be applied in the wider population helping to prevent further spread and finally extinct the outbreak. Similarly, for Malaria, mAbs could be given to travellers or military personnel for protection during their limited stay in an afflicted region, while the endemic populations would benefit of cheaper and long-term protective vaccinations [42]. Such differentiated solutions for mAbs and vaccine are possible for many pathogens. Vaccines and mAbs could also be combined in situations where either fast onset and long duration of protection are needed.

Conclusions

Co-development of mAbs and vaccines not only offers the possibility to leverage synergies across the R&D pipeline and to accelerate vaccine development, but could lay the foundation for a new era of innovative products addressing difficult-to-treat infections in frail target populations. Tailor-made solutions for defined patient populations would help to fight infections that have been elusive so far, and to meet many challenges of our society such as aging, immune-compromised populations and increasingly multi-drug resistant pathogens.

Conflict of interest statement

RR and AS are full-time employees of Glaxosmithkline group of companies.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Behring E, Kitasato S: **Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei**. *Thieren Dtsch Med Wochenschrift* 1890, **49**:1113-1114.

2. Graham BS, Ambrosino DM: **History of passive antibody administration for prevention and treatment of infectious diseases**. *Curr Opin HIV AIDS* 2015, **10**:129-134.

3. • Rappuoli R, Santoni A, Mantovani A: **Vaccines: An achievement of civilization, a human right, our health insurance for the future**. *J Exp Med* 2019, **216**:7-9.

In this review, pioneers in the vaccinology and immunology field, elucidate the importance of vaccine and their fundamental role in achieving a better global health that can help save millions of lives every year.

4. Morens DM: **Antibody-dependent enhancement of infection and the pathogenesis of viral disease**. *Clin Infect Dis* 1994, **19**:500-512.

5. Tristram DA, Welliver RC, Hogerman DA, Hildreth SW, Paradiso P: **Second-year surveillance of recipients of a respiratory syncytial virus (RSV) F protein subunit vaccine, PFP-1: evaluation of antibody persistence and possible disease enhancement**. *Vaccine* 1994, **12**:551-556.

6. Minutello M, Senatore F, Cecchinelli G, Bianchi M, Andreani T, Podda A, Crovari P: **Safety and immunogenicity of an inactivated subunit influenza virus vaccine combined with MF59 adjuvant emulsion in elderly subjects, immunized for three consecutive influenza seasons**. *Vaccine* 1999, **17**:99-104.

7. Weinberger B: **Adjuvant strategies to improve vaccination of the elderly population**. *Curr Opin Pharmacol* 2018, **41**:34-41.

8. Black S, Margarit I, Rappuoli R: **Preventing newborn infection with maternal immunization**. *Sci Transl Med* 2013, **5** 195ps.

9. Bergin N, Murtagh J, Philip RK: **Maternal vaccination as an essential component of life-course immunization and its contribution to preventive neonatology**. *Int J Environ Res Public Health* 2018, **15**.

10. • Traggiai E, Becker S, Subbarao K, Kolesnikova L, Uematsu Y, Gismondo MR, Murphy BR, Rappuoli R, Lanzavecchia A: **An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus**. *Nat Med* 2004, **10**:871-875.

This study shows for the first time the possibility to immortalize human memory B cells isolated directly from subjects who survived natural infection, and to use retrieved neutralizing antibodies to fight emerging infections. This study has been pivotal for the methodological advancement that nowadays allows us to discovery a deluge of fully human mAbs to fight infection diseases.

11. Walker LM, Burton DR: **Passive immunotherapy of viral infections: 'super-antibodies' enter the fray**. *Nat Rev Immunol* 2018, **18**:297-308.

12. Smith MJ, Packard TA, O'Neill SK, Hinman RM, Rihanek M, Gottlieb PA, Cambier JC: **Detection and enrichment of rare antigen-specific B cells for analysis of phenotype and function**. *J Vis Exp* 2017.

13. Zhou T, Xu L, Dey B, Hessell AJ, Van Ryk D, Xiang SH, Yang X, Zhang MY, Zwick MB, Arthos J *et al.*: **Structural definition of a conserved neutralization epitope on HIV-1 gp120**. *Nature* 2007, **445**:732-737.

14. Cohen YZ, Caskey M: **Broadly neutralizing antibodies for treatment and prevention of HIV-1 infection**. *Curr Opin HIV AIDS* 2018, **13**:366-373.

15. Jardine J, Julien JP, Menis S, Ota T, Kalyuzhniy O, McGuire A, Sok D, Huang PS, MacPherson S, Jones M *et al.*: **Rational HIV immunogen design to target specific germline B cell receptors**. *Science* 2013, **340**:711-716.

16. Jardine JG, Kulp DW, Havenar-Daughton C, Sarkar A, Briney B, Sok D, Sesterhenn F, Ereno-Orbea J, Kalyuzhniy O, Deresa I *et al.*: **HIV-1 broadly neutralizing antibody precursor B cells revealed by germline-targeting immunogen**. *Science* 2016, **351**:1458-1463.

17. Escolano A, Steichen JM, Dosenovic P, Kulp DW, Golijanin J, Sok D, Freund NT, Gitlin AD, Oliveira T, Araki T *et al.*: **Sequential immunization elicits broadly neutralizing anti-HIV-1 antibodies in Ig knockin mice**. *Cell* 2016, **166**:1445-1458.e1412.

18. Jardine JG, Ota T, Sok D, Pauthner M, Kulp DW, Kalyuzhniy O, Skog PD, Thinnes TC, Bhullar D, Briney B *et al.*: **HIV-1 VACCINES. Priming a broadly neutralizing antibody response to HIV-1**

using a germline-targeting immunogen. *Science* 2015, **349**:156-161.

19. Abbott RK, Lee JH, Menis S, Skog P, Rossi M, Ota T, Kulp DW, Bhullar D, Kalyuzhniy O, Havenar-Daughton C *et al.*: **Precursor frequency and affinity determine B cell competitive fitness in germinal centers, tested with germline-targeting HIV vaccine immunogens**. *Immunity* 2018, **48**:133-146 e136.

20. Carrillo J, Clotet B, Blanco J: **Antibodies and antibody derivatives: new partners in HIV eradication strategies**. *Front immunol* 2018, **9**:2429.

21. Berry JD, Gaudet RG: **Antibodies in infectious diseases: polyclonals, monoclonals and niche biotechnology**. *N Biotechnol* 2011, **28**:489-501.

22. Fan G, Li J: **Engineering antibodies for the treatment of infectious diseases**. *Adv Exp Med Biol* 2017, **1053**:207-220.

23. Brinkmann U, Kontermann RE: **The making of bispecific antibodies**. *MAbs* 2017, **9**:182-212.

24. •• Rappuoli R, Bottomley MJ, D'Oro U, Finco O, De Gregorio E: **Reverse vaccinology 2.0: human immunology instructs vaccine antigen design**. *J Exp Med* 2016, **213**:469-481.
Here the novel concept named 'reverse vaccinology 2.0' is introduced and analyzed in depth. This work sees the instrumental use of human memory B cells retrieved from infected or vaccinated subjects for high throughput screening of neutralizing antibodies and identification of novel antigens for vaccine development.

25. Mitchell DK, Holmes SJ, Burke RL, Duliege AM, Adler SP: **Immunogenicity of a recombinant human cytomegalovirus gB vaccine in seronegative toddlers**. *Pediatr Infect Dis J* 2002, **21**:133-138.

26. Drulak MW, Malinoski FJ, Fuller SA, Stewart SS, Hoskin S, Duliege AM, Sekulovich R, Burke R, Winston S: **Vaccination of seropositive subjects with CHIRON CMV gB subunit vaccine combined with MF59 adjuvant for production of CMV immune globulin**. *Viral Immunol* 2000, **13**:49-56.

27. Frey SE, Harrison C, Pass RF, Yang E, Boken D, Sekulovich RE, Percell S, Izu AE, Hirabayashi S, Burke RL *et al.*: **Effects of antigen dose and immunization regimens on antibody responses to a cytomegalovirus glycoprotein B subunit vaccine**. *J Infect Dis* 1999, **180**:1700-1703.

28. Pass RF, Duliege AM, Boppana S, Sekulovich R, Percell S, Britt W, Burke RL: **A subunit cytomegalovirus vaccine based on recombinant envelope glycoprotein B and a new adjuvant**. *J Infect Dis* 1999, **180**:970-975.

29. Macagno A, Bernasconi NL, Vanzetta F, Dander E, Sarasini A, Revello MG, Gerna G, Sallusto F, Lanzavecchia A: **Isolation of human monoclonal antibodies that potently neutralize human cytomegalovirus infection by targeting different epitopes on the gH/gL/UL128-131A complex**. *J Virol* 2010, **84**:1005-1013.

30. Hofmann I, Wen Y, Ciferri C, Schulze A, Fuhner V, Leong M, Gerber A, Gerrein R, Nandi A, Lilja AE *et al.*: **Expression of the human cytomegalovirus pentamer complex for vaccine use in a CHO system**. *Biotechnol Bioeng* 2015, **112**:2505-2515.

31. Swanson KA, Settembre EC, Shaw CA, Dey AK, Rappuoli R, Mandl CW, Dormitzer PR, Carfi A: **Structural basis for immunization with postfusion respiratory syncytial virus fusion F glycoprotein (RSV F) to elicit high neutralizing antibody titers**. *Proc Natl Acad Sci U S A* 2011, **108**:9619-9624.

32. Kwakkenbos MJ, Diehl SA, Yasuda E, Bakker AQ, van Geelen CM, Lukens MV, van Bleek GM, Widjojoatmodjo MN, Bogers WM, Mei H *et al.*: **Generation of stable monoclonal antibody-producing B cell receptor-positive human memory B cells by genetic programming**. *Nat Med* 2010, **16**:123-128.

33. McLellan JS, Chen M, Leung S, Graepel KW, Du X, Yang Y, Zhou T, Baxa U, Yasuda E, Beaumont T *et al.*: **Structure of RSV fusion glycoprotein trimer bound to a prefusion-specific neutralizing antibody**. *Science* 2013, **340**:1113-1117.

34. • McLellan JS, Chen M, Joyce MG, Sastry M, Stewart-Jones GB, Yang Y, Zhang B, Chen L, Srivatsan S, Zheng A *et al.*: **Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus**. *Science* 2013, **342**:592-598.
Herein, the use of structural data obtained from the co-crystal structure of a human monoclonal antibodies together with the respiratory syncytial virus (RSV) F-protein has allowed to design, for the first time, a soluble reagent of the F-protein in its prefusion conformation. This event has been paramount for the production of a phenomenal vaccine candidate against RSV which is currently under clinical investigation.

35. Crank MC, Ruckwardt TJ, Chen M, Morabito KM, Phung E, Costner PJ, Holman LA, Hickman SP, Berkowitz NM, Gordon IJ *et al.*: **A proof of concept for structure-based vaccine design targeting RSV in humans**. *Science* 2019, **365**:505-509.

36. Mazur NI, Higgins D, Nunes MC, Melero JA, Langedijk AC, Horsley N, Buchholz UJ, Openshaw PJ, McLellan JS, Englund JA *et al.*: **The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates**. *Lancet Infect Dis* 2018, **18**:e295-e311.

37. Zhu Q, McLellan JS, Kallewaard NL, Ulbrandt ND, Palaszynski S, Zhang J, Moldt B, Khan A, Svabek C, McAuliffe JM *et al.*: **A highly potent extended half-life antibody as a potential RSV vaccine surrogate for all infants**. *Sci Transl Med* 2017, **9**.

38. Domachowske JB, Khan AA, Esser MT, Jensen K, Takas T, Villafana T, Dubovsky F, Griffin MP: **Safety, tolerability and pharmacokinetics of MEDI8897, an extended half-life single-dose respiratory syncytial virus prefusion f-targeting monoclonal antibody administered as a single dose to healthy preterm infants**. *Pediatr Infect Dis J* 2018, **37**:886-892.

39. Geisinger E, Isberg RR: **Interplay between antibiotic resistance and virulence during disease promoted by multidrug-resistant bacteria**. *J Infect Dis* 2017, **215**:S9-S17.

40. •• Tagliabue A, Rappuoli R: **Changing priorities in vaccinology: antibiotic resistance moving to the top**. *Front Immunol* 2018, **9**:1068.
Here the role of vaccines as a tool to fight antimicrobial resistance, the most alarming issue for human global health, has been addressed. The authors stress the importance to identify new strategies to tackle pan-resistant pathogens suggesting the usage of modern technologies and methodologies for the identification of functional human mAbs to discover highly immunogenic antigens and support vaccine development.

41. Ventola CL: **The antibiotic resistance crisis: part 1: causes and threats**. *P T* 2015, **40**:277-283.

42. Cockburn IA, Seder RA: **Malaria prevention: from immunological concepts to effective vaccines and protective antibodies**. *Nat Immunol* 2018, **19**:1199-1211.