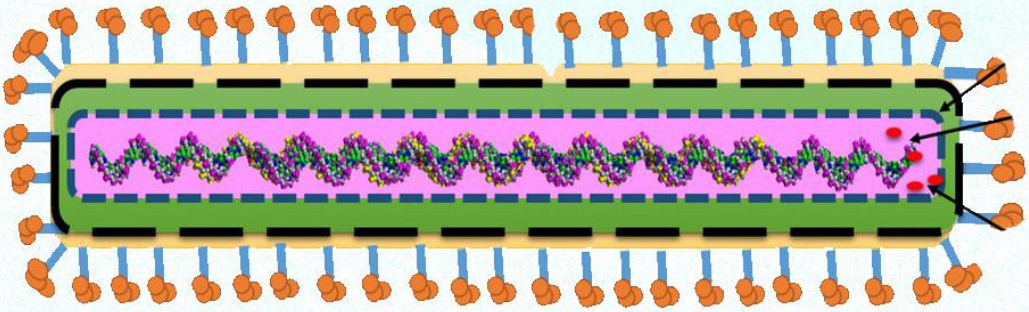
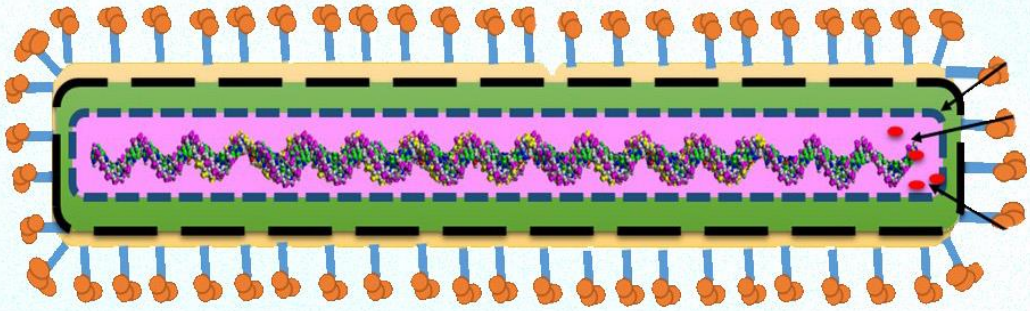
ISSN 2689-0852

BIOTECHNOLOGY KIOSK

AUGUST 2020





Volume 2, Issue 8
www.biotechkiosk.com

Executive Publishers

Megha Agrawal, PhD

(Biotechnology)

Publisher and Editor

Expertise:

Neuroscience, Stroke, Pharmacology, Toxicology, Microbiology and Molecular Biology

Email: megha@biotechkiosk.com
meghaagra@gmail.com

Shyamasri Biswas, PhD

(Biotechnology)

Publisher and Editor

Expertise:

Structural Biology, Enzyme Technology, and Protein Crystallography

Email: shyabiswas@biotechkiosk.com
shyabiswas@gmail.com

Editorial, Sales & Circulation Office

1330 NW 6th Street
Suite A2, Gainesville
FL-32601, USA
Phone: 386-518-9411
Email: publisher@biotechkiosk.com

www.biotechkiosk.com
ISSN 2689-0852
One stop shop for all things biotech

From the Publisher's Desk

Welcome to Biotechnology Kiosk!

The current issue of BK is now online for our readers with the regular features that include research articles by international experts and biotechnology advances around the world. The regular industry and pharma news will appear in the next issue of BK.

This issue contains research articles in the field of COVID-19 along with news and views on the current cutting-edge topics that include latest research breakthroughs in artificial organs and enzyme technology that report on research breakthroughs from around the world.

We look forward to receiving your feedback. We do hope that you will enjoy reading this issue of Biotechnology Kiosk. Please do write to us with your comments. Your suggestions are always appreciated.

Dr. Megha Agrawal and Dr. Shyamasri Biswas

Co Editors-in-Chief, Biotechnology Kiosk

Contents

Volume 2, Issue 8 August 2020

https://www.cardiometabolichealth.org/

https://www.cardiometabolichealth.org/

Research Article

Options for COVID-19 therapeutics: Aerosolized inhalation antibody-conjugated Nanoparticles

*Humphrey Simukoko[a] PhD, Brian Mujuni[b] MSc

[a]University of Zambia, School of Veterinary Medicine, Department of Biomedical Sciences, Africa Center of Excellence for Infectious Diseases of Humans and Animals (ACEIDHA).

[b]Africa Center of Excellence in Materials, Product Development & Nanotechnology (MAPRONANO ACE), P.O Box 7062, College of Engineering Art and Technology, Makerere University.

Abbreviated Names:
Simukoko H., Mujuni, B.

**** Address correspondence to:***

Dr. Humphrey Simukoko, University of Zambia, School of Veterinary Medicine, Department of Biomedical Sciences, P.O. Box 32379, Lusaka, Zambia. Postal code: 10101

Tel/Fax: +260-211-293727

Mobile: +260-975732243

Email: h.simukoko@unza.zm

Article type: *Research Article*

Received: *July 11, 2020*

Revised: *July 20, 2020*

Accepted: *August 4, 2020*

Citation: *Simukoko H. and Mujuni, B.; Options for covid-19 therapeutics: aerosolized inhalation antibody-conjugated nanoparticles, Biotechnology Kiosk, Vol 2, Issue 8, PP: 4-16 (2020); DOI: https://doi.org/10.37756/bk.20.2.8.1*

Abstract

The world is currently faced with a very serious crisis to deal with the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2 or Covid-19) pandemic which started in Wuhan, China in December 2019 and has since spread throughout the world, wreaking havoc in many countries. Several efforts are being made to control the spread of the disease around the world and to find a cure or vaccine. As researchers frantically endeavor to identify remedies for covid-19, there is the need to identify therapies that offer the quickest, safest actions and remedies that are relatively cheap. We propose the use of aerosolized inhalation antibody conjugated nanoparticles for the treatment of covid-19. It is hypothesized in this proposal that the conjugation of nanoparticles with antibodies and delivering the antibody-nanoparticle conjugate as an aerosol via the respiratory tract would provide the quickest and possibly more efficient and relatively cheap remedy against covid-19. The advantage of the inhalation route for delivering antibody conjugated nanoparticles is that since the medication is delivered directly to the affected site, higher doses will be delivered to the site with reduced systemic toxicity and reduced adverse effects on gaseous exchange. Our hypothesis is based on the current knowledge and observations in the areas of monoclonal antibody technology, advances in nanotechnology and Nano medicine as well as advances in inhalation therapeutics.

Key Words: Pandemic, Angiotensin-Converting Enzyme 2 (ACE2) receptor, Respiratory Epithelium, Virus, Nano medicine.

E-Mail: h.simukoko@unza.zm

To cite this article: Simukoko H. and Mujuni, B.; Options for covid-19 therapeutics: aerosolized inhalation antibody-conjugated nanoparticles, Biotechnology Kiosk, Vol 2, Issue 8, PP: 4-16 (2020); DOI: https://doi.org/10.37756/bk.20.2.8.1

Introduction

The World Health Organization (WHO) was informed of a cluster of cases of pneumonia of unknown cause detected in Wuhan City, Hubei Province of China on 31 December 2019. Later the causative agent of the pneumonia was identified by Chinese authorities to be the SARS-CoV-2 and the disease was named coronavirus disease 2019 (COVID-19) by WHO on 11 February 2020. Ever since its identification and characterization, the disease has been spreading world-wide causing high mortalities and morbidities. A group of experts Under the WHO's coordination, with diverse backgrounds has been tasked to work towards the development of vaccines against COVID-19. Other scientists around the world are equally making frantic efforts to develop vaccines and therapeutics against COVID-19 [1].

Efficient and targeted delivery of the envisaged SARS-CoV-2 therapeutics to their sites of action will be cardinal in the treatment of covid-19. We therefore propose the use of aerosolized antibody conjugated nanoparticles via direct inhalation into the respiratory system as one of the potential methods for the treatment of covid-19. Using aerosolized antibody conjugated nanoparticles via direct inhalation has several advantages when compared to other potential remedies against covid-19. First, respiratory tract inhalation delivers medication directly to the site, thus enabling higher doses locally with less systemic toxicity. Second, inhaled drugs are likely to improve or at least have fewer adverse effects on gaseous exchange compared with other systemic routes of administration. On the other hand, systemically delivered drugs may be distributed widely thereby indiscriminately affecting other untargeted tissues which may result in unwarranted injury. Third, direct delivery of the drug to the lungs may permit reduction in the total medication dose and thus potentially lowering cost.

As the world grapples with the COVID-19 pandemic, it is prudent upon the scientific community to consider diligently all potential plausible solutions that may offer even a glimmer of hope now or in the future. Humankind is at the cross-roads for survival and therefore concerted efforts are extremely necessary to combat the COVID-19 pandemic.

Currently there is already an accumulation of knowledge on SARS-CoV-2 and other respiratory diseases. In addition, a number of intervention points for the possible treatment of Covid-19 have been suggested and some possible candidates are already being tested [2, 3]. Most of the potential therapeutic candidates such as hydroxylchloroquine, Ivermectin, remdesivir, interferon β1a and a few others are repurposed remedies that were/are used for the treatment of other conditions. These potential remedies currently being investigated for the possible treatment of SARS-CoV-2 are administered orally, intramuscularly (IM) or intravenously (IV) with a few exceptions such as interferon β1a (SNG001) that is administered via the respiratory route. Moreover, the modes of action of most of these adapted remedies are largely unknown. Based on the current understanding of the structure of the SARS-CoV-2 including its cellular binding and

cellular entry strategies, a number of plausible points for therapeutic interventions have been suggested. Cardinal amongst the therapies being suggested include camostat mesilate, a serine protease inhibitor [3, 4]. In addition, other small molecule drug compounds that might warrant further study have been identified that can limit viral recognition of host cells and/or disrupt host-virus interactions [5, 6]. Efficient and targeted delivery of the suggested SARS-CoV-2 therapeutics to their sites of action will be cardinal in the treatment of covid-19.

We therefore join the scientific community in proposing the use of aerosolized antibody-conjugated nanoparticles via direct inhalation into the respiratory system as one of the potential methods for the treatment of covid-19. The conjugation of nanoparticles to antibodies has been shown to improve efficacy in the treatment of certain diseases because the antibody-nanoparticle conjugate can provide new and enhanced properties as well as stability at the site of intended action [7, 8] The use of nanoparticles in medicine has gained a lot of research interest over the past few decades. Nanoparticles have been used to deliver drugs to specific types of cells including cancer cells [9, 10, 11]. Moreover, nanoparticles can be conjugated to antibodies that target specific epitopes on selected cells. The combination of nanoparticles with specific antibodies can ensure increased efficacy of the conjugate and optimal concentration of the payload at the site of action [12, 13, 14].

We hypothesize that the SARS-CoV 2 can be neutralized within the respiratory tract and its binding capacity to ACE2 receptors of the respiratory epithelium can be curtailed by the nanoparticle-antibody conjugates that would competitively bind to the ACE2 receptors. In this case, the antibody-nanoparticle conjugate will have dual action. On one hand, the nanoparticles will compete with the SARS-C0V-2 for binding to the ACE2 receptors while on the other hand the antibody moieties will bind and immobilize the free viral particles within the respiratory tract. Since the immobilized SARS-CoV-2 cannot effectively bind to the ACE2 receptors as a result of nanoparticle competitive binding and immobilization by the antibodies, the complex that is formed between the antibodies and the viral particles will eventually be eliminated by the mucociliary clearance system which normally clears particulate matter from the respiratory tract. Therefore, we further hypothesize that individuals that would receive the antibody-nanoparticle conjugate inhalation treatment would have a better outcome from a covid-19 infection than those who would not receive the treatment. The hypothesis would be tested by conducting clinical trials in which a group of covid-19 infected individuals would receive the therapy while another group would receive a placebo.

In this article, the hypothesis on the use of antibody-nanoparticle conjugate inhalation treatment is contextualized.

Evaluation of the hypothesis

Inhalation of aerosolized medicines through the oral route is one of the most effective ways to get lifesaving medications to people with lung infections. The envisaged end product for our proposed covid-19 treatment is a hand-held covid-19

pressurized metered-dose aerosol inhaler or nebulizer. The user puts the mouth-piece of the inhaler in the mouth and presses down on the canister to release the medicine while breathing in to allow the medication to get into the respiratory tract (Figure 1). The hand-held inhaler is a small canister that works like a spray can.

Figure 1: *Demonstration of the use of a handheld metered-dose inhaler for the treatment of COVID-19. The user puts the mouth-piece of the inhaler in the mouth and presses down on the canister to release the medicine while breathing in to allow the medication to get into the respiratory tract.*

When a plunger on the inhaler is pushed down, the medicine is ejected in form of an aerosol which the individual breathes in (Figure 2). The inhaler/nebulizer in our case would contain antibody-conjugated nanoparticles that target the ACE2 receptors on the respiratory cells and will at the same time bind the free SARS-CoV-2 spike proteins. The SARS-CoV-2 spike protein monoclonal antibody will be conjugated to the nanoparticle that has an epitope for binding to ACE2 receptors on respiratory epithelial cells (Figure 3).

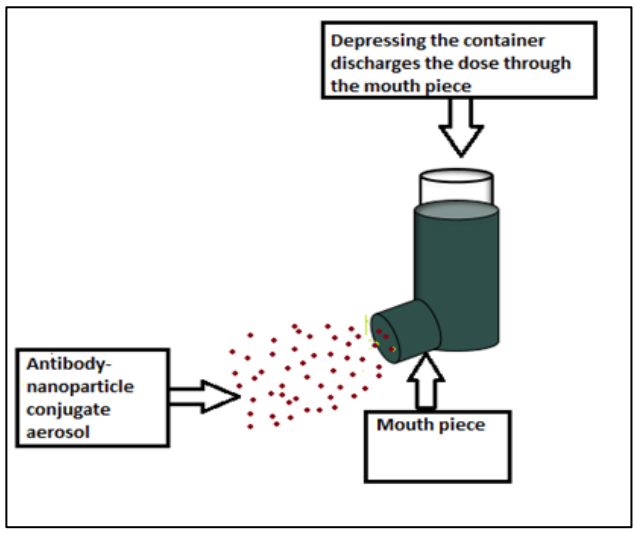


Figure 2: *Function of the pressurized hand-held metered-dose inhaler. The handheld metered-dose inhaler is a small canister that works like a spray can. When a canister on the inhaler is pushed down, the medicine is ejected in form of an aerosol which the individual breathes in. In the case of our proposed therapy, the aerosol would contain SARS-CoV-2 spike protein monoclonal antibody-nanoparticle conjugates*

When the medication containing the SARS-CoV-2 spike protein monoclonal antibody-nanoparticle conjugates is inhaled into the respiratory tract, the conjugates will competitively bind to the ACE2 receptors against the SARS-CoV-2 virus. In this case the conjugates would have a dual functionality. On one end, the free nanoparticle moiety would interact and bind to the ACE2 receptors while the free epitope on the monoclonal antibody would interact and bind any free SARS-CoV-2 viral particles. Thus, the viral particles would have insufficient or no ACE2 receptors to bind (Figure 4 and Figure 5).

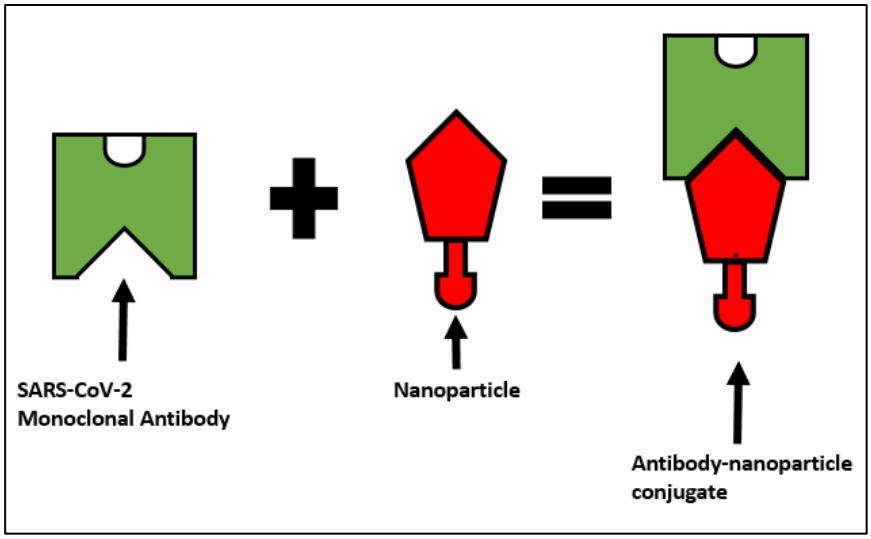


Figure 3: *Monoclonal antibody-nanoparticle conjugation. The inhaled aerosol contains SARS-CoV-2 monoclonal antibody-nanoparticle conjugates. The SARS-CoV-2 spike protein monoclonal antibody is conjugated to the nanoparticle that has an epitope for binding to ACE2 receptors on respiratory epithelial cells.*

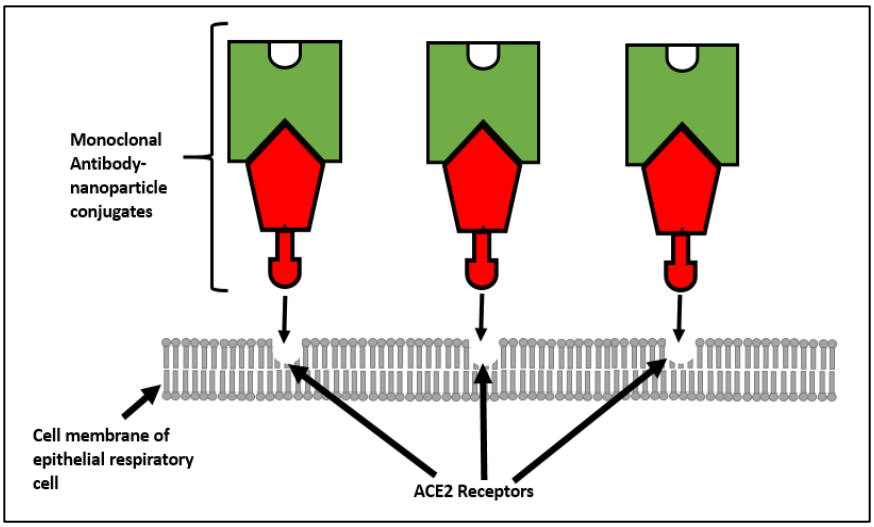


Figure 4: *SARS-CoV-2 spike protein monoclonal antibody-nanoparticle attachment to ACE2 receptors. When the medication containing the SARS-CoV-2 spike protein monoclonal antibody-nanoparticle conjugates is inhaled into the respiratory tract, the conjugates will competitively bind to the ACE2 receptors against the SARS-CoV-2 virus. In this case the conjugates would have a dual functionality. On one end, the free nanoparticle moiety would interact and bind to the ACE2 receptors while the free epitope on the monoclonal antibody would interact and bind any free SARS-CoV-2 viral particles. Thus, the viral particles would have insufficient or no ACE2 receptors to bind.*

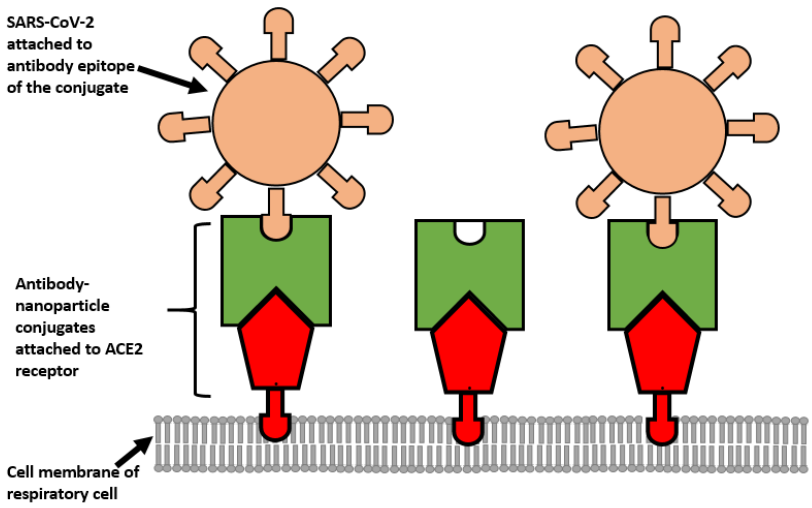


Figure 5: *Capture of the SARS-CoV-2 by the monoclonal antibody-nanoparticle conjugate. Since the viral particles cannot effectively bind to the ACE2 receptors which have been competitively bound by the nanoparticle moieties, the free viral particles get bound and are captured by the epitopes on the monoclonal antibodies. The viral particles together with the monoclonal antibody-nanoparticle complexes are then removed from the respiratory tract by the mucociliary clearance escalator*.

How SARS-CoV-2 enters respiratory cells

Recent studies have shed more light on how the SARS-CoV-2 invades respiratory tract cells. SARS- CoV-2 gets into respiratory tract cells via the ACE2 receptor [15] The ACE2 receptor appears to be the passage for the virus into respiratory epithelial cells. The structural characteristics of the SARS-CoV-2 virus are similar to other coronaviruses. The virus contains four structural proteins known as the spike, envelope, membrane and nucleocapsid proteins [16].

The protein that interacts with the host cell receptors is chiefly the spike protein. In the initial step of receptor binding, the viral spike protein becomes cleaved into S1 and S2 by the host cell protease [15, 16, 17, 18, 19]. One of the host cell proteases is the transmembrane protease serine 2 (TMPRSS2). The main function of the S1 subunit is to bind with the host cell surface receptors, while the function of the S2 subunit is to effect fusion of the virus with the host cell membrane [16, 17, 18, 20, 21].

Therefore, there are two potential therapeutic approaches against SARS-CoV-2. The first approach would be to either develop a vaccine that contains antigens derived from the spike protein, which can boost recognition of the virus by the immune cells or to develop monoclonal antibodies that bind to the coronavirus spike protein S1 subunit and block the interactions with the human cells ACE2 receptors. The second potential approach would be to target the transmembrane protease serine 2 which is responsible for entry and viral spread of the SARS-CoV-2. In our hypothesis, the aim is to produce monoclonal antibodies that bind the coronavirus spike protein.

Nanoparticles in medicine (Nanomedicine) and potential use in covid-19 treatment

Nanotechnology deals with matter generally in the 1-100 nm scale. The application of nanotechnology to medicine is known as nanomedicine. It involves the use of engineered materials of size 1-100nm length to develop novel therapeutic and diagnostic strategies [8]. Nanoparticles that are targeted to specific diseased cells or healthy cells of interest can reduce possible side effects. Moreover, nanoparticles can deliver a much higher payload to the intended site of action. If a drug is delivered in its conventional form, it is estimated that less than 1% of the drug actually makes it to the target site with 99% of the drug going to other parts of the body [9,10,11,12,13,14]. Nanotechnology is already used in many commercial medicinal products but many of them are not available to the consumer [14]. Given the urgent need for a Covid-19 remedy, nanotechnology offers grain of hope and may act as an enabling tool to skew promising medical therapeutics.

SARS-CoV-2 spike protein nanoparticles

Methods for generating SARS-CoV-2 full-length spike protein nanoparticles are available. The spike protein nanoparticles can be obtained and characterized by cleaving off the spike glycoproteins of the SARS-CoV-2 which reside on the surfaces of the virions. The purified full-length SARS-CoV-2 proteins have been determined to have molecular weight of approximately 160kDa by SDS-PAGE and approximately 25 nm in diameter [20, 21]. Moreover, recombinant spike protein nanoparticles that can bind to ACE2 receptors are available YP_009724390.1 [22]

In our hypothesized therapy, the spike protein nanoparticles will be conjugated to monoclonal antibodies against SARS-CoV-2.

Monoclonal SARS-CoV-2 antibodies

Monoclonal antibodies (Mabs) against coronaviruses or SARS-associated coronaviruses can be developed and characterized for reactivity to the SARS-CoV-2 spike protein (S), nucleocapsid protein (N), membrane protein (M), and envelop protein (E) using enzyme-linked immunoabsorbent assay (ELISA), radioimmunoprecipitation, immunofluorescence, Western Blot and microneutralization assays (32). Indeed, single stranded DNA (ssDNA) aptamers with high binding affinity to the SARS-CoV proteins can be identified from DNA libraries [22, 23, 24. 25.]. In our hypothesis, the aptamers that bind to the nucloecapsid protein will be used.

Antibody-nanoparticle conjugation

Antibody-conjugated nanoparticles offer great opportunities to overcome limitations found in conventional therapeutics [14]. They combine the advantages given by the nanoparticles with the ability to bind to their target with high affinity and improve cell penetration given by the antibodies [8,9,10]. Antibody-conjugated nanoparticles could play a critical role in medical therapeutics [14]. In biotechnology, antibodies could be used to carry several elements such as drugs and nanoparticles in diagnostic procedures or even in therapy to bind to specific targets. The conjugation of

antibodies to nanoparticles can generate products that combine the properties of the antibody and nanoparticle whereby the hybrid product would show adaptability and specificity. By conjugating different moieties to the nanoparticles, their application can be widened in different fields including therapies for covid-19 and can provide them with novel or boosted properties [7,8].

Aerosolized inhalation antibody-conjugated nanoparticles and potential in covid-19 treatment

In many instances, respiratory diseases are treated using inhalation therapy. Currently there is significant research in pulmonary drug delivery using solid colloidal nanoparticles in the treatment of many respiratory diseases [24].

The inhalation drug administration route is often used for the management of respiratory diseases. Compared with other routes of administration, inhalation offers a number of advantages in the treatment of these diseases. For example, via inhalation, a drug is directly delivered to the target organ, delivering high drug concentrations but low systemic drug concentrations. Therefore, drug inhalation is typically associated with high pulmonary efficacy and minimal systemic side effects [24, 25. 26, 27, 28, 29] and would thus be useful in covid-19 therapy.

HYPOTHESIS TESTING

In order to evaluate the effectiveness and safety of the medication, clinical trials will be performed by monitoring the effects on groups of people. The four phases of the classical clinical trial will be followed.

Phase I trial will be done to assess safety and side effects as well as to determine the correct drug dosage. The drug will be administered to 20 but less than 100 healthy individuals.

Phase II trial. In this trial phase, about 100 to 300 volunteers will be given the drug in order to assess the effectiveness of the durg. In this phase the aim will be to obtain preliminary data on whether the drug works in people who have COVID-19. In addition, this phase will continue to assess the safety, including short-term side effects.

Phase III trial. In this phase, more people volunteers (500-1000) will be involved in order to gather more information about safety and effectiveness and to study different populations and different dosages as well as in combination with other drugs.

Phase IV trial. If the drug is approved by the relevant regulatory agencies, the drug's effectiveness and safety will be monitored in large, diverse populations.

Potential Advantages and Disadvantages of Aerosolized Inhalation SARS-CoV-2 therapy

Inhalation route has many important advantages compared to other routes of drug administration and should be seriously considered in potential SARS-CoV-2 therapies. First, respiratory tract inhalation delivers medication directly to the site, thus enabling higher doses locally with less systemic toxicity. Second, inhaled drugs are likely to improve or at least have fewer adverse effects on gaseous exchange compared with other systemic routes of administration. On the other hand, systemically delivered drugs may be

distributed widely thereby indiscriminately affecting other untargeted tissues which may result in unwarranted injury. Third, direct delivery of the drug to the lungs may permit reduction in the total medication dose and thus potentially lowering cost.

The inhaled route may also have some disadvantages. Inhaled aerosolized SARS-CoV-2 therapies may not be tolerated by some individuals due to sensitization or direct irritation on the respiratory airways by the drug or the excipients. In addition, the dosage may not be very precise because of variances in breathing patterns and the challenges in determining exactly how much medication reaches the targeted regions of the lungs. Also, the cumbersomeness and difficulty in effective operation of inhalation devises by the user may cause erroneous dose administration [26, 27, 29].

Potential cytotoxicity of the proposed treatment and possible remedial actions

The main active ingredients of the proposed treatment will be the antibodies conjugated with the SARS-CoV-2 recombinant spike protein nanoparticles. The active ingredients will be contained in an inert non-toxic excipient. The recombinant SARS-CoV-2 spike protein nanoparticles that will be used in the proposed treatment have been assessed by others for safety and binding affinity to ACE2 receptors [22]. Therefore, no major toxicity is expected from the nanoparticles. Also the antibodies (aptamers) that will be used in the proposed treatment will be ssDNA whose affinity and safety are already known. The aptamers will be identified from DNA libraries [22,23,24,25]. Thus, the active ingredients in the proposed treatment will be compatible to the tissues within the respiratory tract. Qualified medical personnel will be on hand in cases of inadvertent adverse reactions to either the excipient or any of the active ingredients and appropriate medical interventions will be immediately implemented.

Conclusion

The advantages of using inhaled aerosolized antibody-conjugated SARS-CoV-2 therapies would seem to outweigh other routes of administration. It is therefore the contention of the author that potential remedies for the treatment of SARS-CoV-2 should be administered preferably through the respiratory tract route as inhalation aerosols conjugated to nanoparticles. Respiratory route administration of potential SARS-CoV-2 therapies would be advantageous compared to other routes such as the oral route, IM or IV routes. The respiratory tract route would provide a quick and relatively safe route for SARS-CoV-2 therapies preferably using aerosolized antibody-conjugated nanoparticles.

However, researchers in SARS-CoV-2 therapeutics before considering clinical trials for aerosolized antibody-nanoparticle conjugates should ensure the following: (i) Identification of appropriate nanoparticles for conjugation with SARS-Cov-2 spike antibodies [30, 31, 32, 33, 34, 35] (ii) Production of monoclonal antibodies that are specific to the SARS-CoV-2 spike proteins (iii) Conjugation of monoclonal antibodies with nanoparticles and , (iv) Production and optimization of aerosolized antibody-

nanoparticle conjugates using easy to use inhalation delivery systems.

Acknowledgements

The authors are grateful to the University of Zambia for providing unlimited internet access and library facilities.

Conflict of interest statement

There is no conflict of interest to disclose

References

1. WHO. Emergencies preparedness, responses: Pneumonia of unknown cause, diseases outbreak news. https://www.who.int/csr/don/05-january-2020-pneumonia-of-unkown-cause-china/en/. 2020. Accessed on 08/05/2020.
2. Guo Y, Cao Q, Hong Z, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. Mil Med Res. 2020; 7:11, https://doi.org/10.1186/s40779-020-00240-0
3. Mahase E. Covid-19: What treatments are being investigated? BMJ. 2020; 368: doi: https://doi.org/10.1136/bmj.m1252
4. Liu C, Zhou Q, Li Y, et al. Research and Development on Therapeutic Agents and Vaccines for COVID-19 and Related Human Coronavirus Diseases. ACS Cent. Sci. 2020; 6, 3: 315-31, https://pubs.acs.org/doi/10.1021/acscentsci.0c00272.
5. Smith M & Smith CJ. Repurposing Therapeutics for COVID-19: Supercomputer-Based Docking to the SARS-CoV-2 Viral Spike Protein and Viral Spike Protein-Human ACE2 Interface. ChemRXIV. 2020, DOI: 10.26434/chemrxiv.11871402.v3
6. Kruse RL. Therapeutic strategies in an outbreak scenario to treat the novel coronavirus originating in Wuhan, China. F1000Res. 2020. 9:72, https://doi.org/10.12688/f1000research.22211.2
7. Mullen DG, Fang M., Desai A, Baker JR, Orr BG, Banaszak HM M. A quantitative assessment of nanoparticle-ligand distributions: implications for targeted drug and imaging delivery in dendrimer conjugates. ACS nano. 2010; 4(2): 657–70, DOI: 10.1021/nn900999c
8. Zhang L, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC. Nanoparticles in medicine: therapeutic applications and developments. Clin Pharmacol Ther. 2008; 83(5):761-69, https://doi.org/10.1038/sj.clpt.6100400
9. Ma X, Xiong Y, Lee, LTO. Application of Nanoparticles for Targeting G Protein-Coupled Receptors. Int. J. Mol. Sci. 2018; 19(7): 2006, DOI: 10.3390/ijms19072006
10. Yameen B, Choi WI, Vilos C, Swami A, Shi JJ, Farokhzad OC. Insight into nanoparticle cellular uptake and intracellular targeting. J. Control. 2014; 190: 485–99, DOI: 10.1016/j.jconrel.2014.06.038
11. Jain R.K & Stylianopoulos T. Delivering nanomedicine to solid tumors. Nat. Rev.

Clin. Oncol. 2010; 7: 653–64, DOI: https://doi.org/10.1038/nrclinonc.2010.139

12. Richard S, Boucher M, Saric A, et al. Optimization of pegylated iron oxide nanoplatforms for antibody coupling and bio-targeting. J. Mater. Chem. B. 2017; 5: 2896–907, https://doi.org/10.1039/C6TB03080G
13. Qinqin H, Hua L, Lihua W, Hongzhou G, Chunhai F. DNA Nanotechnology-Enabled Drug Delivery Systems. Chem Rev. 2019; 119 (10): 6459-506, https://doi.org/10.1021/acs.chemrev.7b00663
14. Murthy SK. Nanoparticles in modern medicine: state of the art and future challenges. Int J Nanomedicine. 2007; 2(2): 129–41, PMCID: PMC2673971
15. Gallagher TM & Buchmeier MJ. Coronavirus spike proteins in viral entry and pathogenesis. Virology. 2001; 279(2): 371–4, https://doi.org/10.1006/viro.2000.0757
16. Belouzard S, Millet JK, Licitra BN. Whittaker GR. Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses. 2012;4 (6): 1011-1033, https://doi.org/10.3390/v4061011
17. Heald-Sargent T & Gallagher T. Ready, Set, Fuse! The Coronavirus Spike Protein and Acquisition of Fusion Competence. Viruses. 2012. 4(4): 557–580, https://doi.org/10.3390/v4040557
18. Simmons G, Zmora P, Gierer S, Heurich A, Pöhlmann S. Proteolytic activation of the SARS-coronavirus spike protein: Cutting enzymes at the cutting edge of antiviral research. Antiviral Res. 2013; 100(3): 605–14, DOI: DOI: 10.1016/j.antiviral.2013.09.028
19. Belouzard S, Chu VC, Whittaker GR. Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. Proc. Natl. Acad. Sci. U.S.A. 2009; 106(14): 5871–6, https://doi.org/10.1073/pnas.0809524106
20. Lan J, Ge J, Yu J, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor [published online ahead of print, 2020 Mar 30], Nature 581, 215–220 (2020), https://doi.org/10.1038/s41586-020-2180-5
21. Luan J, Lu Y, Jin X & Zhang L. Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection. Biochemical and biophysical research communications. 2020. 526(1), 165–169, DOI: https://doi.org/10.1016/j.bbrc.2020.03.047.
22. Groff K, Brown J, Clippinger AJ. Modern affinity reagents: Recombinant antibodies and aptamers. Biotechnol Adv. 2015;33(8):1787-1798, DOI: 10.1016/j.biotechadv.2015.10.004
23. Xu Y, Lou Z, Liu Y, et al. Crystal structure of severe acute respiratory syndrome coronavirus spike protein fusion core. J Biol Chem. 2004. 279(47):49414-49419, DOI: 10.1074/jbc.M408782200
24. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020.

367(6483):1260-1263, https://doi.org/10.1126/science.abb2507

25. Cho SJ, Woo HM, et al. Novel system for detecting SARS coronavirus nucleocapsid protein using an ssDNA aptamer. J. Biosci. Bioeng. 2011. 112:e1347-e4421, DOI: https://doi.org/10.1016/j.jbiosc.2011.08.014
26. Aysu Y, Mesut A, Mine O. Nanopharmaceuticals: Application in inhaler systems. In: Emerging Nanotechnologies in Immunology. The Design, Applications and Toxicology of Nanopharmaceuticals and Nanovaccines Micro and Nano Technologies. 1st Edition. (Ranjita Shegokar and Eliana B. Souto, eds.). 2018. pp: 165-201.
27. Labiris NR. & Dolovich MB. Pulmonary drug delivery. Part II: the role of inhalant delivery devices and drug formulations in therapeutic effectiveness of aerosolized medications. Br J Clin Pharmacol. 2003; 56(6): 600–12, https://doi.org/10.1046/j.1365-2125.2003.01893.x
28. Borghardt MJ, Kloft C, Sharma A. Inhaled Therapy in Respiratory Disease: The Complex Interplay of Pulmonary Kinetic Processes. Can Respir J. 2018, https://doi.org/10.1155/2018/2732017
29. Hill NS, Preston IR, Roberts KE. Inhaled Therapies for Pulmonary Hypertension. Resp Care. 2015. 60 (6): 794-805, DOI: 10.4187/respcare.03927
30. Coleman CM, Liu YV, Mu H, et al. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine. 2014. 32(26), 3169–3174, DOI: 10.1016/j.vaccine.2014.04.016
31. Liu YV. Chimeric severe acute respiratory syndrome coronavirus (SARS-CoV) S glycoprotein and influenza matrix 1 efficiently form virus-like particles (VLPs) that protect mice against challenge with SARS-CoV. Vaccine. 2011. 29(38): 6606–6613, DOI: 10.1016/j.vaccine.2011.06.111
32. Li J. Immunogenicity and protection efficacy of monomeric and trimeric recombinant SARS coronavirus spike protein subunit vaccine candidates. Viral Immunol. 2013. 26(2):126–132, http://doi.org/10.1089/vim.2012.0076
33. He Y. Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implication for developing subunit vaccine.Biochem Biophys Res Commun. 2004. 324(2):773–781, https://doi.org/10.1016/j.bbrc.2004.09.106
34. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV--a target for vaccine and therapeutic development. Nat Rev Microbiol. 2009. 7(3):226-236, https://doi.org/10.1038/nrmicro2090
35. Tripp RA, Haynes LM, Moore D et al. Monoclonal antibodies to SARS-associated coronavirus (SARS-CoV): identification of neutralizing and antibodies reactive to S, N, M and E viral proteins J. Virol. Methods, 128 (2005), pp. 21-28, https://doi.org/10.1016/j.jviromet.2005.03.021

http://ishatherapeutics.com/

ISHA Therapeutics

CONTRIBUTING TO THE SPIRIT OF DRUG DEVELOPMENT

"Contributing to the spirit of translational drug development"

ISHA Therapeutics, is a unique organization with its primary location based out of New Jersey, exclusively focused on supporting clinical and translational development programs spanning from very early on to late stage developments. We provide consulting services to our esteemed clients who aspire to develop safe and efficacious new drugs, devices and biologicals. We also have our in-house innovative drug development pipeline.

ISHA, specializes in providing translational drug development expertise (non-clinical to clinical, regulatory expertise) to our esteemed clients.

Therapeutic area expertise:

We have interacted with the US-FDA, Division of Infectious diseases, cardiovascular diseases, CNS (Huntington Disease, Autism), Oncology, Dermatology, Pharmaceutical Quality etc. for pre-IND, IND communications.

Insights of isha therapeutics:

- We provide innovative, realistic solutions with holistic approach
- Implementing "team science" approach
- Concept to client satisfaction
- Project specific personal interaction with clients
- Consistent high quality
- Resourcefulness, rationality, transparency and ethics.

If you plan to catalyze your drug development program and need an unbiased efficient scientific strategy do reach out to ISHA's team of experts.

Contact us:
Isha Therapeutics LLC,
300, Parsippany Road,
Parsippany, New Jersey 07054, USA.
Website: http://ishatherapeutics.com/
Email: manoj.jadhav@ishatherapeutics.com
Cell: 352-507-9092

Mini Review

Effective countermeasure for Filovirus infections: focusing monoclonal antibodies as passive prophylaxis shield against Ebola Virus disease.

Pradeep Kumar Singh[a], Shweta Kushmakar Singh[b]

[a]Microbiology Department, All India institute of medical sciences. New Delhi, India

[b]Department of Pediatrics, All India institute of medical sciences. New Delhi, India

Abbreviated Names:
Singh P.K., Singh S.K.

**** Address correspondence to:***

Dr. Pradeep Kumar Singh, Department of Microbiology, All India Institute of Medical Sciences. New Delhi, India

Mobile: +91 96544 02313

Email: pksingh1976a@gmail.com

Article type: *Mini Review*

Received: *June 14, 2020*

Revised: *July 01, 2020*

Accepted: *July 15, 2020*

Citation: *Singh P and Singh S; Effective countermeasure for Filovirus infections: focusing monoclonal antibodies as passive prophylaxis shield against Ebola Virus disease, Biotechnology Kiosk, Vol 2, Issue 8, PP: 18-35 (2020); DOI: https://doi.org/10.37756/bk.20.2.8.2*

Abstract

In 2020 the pandemic of COVID 19 by SARS-COV 2 infected more than 27 million with more than 875,000 deaths. Present day world is more compact with quick mode of transport between far locations, this makes spread of new viral infections at alarming rates. Similarly, Filovirus is a family of extremely dangerous Marburgvirus and Ebolavirus with up to 90% mortality rate. Since first Filovirus was discovered in 1967, many outbreaks were reported from African countries. Increased number of infected human population in other outbreaks in 2014 – 2017 poses a question of our understanding of Filovirus reservoirs. We are beginning to understand the relation between virus and ecological agents and their role in the spread of disease, but it is still a long road ahead. To counteract and containment of Filovirus infection, it is utmost requirement to understand the viral life cycle patterns, agents involved and type of circulating strains in different geographical locations. This information will provide the basis to develop viable therapies to counteract future outbreaks. In these outbreaks' magnitude of population and geographical area affected creates the urgency to generate effective vaccines and prophylactic agents so that mortalities can be controlled during future outbreaks. Therapies are required for pre-infection acute phase and post-infection. Here, we summarize recent advances in immunotherapy strategies that can be used as passive prophylaxis. We focused on development of recent monoclonal antibodies and cocktails that can be used as neutralizing agents or immunotherapy for Ebola infected patients During the pre-outbreak period it is required to vaccinate susceptible populations that will allow limiting the infection and mortalities. Furthermore, during the acute phase to neutralize virus and limiting disease symptoms, passive prophylaxis mean neutralizing antibodies are required. In the recent past few promising therapies are developed, some of these are on the clinical trial phase. Here we will review these therapies with their advantages in protecting against Filovirus.

Key Words: Pandemic, COVID 19, neutralizing antibodies

E-Mail: pksingh1976a@gmail.com

To cite this article: Singh P and Singh S; Effective countermeasure for Filovirus infections: focusing monoclonal antibodies as passive prophylaxis shield against Ebola Virus disease, Biotechnology Kiosk, Vol 2, Issue 8, PP: 18-35 (2020); DOI: https://doi.org/10.37756/bk.20.2.8.2

Introduction

As most of the world is now connected with air, land, and sea routes, the COVID-19 pandemic will become a classic example of how a single virus can affect the whole world. This pandemic possesses a real threat to human life and the world economy. COVID 19 situation brings the focus of whole scientific community on Corona CoV 2 testing, treatment, and vaccination strategies. We are living in a world with the continuous threat of new disease and infection, here we have focused on available effective countermeasures for Filovirus infections, focusing on monoclonal antibodies.

On May 11, 2017, the Ministry of Public Health of the Democratic Republic of the Congo notified international public health agencies of a cluster of suspected cases of Ebola Virus Disease (EVD) in the Likati health zone of the province of Bas Uélé. Teams from international agencies, including CDC, WHO, MSF (Doctors without Borders), and others, supported the Ministry of Public Health's epidemiologic, diagnostic, clinical, and communications efforts to respond to the outbreak. The response faced challenging logistical obstacles, including the remoteness of the area and limited services. Mobile diagnostic laboratories provided testing of samples in the affected areas. Following a period of 42 days since the second negative laboratory diagnostic test of the last confirmed patient, WHO declared an end to the outbreak on July 2, 2017. Summarizing the total 8 cases (probable or confirmed) 5 were laboratory confirmed and 4 died (50%) (https://www.cdc.gov/vhf/Ebola/outbreaks/history/chronology.html).

Earlier Ebola Virus Disease (EVD) was assumed to be too lethal to spread to large geographical areas as the disease was associated with high mortality rates of upto 100% cases (CDC, USA 2020). However, after 2013 outbreak primarily in Guinea, Liberia and Sierra Leone with 28,616 human cases, health agencies were forced to make fundamental changes in general view regarding the potential of EVD as a global threat.

The global health community recognizes the urgent need to develop strategies that can be used before, during and after any future Filovirus outbreak. These include development of vaccines and immunotherapy. The information regarding the eco - biology of Filovirus greatly hinder the development of accurate therapies. Even after more than 50 years, our understanding of Filovirus natural reservoirs is very limited. The limited information on natural host and pathology of Filovirus is one of the major causes of the limited development of new therapies.

Here, we summarize recent advances in immunotherapy strategies, focusing on Ebola targeting monoclonal antibodies (mAbs) that can be used for neutralization of virus and their potential use as passive prophylaxis.

In past few years, many epitopes on Ebola surface glycoproteins (GP) are identified. GP proteins play central role in virus entry inside host cells and pan out as effective target for mAb mediated neutralization. Various cocktails and mono-

immunotherapy have been suggested with successful results in various laboratory organisms including rodents like mice and non-human primates (macaques). Studies postulated multiple potential targets for interference to inhibit viral entry. These sites are associated with conformation and proteolytic cleavage-based activation of GP protein and interaction with its endosomal receptor, Niemann Pick C1 (NPC1) (Saphire and Aman, 2016). Peripheral B-cells isolated from past Ebola virus outbreak human survivors followed by transformation of B-cells and screening Ebola binding antibodies, is one of the best methods for identification of immunologically safer anti-Ebola mAbs. One of the first mAb developed KZ52 was also used to characterize neutralizing epitope within EBOV GP, consisting of residues at the GP1-GP2 interface (Lee et al., 2008). Similarly, cocktail of 3mAbs with trade name ZMappTM is also shown to be promising in clearance of viremia (Murin et al., 2014). Recently Zhao et al., 2017 identified several cross-neutralizing epitopes suggesting that pan Ebola or broad neutralizing antibodies (bNAbs) and cross-protective vaccines might be developed. In 2020 Fan P. et al., also reported various human origin monoclonal antibodies showing neutralizing effect.

Filovirus

The family Filoviridae is subclassified into three genera i.e. Ebolavirus, Marburgvirus and Cuevavirus (Kuhn et al. 2014). The genus Ebolavirus consists of five species: Zaire Ebolavirus (now known as EBOV), Sudan Ebolavirus (SUDV), Bundibugyo Ebola¬virus (BDBV), Tai Forest Ebolavirus (TAFV) and Reston Ebolavirus (RESTV).

The Marburgvirus genus currently consists of a two closely related virus types, Marburg Marburgvirus (MARV) and Ravn virus (RAVV) both classified as single recognized species Marburg Marburgvirus I (Amarasinghe et al. 2017). The Cuevavirus genus includes a single species, Lloviucuevavirus, with one member, Lloviu virus. Although genome of Cuevaviruswas dem¬onstrated to be present in bats in northern Spain still virus is not isolated (Negredo et al. 2011).

Disease symptoms

Ebola Virus infection is followed by initial nonspecific symptoms, such as fever, severe fatigue, weakness, and headache, sometime accompanied with a maculopapular rash. In the later phase other symptoms including nausea, vomiting, diarrhea, abdominal pain, and other appears. In severe cases lethal unexplained hemorrhage starts. Symptoms may appear anywhere from 2 to 21 days after exposure to Ebola, but the average is 8 to 10 days (Brown et al. 2017; CDC 2020).

Epidemiology

Ebola virus is one of the world's most feared diseases with mortality rates up to 25% to 90%.First discovered in 1976 with simultaneous outbreaks in Nzara, South Sudan, and Yambuka, Democratic Republic of the Congo (previously called Zaire), by 2013 there had been more than 1700 cases with a case fatality rate ranging from 25% to 90%. Of the 5 strains of EBV 4 strains of virus infect humans, with Zaire Ebolavirus as the most commonly found causative agent in outbreaks (CDC).

EVD is so fatal that until 2014 it was thought to be a localized disease with limited

risk of converting to large outbreak. The 2013 to 2016 EVD outbreak in West Africa turned out otherwise and made scientific community to rethink the potential of EVD. During 2013-2016 reports of cases imported in different countries were also very high. For the first time in the history of Ebola virus outbreaks, world witness the deadly potential of Ebola in term of population infected and area involved. Modern world easy air, land, and water connectivity helps the spread of the disease at easier and faster rate. Cases were imported into, some with localized onward transmission in Mali (8), Nigeria (20), the United States (2), the United Kingdom (1), Senegal (1), and Spain (1), along with the repatriation of a further 24 patients to the United States and Europe. World Health Organization (WHO) declared the conclusion of the Public Health Emergency of International Concern in March 2016, more than 28,000 people had been infected, with more than 11,00 deaths (CDC).

Host animals

Limited numbers of studies are available to understand the ecology of Filovirus Studies suggest that Filovirus are zoonotic. Although Filovirus natural reservoir (s) still not clear, but recently Marburgvirus and Ebolavirus were detected in fruit bats in Africa. Marburgvirus has also been isolated in several occasions from Rousettus bats in Uganda. To date, the only wild nonhuman primates found infected with Ebola viruses were African great apes: western lowland gorillas (Gorilla gorilla gorilla) and central chimpanzees (Pan troglodytes troglodytes) with EBOV and western chimpanzees (Pan troglodytes verus) with Taï Forest Ebolavirus (TAFV) (Leroy et al. 2004a, 2004b, 2005). Mortalilty is also reported by Reston Ebolavirus (RESTV) in long-tailed macaques (Macacafascicularis) held under captivity (Gogarten et al. 2017).

Human Transmission

Lawrence et al. (2017) reviewed our current understanding of Ebola virus transmission in humans. EBOV has been detected as virus particle or its RNA in a range of bodily fluids including blood, stool, semen, breast milk, saliva, sweat and tears. Transmission of disease from infected to healthy individual is believed to be via contact of these body fluids, fomites, droplets and aerosols. Experimenal models using NHPs have shown that EBOV is both highly infectious and contagious; it can use many administration routes including oral, conjunctival, submucosal and respiratory routes. EBOV have been detected in semen of survivors at least up to around 500 days (Diallo et al. 2016).

Viral genome and proteins

Ebolavirion contains non segmented around 19kb of linear, negative-sense, single-stranded RNA. Capping and tailing of RNA absent, although 3’ and 5’ UTRs are present. The viral genome encodes a message for 7 structural proteins and 1 non-structural protein in gene order of 3′ –leader (UTR)– NP – VP35 – VP40 – GP/sGP – VP30 – VP24 – L – trailer (UTR) – 5′ (figure 1). UTR regions contain sequences responsible for gene regulation and new virus assembly.

Understanding of Ebola Virus pathogenesis is used as a hallmark of Filovirus pathogenesis. Most of the information comes from experiments involving non-human primates. The disease

severity in humans and NHPs appears to be an outcome of aggressive virus replication and inflammatory response by the host defense system. Possibly, various cytokines and cytotoxic molecules are released during Filovirus infection that results in disease symptoms, including high fever, vascular leakage and coagulopathy (Messaoudi et al. 2015).

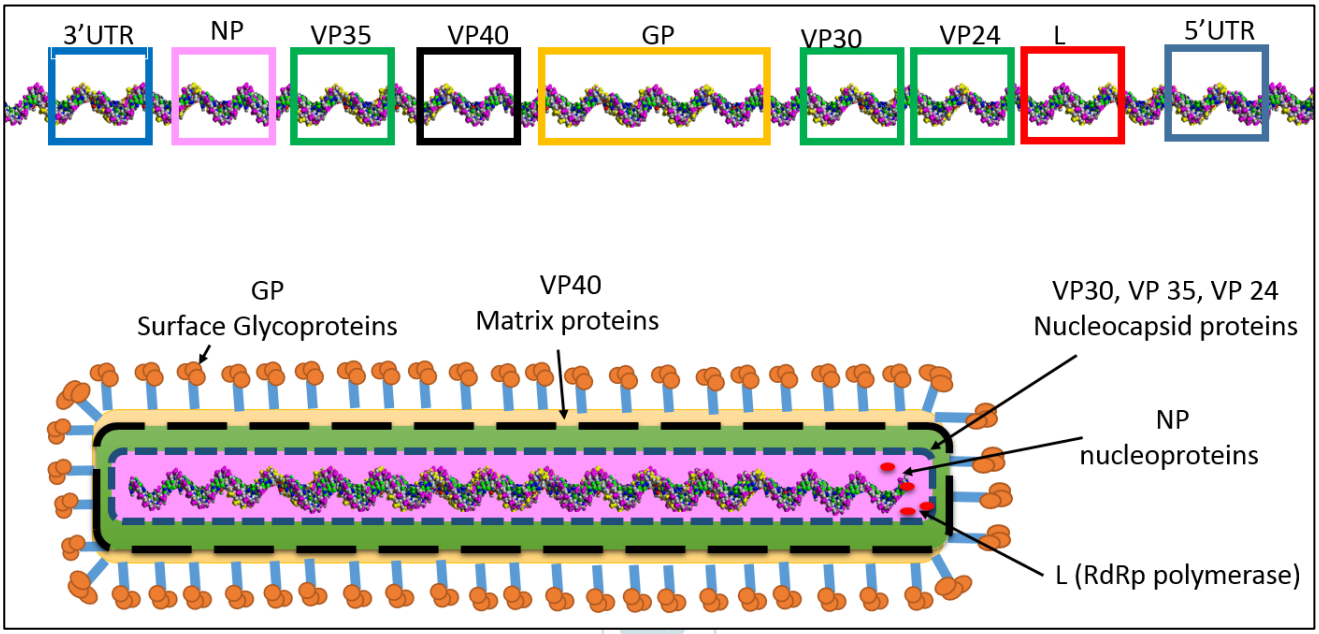


Figure1: *Ebola Genome and corresponding products of gene products: Ebola genome is around 19kb long ss(—) RNA with 7 protein coding genes flanked by 3' and 5' untranslated regions UTRs. 5' m7G capping and 3' polyA tailing is absent.*

Ebola genomic RNA is packed in nucleocapsid consisting of multiple proteins: NP (nucleoprotein), L ('large', polymerase), and viral proteins VP35, VP30, and VP24. Host derived membrane envelope has two transmembrane proteins i.e glycoprotein (GP) spike trimers and VP40 matrix protein (Beniac et al. 2017). GP trimers is a class I fusion protein and play role in attachment to host cells, endosomal entry, and membrane fusion. Many researchers have discovered antibodies targeting GP proteins and convincingly demonstrated the importance of GP protein as target for immunotherapy (Beniac et al. 2017).

Experiments with GP expression in Ebola infected Vero E6 cell reveals that after transcription of GP gene it produces three type of GP proteins using post transcription RNA editing. Unedited mRNA (71%)istranslated to 364-residue soluble GP protein that later forms disulfide bridged dimers and a smaller peptide fragment called Δ (Delta) resulted due to furin cleavage of larger precursor of soluble GP. Around 24% GP mRNA are edited causing +1 shift in reading frame. The resulting +1 ORF translated to 676-residue full length GP that is further cleaved by Furin, forming membrane bound GP1/GP2 trimers. GP1

consists of RBS while GP2 wrap GP1 like a ribbon. The trimer structure forms chalice like structure and consists of glycan cap, receptor binding site (RBS), IFL cathepsin loop, Furin cleavage site, transmembrane domain, mucin like domain (M), TNF-α converting enzyme (TACE) cleavage site. Another 5% with +2 frameshift translated to much smaller soluble GP (ssGP). Both soluble GPs are N-linked glycosylated and present in easily detectable quantities body fluids. Δ peptide is O- linked glycosylated while GP1/GP2 trimers are both N- and O-linked glycosylated (Ning et al. 2016; Beniac et al. 2017). GP1/GP2 trimers are further cleaved to soluble GP trimers (GPCL), particles seen as GP shedding.

Virus particles attach to host cell through two types of relatively non-specific receptors: 1. Carbohydrate interacting C-type lectins (CLECs) and 2. Phosphatidylserine (PtdSer) receptors that interact with the viral envelope Phosphatidylserine. Other receptors like CLECs (LSECTin, DC-SIGN [dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin], L-SIGN [liver/lymph node-specific ICAM-3 grabbing nonintegrin], MBL mannose-binding lectin, and hMGL [human macrophage galactose- and N-acetylgalactosamine-specific C-type lectin]) are also thought to play role in viral glycans attachment. Micropinocytosis is thought to be primary entry route of Ebola virus. Initially virus particles are trapped in endosomes followed by environmental changes in form of lowering of pH. Lower pH and possibly activation of some hydrolytic enzymes triggers conformation changes in GP1/GP2 trimers leading to exposure of receptor binding domains on GP1.

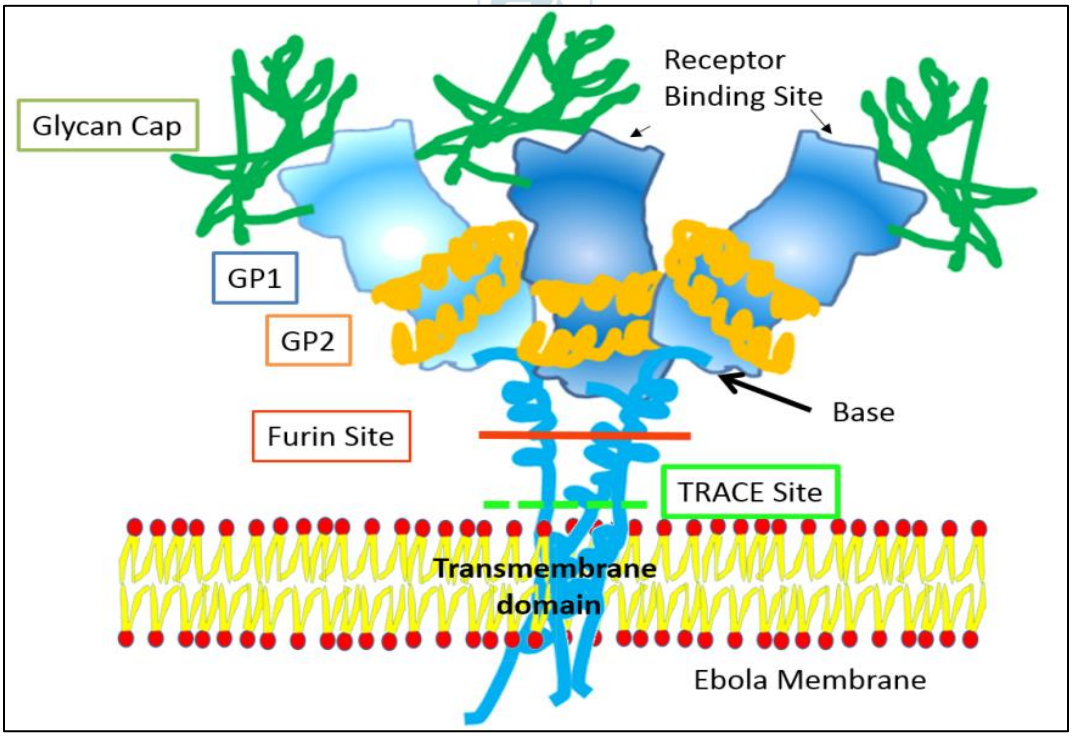


Figure 2: *Structure of Ebola Glycoprotein (GP) trimers.*

In endosome Nieman-Pick disease type C1 (NPC-1)- protein is demonstrated as receptors for GP. Interaction with NPC-1 promotes further conformation changes in GP2 leading to exposure of fusion loop that embedded in endosomal membrane leading to fusion of viral and endosomal membrane. The viral genomics released in cytosol to proceed next stages of virion replication cycles leading to generation of new virus particle (Bornholdt et al., 2016a; Miller et al., 2012; Wang et al., 2016a).

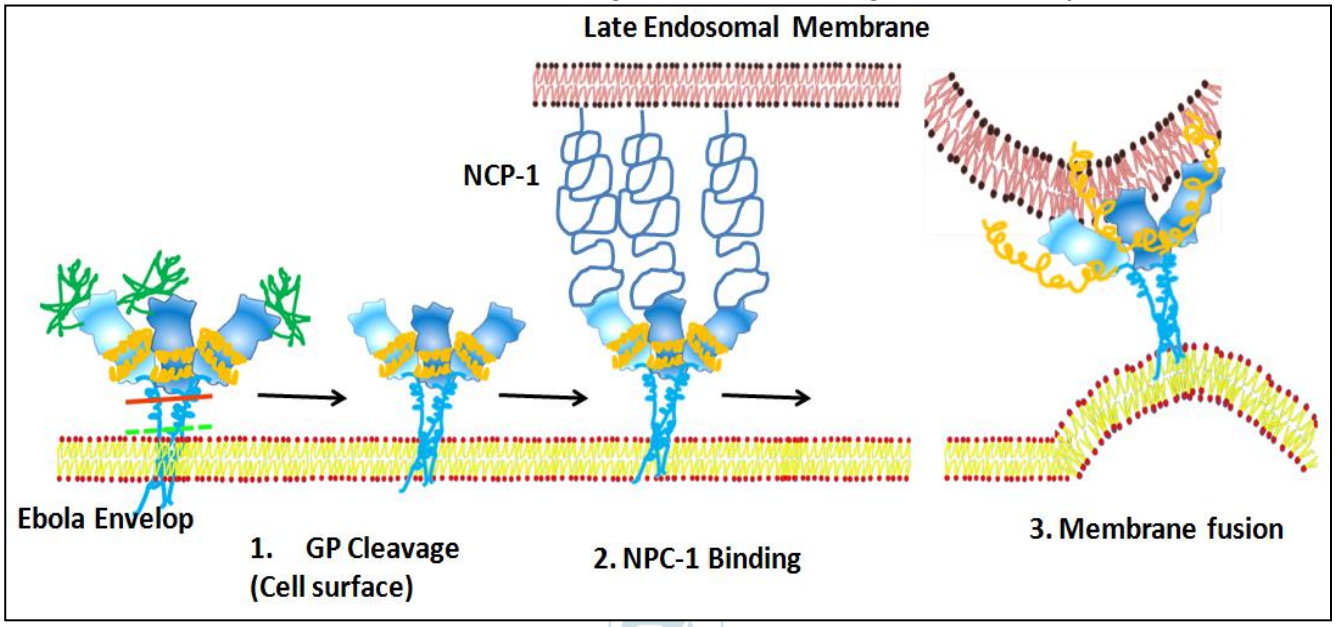


Figure 3: *Role of GP protein in viral membrane fusion with endosome membrane.*

Diagnostic methods

Initial phase of Ebola infection causes generalized symptoms like fever, making it difficult to suspect Ebola symptomatically. Suspected patients can me diagnosed by any of the available methods as prescribed by CDC, USA. The test includes: 1. for within a few days after symptoms begin: Antigen-capture enzyme-linked immunosorbent assay (ELISA) testing, IgM ELISA, Polymerase chain reaction (PCR) and Virus isolation; 2. Later in disease course or after recovery: IgM and IgG antibodies and 3. Retrospectively in deceased patients: Immunohistochemistry testing, PCR and Virus isolation (CDC, USA).

Immunotherapy

Importance of GP in virus entry make it most important target for the development of immunotherapy. Currently, three modes of viral targets are identified: 1. Inhibition of receptor binding, 2. Cathepsin-mediated cleavage inhibition, and 3. Blocking structural rearrangements of GP2 involved in formation of fusion loop. Monoclonal antibodies are currently most useful immunotherapy agents (Saphire and Aman 2017). In the past few years, many different antibodies were generated using EBOV specific B-cells

isolated from human survivors. Other approaches for generation of mAbs using naïve antibody repertoire or somatic mutations were also successfully utilized. Summary of some antibodies are given in table 1. Similarly, epitopes discovered on GP1- GP2 trimer is given in Figure 4. One of the first antibodies developed was KZ52 that interacts with GP in its trimeric, pre-fusion conformation (GP1+GP2). The Ab was derived from B- cells of a human survivor of the 1995 Kikwit outbreak. KZ52 specific epitope were mapped to base of GP1/GP2 trime - the assembly responsible for fusion of viral membrane to endosome. KZ52 proved to be neutralizing ab in rodents but failed to protect EBOV-infected non-human primates (NHPs) (Lee et al. 2008).

Soon after the initial reports on anti EBOV antibody, idea of using cocktails of different antibodies were materialized. One of the first successful cocktails was developed by trade name ZMapp™, it consists of three monoclonal antibodies (mAbs). The mAbs 2G4 and 4G7, binds to epitope present at base of GP1/GP2 trimer, while mAb3C6 binds carbohydrate cap. ZMapp™ provides 100% protection of NHPs 5 days post-infection. ZMapp™ is the first successful candidate immunotherapy suggesting importance of combination of different target binding Ab, a key possible treatment for patients infected with EBV. This also shows importance of GP1/GP2 in viral entry and their importance as target for generation of neutralizing antibody (Wong et al. 2014).

Various other antibodies were also developed that specifically binds to epitopes present on different part of GP1/GP2 trimers. Here we tried to summarize some of these antibodies with information on their targets in very brief.

Furuyama et al. (2016) reported neutralizing mAb (6D6) that targets internal fusion loop in GP molecule thereby hindering membrane fusion of the viral envelope with endosomal membranes. The mAb recognize Ebolavirus glycoprotein, it effectively inhibits cellular entry all known Ebolavirus species in vitro. The mAb was successful in mouse models.

Flyak et al. (2016) isolated several antibodies specific to glycan cap that neutralized multiple Ebolaviruses, including SUDV as demonstrated in guinea pigs. The ab isolate from transformed B- cell isolated from human survivors of 2007 Uganda BDBV outbreak.

Bornholdt et al. (2016b) isolated and characterized 349 GP-specific monoclonal antibodies (mAbs). Antibodies were prepared from in vitro Epstein Barr virus mediated transformed B cells isolated from survived 2014 EBOV Zaire outbreak. They show 77% of the mAbs neutralize live EBOV, and several mAbs exhibit unprecedented potency. GP1/GP2 trimer stalk regions were appear to be the primary target for antibodies leading to inhibition of membrane fusion. The successful results were seen in mice.

Keck et al. (2016) identifies a set of pan-Ebolavirus and pan-Filovirus monoclonal antibodies (FVM02) derived from cynomolgus macaques. The macaques were challenged with mixture of GP and virus-like particles representing three different Filovirus species. Many different antibodies were isolated with different epitopes on GP. Antibody binding to a highly conserved

epitope within the fusion loop of Ebolavirus and Marburgvirus species was also identified. Significant success in neutralizing EBOV was reported in mouse model of EBOV infection.

Everardo González-González (2020) describe the development of HEK293T cells engineered for stable expression of mAb13C6, a neutralizing anti-EBOV monoclonal antibody. The produced antibodies exhibited the expected functionality; they recognized the GP glycoprotein of the Ebola virus in both ELISA assays and cell binding experiments using HEK293T cells engineered to express the EBOV GP at their membrane surface.

Xiaoyan Tian (2020) reported the induction and isolation of two monoclonal antibodies that specifically recognized the glycoprotein (GP) and secreted glycoprotein (sGP) of the Ebola virus. Plasmids encoding either GP or sGP were constructed and immunized BALB/c mice, accordingly purified sGP was boosted. The antisera were analyzed for binding activity against sGP protein in enzyme-linked immunosorbent assay (ELISA) and neutralization activity in a pseudo typed virus neutralization assay.

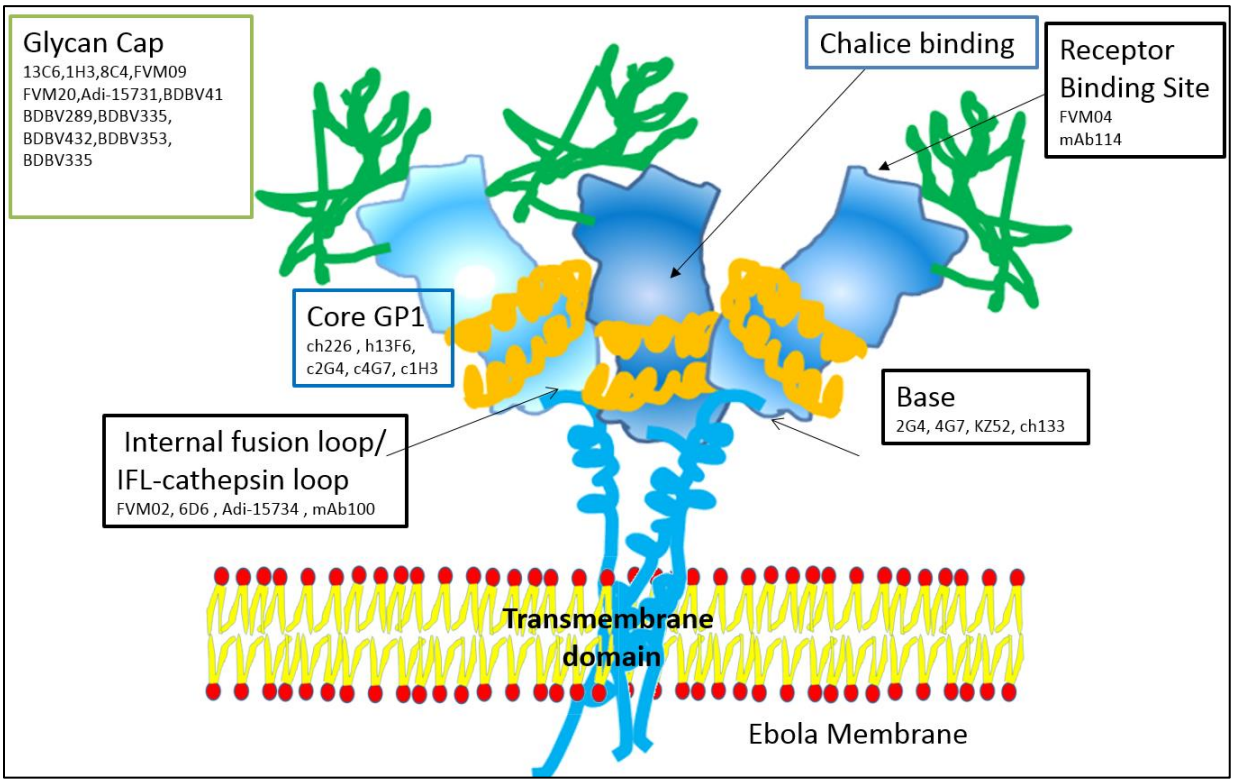


Figure 4: *GP1/GP2 associated epitopes.*

Table 1. Table: Summary of mAbs identified as agents of immunotherapy against Ebola virus

Antibody	source	Target Epitope	results	reference
First Antibody used				
KZ52 (monoclonal)	1995 human survivor	residues within both GP1 and GP2 at the base of the trimer	neutralization and protection of rodents from EBOV	Lee, J.E. et al. (2008)
Engineered Antibody				
bispecific antibody (bsAb)	engineered	'Trojan horse' mechanism binds both NCP-1 and GP	neutralization all EBOV, Mice	Wec et al. 2016
Cocktails of mAbs				
MB-003 (MappBio) c13C6, h13F6, c6D8	vaccinated mice	GP core, glycan cap, mucin-like domain	non neutralizer in absence of complement	Olinger GG Jr, et al. (2012)
ZMAb (Defyrus) c2G4, c4G7, c1H3	vaccinated mice	GP core	Neutralizing, non-neutralizing (c1H3)	Qiu et al. (2012)
ZMapp : c13C6, c2G4, and c4G7	vaccinated mice	trimeric GP chalice	100% protection of NHPs 5 days post-infection	Wong et al. 2014
Next gen Cocktail: ADI-15878, ADI-15946, and/or CA45	2014 Zaire outbreak and Macaques	internal fusion loop with the N-terminus GP, glycan cap, soluble GP (sGP), regions of the trimeric GP spike including	Not evaluated in combination	Zhao et al. (2017)

Others				
2G1 + others	Monoclonal antibodies from healthy person vaccinated with Ad5-EBOV	Against GP	Neutralizer	P. Fan et. al. 2020
mAb13C6	Genetic engineered expression in HEK293T cells	Against GP	Neutralizer	Gonzalez-Gonzalez et. al. 2020
T231 and T242	Monoclonal antibodies	Against glycoprotein (GP) and secreted glycoprotein (sGP) of the Ebola virus.	Variable	X tian et al. 2020
6D6	mouse hybridoma	against the internal fusion loop (IFL)	neutralizer	Furuyama et al. 2016
'stalk-binders' 57 antibodies	survivors of 2007 Uganda BDBV infection	GP \glycan cap	variable	Flyak et al 2016
349 Abs including 2G4 or 4G7	2014 Zaire outbreak	mucin-like domain, glycan cap	variable	Bornholdt et al 2016
pan-filovirus antibody (FVM02)	cynomolgus macaques	core GP, Glycan Cap, fusion loop	variable pan Ebola Pan Filovirus	Keck et al.2015
mAb100, mAb114, mAb165, and mAb166	human survivor of the 1995 Kikwit outbreak	GP2 IFL as well as residues at the GP1 N terminus	moderate neutralizer	corti et. al. 2016
mAb114	human survivor of the 1995 Kikwit outbreak	the glycan cap and inner chalice of GP	human survivor of the 1995 Kikwit outbreak	Misasi et al. 2016
pan-ebolavirus mAb, termed FVM04	macaques	RBS	pan-Ebola and SUDV	Howell et al 2016

CA45	macaques	internal fusion loop with the N-terminus GP	pan ebola	Zhao et al. (2017)
m21D10	mouse	GP		Holtsberga et al. 2016
m16G8	mouse	GP2		Holtsberga et al. 2016
m8C4	mouse	glycan cap and possibly neighboring residues within the core GP1 and cathepsin cleavage site	EBOV and SUDV	Holtsberga et al. 2016
m17C6	mouse	GP		Holtsberga et al. 2016
m16G8, m8C4, m17C6, and m4B8	mouse	GP	variable	Holtsberga et al. 2016
ADI-15878, ADI-15946, and ADI-15742	2014 Zaire outbreak	glycan cap, soluble GP (sGP), regions of the trimeric GP spike including the base, internal fusion loop (IFL) and the stalk	ADI-15878 and ADI-15742 shown highly potent neutralizing activity against all five known Ebolaviruses	Wec et al. (2017)

Corti et al. (2016) isolated peripheral B-cells from a survivor of the 1995 Kikwit, Democratic Republic of the Congo outbreak. One antibody (mAb100) provides protection in macaques up to 5 days post-infection of EBOV. Misasi et al. using crystal structures of antibodies bound GP reveals epitope near the tip of the GP2 IFL as well as residues at the GP1 N-terminus, which mediates viral cell entry. Interestingly mAb114 was shown interacting with the glycan cap and inner chalice of GP trimer. Even after removal of carbohydrate cap mAb114 remains associated. This binding may cause hindrance in interaction between GP RBS and NPC-1 leading to failure of membrane fusion.

Another pan Ebolavirus mAb, (FVM04) isolated from vaccinated macaque, identified by Howell *et al. (2016),* binds to the tip of the RBS crest and blocks NPC-1 binding . Wec et al. (2017) screened their

previously published 349 mAb library for broad neutralizers. Two mAbs were identified that could potently neutralize all five Ebolaviruses. The antibody targets the glycan cap, soluble GP (sGP), regions of the trimeric GP spike including the base, internal fusion loop (IFL) and the stalk. Prominent mAbs identified are the base and internal fusion loop (IFL) binders ADI-15878, ADI-15946, and ADI-15742, of these ADI-15878 and ADI-15742 shown highly potent neutralizing activity against all five known Ebolaviruses. ADI-16061 is the stalk binder.

Zhao et al. (2017) characterizes antibody (CA45) isolated immunized macaque. CA45 targets the internal fusion loop with the N-terminus GP. It potently neutralizes Ebola, Sudan, Bundibugyo, and Reston viruses; therefore, it is a candidate immunotherapy agent that can be used alone or in combination with other antibodies. It provided full protection against all pathogenic Ebolaviruses in mice, guinea pigs, and ferrets. Zhao et al. suggested that next generation cocktails of mAbs can comprise ADI-15878, ADI- 15946, and/or CA45.

Interestingly Wec et al. (2016) describe the use of 'Trojan horse' strategy using bispecific antibody, in which mAbs specific for NPC1 or the GP receptor-binding site are coupled to a mAb against a conserved, surface-exposed GP epitope. In mice, bispecific antibodies neutralized all ebolaviruses types.

Conclusion

In summary, these studies demonstrated the importance of GP proteins in viral entry into host cell. Various neutralizing epitopes are discovered on GP1, GP2 and glycan cap. Many different mAbs are identified and suggested to being used as monotherapy or cocktail. Antibodies like KZ52, 2G4, 4G7, 13C6, 6D6, FVM02, mAb100, mAb114, FVM04, CA45 and others successfully demonstrate the neutralization potential of these mAbs. Shedding of GP in body fluids may provide additional problem in use of mAbs. As most of the identified mAbs recognize epitopes on parts of GP trimer that is subject to cleaved during viral entry (GP shedding), this will provide the competition to circulating antibody and may lower the avidity of neutralizing antibodies.

It is suggested that cocktail of mAbs has higher potential to neutralize Ebola virus as compared to use of single mAb type. It is understandable that use of multiple antibodies that recognizes different target, have better chance than single antibodies. We should also explore these antibodies for their role in controlling the symptoms of disease in infected animals. This information will provide us the true potential of mAbs as agent of neutralization and passive prophylaxis in Ebola infected patient. At the moment we require a robust reagent that not only neutralize the Ebola Virus but also help in controlling disease associated symptoms. Use of neutralizing antibodies as passive prophylactic can be of great use to reduce mortality rates during future outbreaks. New approach like 'Trojan horse' bispecific antibodies have potential as broad anti-filovirus immune therapeutics. We should never underestimate the potential of other relatively silent Filovirus as agent of future outbreaks. Development of pan Ebola or pan Filovirus mAbs will surely be a step-in preparation for future.

References

1. Amarasinghe, Gaya K., YīmíngBào, Christopher F. Basler, SinaBavari, Martin Beer, NicolásBejerman, Kim R. Blasdell et al. "Taxonomy of the order Mononegavirales: Update 2017." *Archives of Virology* (2017): 1-12. doi: 10.1007/s00705-017-3311-7
2. Baseler, Laura, Daniel S. Chertow, Karl M. Johnson, Heinz Feldmann, and David M. Morens. "The pathogenesis of Ebola virus disease." *Annual Review of Pathology: Mechanisms of Disease* 12 (2017): 387-418. DOI: 10.1146/annurev-pathol-052016-100506
3. Beniac, Daniel R., and Timothy F. Booth. "Structure of the Ebola virus glycoprotein spike within the virion envelope at 11 Å resolution." *Scientific reports* 7 (2017): 46374.https://doi.org/10.1038/srep46374
4. Bornholdt, Zachary A., Esther Ndungo, Marnie L. Fusco, Shridhar Bale, Andrew I. Flyak, James E. Crowe, Kartik Chandran, and Erica Ollmann Saphire. "Host-primed Ebola virus GP exposes a hydrophobic NPC1 receptor-binding pocket, revealing a target for broadly neutralizing antibodies." *MBio* 7, no. 1 (2016a): e02154-15. doi: 10.1128/mBio.02154-15
5. Bornholdt, Zachary A., Hannah L. Turner, Charles D. Murin, Wen Li, Devin Sok, Colby A. Souders, Ashley E. Piper et al. "Isolation of potent neutralizing antibodies from a survivor of the 2014 Ebola virus outbreak." *Science* 351, no. 6277 (2016b): 1078-1083. DOI: 10.1126/science.aad5788
6. Brown, Colin S., Stephen Mepham, and Robert J. Shorten. "Ebola Virus Disease." *Clinics in Laboratory Medicine* 37, no. 2 (2017): 269-284. DOI: 10.1016/j.cll.2017.01.003
7. CDC: Centers for Disease Control and Prevention. Outbreaks chronology: Ebola hemorrhagic fever [Internet]. Atlanta (GA): CDC; 2014. Available at: http://www.cdc.gov/vhf/ebola/resources/outbreak-table.html. Accessed September 2, 2020.
8. Corti, Davide, John Misasi, SabueMulangu, Daphne A. Stanley, Masaru Kanekiyo, Suzanne Wollen, AuréliePloquin et al. "Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody." *Science* 351, no. 6279 (2016): 1339-1342. DOI: 10.1126/science.aad5224
9. Diallo, Boubacar, DaoudaSissoko, Nicholas J. Loman, HadjaAïssatou Bah, Hawa Bah, Mary Claire Worrell, RamataSacko et al. "Resurgence of Ebola virus disease in Guinea linked to a survivor with virus persistence in seminal fluid for more than 500 days." *Clinical infectious diseases* 63, no. 10 (2016): 1353-1356. https://doi.org/10.1093/cid/ciw601
10. Fan, Pengfei, et al. "Potent neutralizing monoclonal antibodies against Ebola virus isolated from vaccinated donors." Mabs. Vol. 12. No. 1. Taylor & Francis, 2020. https://doi.org/10.1080/19420862.2020.1742457
11. Flyak, A. I., X. Shen, C. D. Murin, H. L. Turner, J. A. David, M. L. Fusco, R. Lampley et al. "Cross-reactive and potent neutralizing antibody responses in human survivors of natural Ebolavirus infection. Cell164: 392–405." (2016). DOI: 10.1016/j.cell.2015.12.022

12. Furuyama, W., A. Marzi, A. Nanbo, E. Haddock, J. Maruyama, H. Miyamoto, M. Igarashi et al. "Discovery of an antibody for pan-ebolavirus therapy. Sci Rep 6: 20514." (2016). DOI: https://doi.org/10.1038/srep20514
13. Gogarten, Jan F., Sebastien Calvignac-Spencer, and Fabian H. Leendertz. "Ebola Virus Disease." *The International Encyclopedia of Primatology* (2017). https://doi.org/10.1002/9781119179313.wbprim0390
14. Gonzalez-Gonzalez E, Palestino-Diaz I, Lopez-Pacheco F, Marquez-Ipiña AR, Lara-Mayorga IM, Trujillo-de Santiago G, Alvarez MM. Rapid and cost-effective development of stable clones for the production of anti-Ebola monoclonal antibodies in HEK293T cells. bioRxiv. 2020 Jan 1. DOI: 10.1101/2020.04.21.054429
15. Howell, Katie A., XiangguoQiu, Jennifer M. Brannan, Christopher Bryan, Edgar Davidson, Frederick W. Holtsberg, Anna Z. Wec et al. "Antibody treatment of Ebola and Sudan virus infection via a uniquely exposed epitope within the glycoprotein receptor-binding site." *Cell reports* 15, no. 7 (2016): 1514-1526. doi: 10.1016/j.celrep.2016.04.026
16. Keck, Zhen-Yong, Sven G. Enterlein, Katie A. Howell, Hong Vu, Sergey Shulenin, Kelly L. Warfield, Jeffrey W. Froude et al. "Macaque monoclonal antibodies targeting novel conserved epitopes within filovirus glycoprotein." *Journal of virology* 90, no. 1 (2016): 279-291. https://doi.org/10.1128/JVI.02172-15
17. Kuhn, Jens H., Kristian G. Andersen, Sylvain Baize, YīmíngBào, SinaBavari, Nicolas Berthet, Olga Blinkova et al. "Nomenclature-and database-compatible names for the two Ebola virus variants that emerged in Guinea and the Democratic Republic of the Congo in 2014." *Viruses* 6, no. 11 (2014): 4760-4799. doi: 10.3390/v6114760
18. Kuhn, Jens H., YīmíngBào, SinaBavari, Stephan Becker, Steven Bradfute, Kristina Brauburger, J. Rodney Brister et al. "Virus nomenclature below the species level: a standardized nomenclature for filovirus strains and variants rescued from cDNA." *Archives of virology* 159, no. 5 (2014): 1229-1237. doi: 10.1007/s00705-013-1877-2
19. Lawrence, Philip, Nicolas Danet, Olivier Reynard, Valentina Volchkova, and Viktor Volchkov. "Human transmission of Ebola virus." *Current opinion in virology* 22 (2017): 51-58. DOI: 10.1016/j.coviro.2016.11.013
20. Lee, Jeffrey E., Marnie L. Fusco, Ann J. Hessell, Wendelien B. Oswald, Dennis R. Burton, and Erica Ollmann Saphire. "Structure of the Ebola virus glycoprotein bound to a human survivor antibody." *Nature* 454, no. 7201 (2008): 177. DOI: 10.1038/nature07082
21. Leroy, E. M., P. Telfer, B. Kumulungui, P. Yaba, P. Rouquet, P. Roques, J-P. Gonzalez, T. G. Ksiazek, P. E. Rollin, and E. Nerrienet. "A serological survey of Ebola virus infection in central African nonhuman primates." *The Journal of infectious diseases*190, no. 11 (2004): 1895-1899. DOI: 10.1086/425421

22. Leroy, Eric M., Brice Kumulungui, Xavier Pourrut, Pierre Rouquet, Alexandre Hassanin, Philippe Yaba, André Délicat, Janusz T. Paweska, Jean-Paul Gonzalez, and Robert Swanepoel. "Fruit bats as reservoirs of Ebola virus." *Nature* 438, no. 7068 (2005): 575-576. DOI: 10.1038/438575a
23. Leroy, Eric M., Pierre Rouquet, Pierre Formenty, Sandrine Souquière, Annelisa Kilbourne, Jean-Marc Froment, Magdalena Bermejo et al. "Multiple Ebola virus transmission events and rapid decline of central African wildlife." *Science* 303, no. 5656 (2004): 387-390. DOI: 10.1126/science.1092528
24. Messaoudi, Ilhem, Gaya K. Amarasinghe, and Christopher F. Basler. "Filovirus pathogenesis and immune evasion: insights from Ebola virus and Marburg virus." *Nature reviews. Microbiology* 13, no. 11 (2015): 663. DOI: 10.1038/nrmicro3524
25. Miller, Emily Happy, GregorObernosterer, MatthijsRaaben, Andrew S. Herbert, Maika S. Deffieu, Anuja Krishnan, Esther Ndungo et al. "Ebola virus entry requires the host-programmed recognition of an intracellular receptor." *The EMBO journal* 31, no. 8 (2012): 1947-1960. doi: 10.1038/emboj.2012.53
26. Misasi, John, Morgan SA Gilman, Masaru Kanekiyo, Miao Gui, Alberto Cagigi, SabueMulangu, DavideCorti et al. "Structural and molecular basis for Ebola virus neutralization by protective human antibodies." *Science* 351, no. 6279 (2016): 1343-1346. doi: 10.1126/science.aad6117
27. Murin, Charles D., Marnie L. Fusco, Zachary A. Bornholdt, XiangguoQiu, Gene G. Olinger, Larry Zeitlin, Gary P. Kobinger, Andrew B. Ward, and Erica Ollmann Saphire. "Structures of protective antibodies reveal sites of vulnerability on Ebola virus." *Proceedings of the National Academy of Sciences* 111, no. 48 (2014): 17182-17187. https://doi.org/10.1073/pnas.1414164111
28. Negredo, Ana, Gustavo Palacios, Sonia Vázquez-Morón, Félix González, HernánDopazo, Francisca Molero, Javier Juste et al. "Discovery of an ebolavirus-like filovirus in europe." *PLoS pathogens* 7, no. 10 (2011): e1002304.
29. Ning, Yun-Jia, Fei Deng, Zhihong Hu, and Hualin Wang. "The roles of ebolavirus glycoproteins in viral pathogenesis." *VirologicaSinica* (2016): 1-13. doi: 10.1371/journal.ppat.1002304
30. Olinger, Gene Garrard, James Pettitt, Do Kim, Cara Working, OgnianBohorov, Barry Bratcher, Ernie Hiatt et al. "Delayed treatment of Ebola virus infection with plant-derived monoclonal antibodies provides protection in rhesus macaques." *Proceedings of the National Academy of Sciences* 109, no. 44 (2012): 18030-18035. doi: 10.1073/pnas.1213709109
31. Qiu, Xiangguo, Jonathan Audet, Gary Wong, Stephane Pillet, Alexander Bello, Teresa Cabral, Jim E. Strong et al. "Successful treatment of Ebola virus–infected cynomolgus macaques with monoclonal antibodies." *Science translational medicine* 4, no. 138 (2012): 138ra81-138ra81.

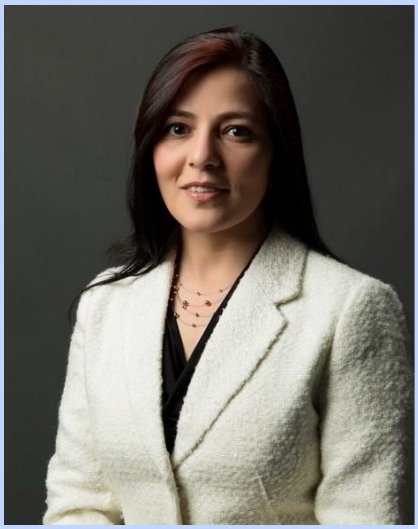
https://doi.org/10.1126/scitranslmed.3003876

32. Saphire, Erica Ollmann, and M. JavadAman. "Feverish Quest for Ebola Immunotherapy: Straight or Cocktail?." *Trends in microbiology* 24, no. 9 (2016): 684-686. DOI: 10.1016/j.tim.2016.05.008
33. Tian X, Chen D, Wang H, Xu S, Zhu L, Wu X, Wu Z. The induction and characterization of monoclonal antibodies specific to GP of Ebola virus. Journal of medical virology. 2020 Aug;92(8):996-1006. https://doi.org/10.1002/jmv.25615
34. Wang, Han, Yi Shi, Jian Song, Jianxun Qi, Guangwen Lu, Jinghua Yan, and George F. Gao. "Ebola viral glycoprotein bound to its endosomal receptor Niemann-Pick C1." *Cell* 164, no. 1 (2016): 258-268. DOI: 10.1016/j.cell.2015.12.044
35. Wec, Anna Z., Andrew S. Herbert, Charles D. Murin, Elisabeth K. Nyakatura, Dafna M. Abelson, J. Maximilian Fels, Shihua He et al. "Antibodies from a Human Survivor Define Sites of Vulnerability for Broad Protection against Ebolaviruses." *Cell* 169, no. 5 (2017): 878-890. doi: 10.1016/j.cell.2017.04.037
36. Wec, Anna Z., Elisabeth K. Nyakatura, Andrew S. Herbert, Katie A. Howell, Frederick W. Holtsberg, Russell R. Bakken, Eva Mittler et al. "A “Trojan horse” bispecific-antibody strategy for broad protection against ebolaviruses." *Science* 354, no. 6310 (2016): 350-354. https://doi.org/10.1126/science.aag3267
37. Wong, Gary, XiangguoQiu, Gene G. Olinger, and Gary P. Kobinger. "Post-exposure therapy of filovirus infections." *Trends in microbiology* 22, no. 8 (2014): 456-463. DOI: 10.1016/j.tim.2014.04.002
38. Zhao, Xuelian, Katie A. Howell, Shihua He, Jennifer M. Brannan, Anna Z. Wec, Edgar Davidson, Hannah L. Turner et al. "Immunization-elicited broadly protective antibody reveals ebolavirus fusion loop as a site of vulnerability." *Cell* 169, no. 5 (2017): 891-904. DOI: 10.1016/j.cell.2017.04.038

Biotechnology Advances around the World

Editor's Picks

Every issue of Biotechnology Kiosk presents select latest research news picked by the editors-in-chief on significant research breakthroughs in different areas of biotechnology around the world. The aim is to promote further R&D in all of these cutting edge areas of biotechnology. The editors have compiled and included the following innovations and breakthroughs to highlight the latest biotechnology advances.



Dr. Megha Agrawal
Co Editor-in-Chief

Dr. Shyamasri Biswas
Co Editor-in-Chief

Enzyme Technology

An innovative super enzyme system for recycling plastic infinitely and reduce global plastic pollution

It is widely known that plastics pollution poses a crisis and serious threat to the global environment health that needs to be addressed urgently. Research in this direction has focused on microbes to leverage their capacity to utilize synthetic polymers as carbon and energy sources. Polyethylene terephthalate 'PET' is an important engineering polymeric material, which is the most common thermoplastic. This material is frequently used to make single-use drinks bottles, clothing and carpets. The biodegradation of PET is extremely slow and it takes hundreds of years to break down in the environment.

However, a recently developed enzyme PETase was demonstrated in shortening this time to days. In a previous discovery, the enzyme PETase was shown to be capable of breaking down PET back into its building blocks. This breakthrough created an opportunity to recycle plastic infinitely and reduce plastic pollution. This include reducing the greenhouse gases driving climate change. This initial discovery paved the way to revolutionize the plastic recycling industry by creating a potential low-energy solution to tackle plastic waste. The engineered natural PETase enzyme showed about 20 percent faster at breaking down PET. While PETase based approach was shown not fast enough to make the process commercially viable to handle the tons of discarded PET bottles littering the planet, nevertheless, this provided the first hope and a potential solution to the global plastic pollution problem.

Now, a transatlantic team of scientists recently demonstrated re-engineered plastic-eating enzyme PETase to create a two-enzyme system or an enzyme 'cocktail' that can digest plastic up to six times faster compared to just 20 percent breaking down action provided by PETase alone. Their study was published in the journal Proceedings of the National Academy of Sciences (Characterization and engineering of a two-enzyme system for plastics depolymerization, Proceedings of the National Academy of Sciences, 2020; 202006753 DOI: 10.1073/pnas.2006753117).

In their study, researchers employed a second enzyme called MHETase, which is usually found in the same rubbish dwelling bacterium that lives on a diet of plastic bottles. Subsequently, they combined the second enzyme with PETase to speed up the breakdown of plastic. This approach involved simply mixing of PETase with MHETase that doubled the speed of PET breakdown. They showed that engineering a connection between the two enzymes can create a super-enzyme that increase this activity by a further three times.

The mechanism of working of PETase and the newly combined MHETase-PETase super enzyme both work by digesting PET plastic, and subsequently returning it to its original building blocks. This process can be leveraged for plastics to be made and reused infinitely that can greatly reducing dependence on fossil resources such as oil

and gas. The research team solved the 3D crystal structure of the MHETase enzyme by using the Diamond Light Source Oxfordshire in the U.K. This allowed the researchers to see individual atoms and the molecular blueprints to begin engineering a faster enzyme system. The combination of structural, computational, biochemical and bioinformatics approaches revealed molecular insights into its structure and how it functions.

Artificial Organs

Artificial pancreas for controlling type 1 diabetes in children

The artificial pancreas technology represents a closed-loop control system that provides an all-in-one diabetes management for patients. This technology is for tracking blood glucose levels using a continuous glucose monitor (CGM) that automatically delivers the insulin when needed using an insulin pump. The system offers superior performance compared to the standard testing by finger stick. It allows delivery of insulin by multiple daily injections that is done by a pump controlled by the patient or caregiver.

Previous studies have shown that the existing treatment allows to successfully keep the blood glucose in a healthy range only for fewer than 1 in 5 children with type 1 diabetes. Thus, this medical limitation poses serious consequences on the long-term health and quality of life for children who have type 1 diabetes. Further, previous research showed that the efficiency of the diabetes management system was safe and effective only for people ages 14 and older. Thus, a better diabetes management is needed for younger children.

Recently, a clinical trial at four pediatric diabetes centers in the United States was conducted that reported a new artificial pancreas system. This system was shown to be able to automatically monitor and regulates blood glucose levels. More importantly, it was shown that the system was safe and effective at managing blood glucose levels in children as young as age six with type 1 diabetes.

The trial was conducted by the scientists at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), part of the National Institutes of Health. The results from the trial were published August 26 in the New England Journal of Medicine (A Randomized Trial of Closed-Loop Control in Children with Type 1 Diabetes, New England Journal of Medicine, 2020; 383 (9): 836 DOI: 10.1056/NEJMoa2004736).

In their clinical trials, researchers found that youth using the artificial pancreas system developed by them showed 7% improvement in keeping blood glucose in range during the daytime. Whereas, a 26% improvement in nighttime control compared to the control group was demonstrated. It is important to note that the nighttime control is crucial for people with type 1 diabetes due to the fact that severe, unchecked hypoglycemia could potentially lead to seizure, coma or even death. The artificial pancreas system reflected a nearly 11%

improvement in the overall time-in-range goal that translated to 2.6 more hours per day in range. The demonstrated improvement in blood glucose control in very promising that could lead to breakthroughs in diabetes management for children especially during the overnight hours. This could let parents and caregivers sleep better at night knowing their kids are safer.

The artificial pancreas technology included the Control-IQ system containing an insulin pump that was programmed with advanced control algorithms based on a mathematical model using the person's glucose monitoring information to automatically adjust the insulin dose.

This breakthrough in artificial pancreas technology that is safe and effective for children with type 1 diabetes is a major step going forward in improving the quality of life and disease management in children as young as age six.

Compiled and Edited by Dr. Megha Agrawal & Dr. Shyamasri Biswas

For all production related questions or sending your ads, please e-mail or call our production department: E-mail: sales@biotechkiosk.com; Phone: 386-518-9411.

THANK YOU!