UNIVERSITY OF JOHANNESBURG

FACULTY OF SCIENCE

DEPARTMENT OF BIOTECHNOLOGY AND FOOD TECHNOLOGY

ADVANCED DIPLOMA IN BIOTECHNOLOGY

MODULE BTN7X01

APPLIED DISEASE AND IMMUNE RESPONSE

CAMPUS **DFC**

MAIN SUMMATIVE ASSESSMENT NOVEMBER 2020 MEMORANDUM

AVAILABLE DATE: 12/10/2020 **DUE DATE: 12/11/2020**

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EXTERNAL MODERATOR **PROF AN TRAORE-HOFFMAN**

MARKS: 65 (+5 BONUS)

NUMBER OF PAGES: 3

INSTRUCTIONS TO STUDENTS:

1. **COMPLETE THE ACADEMIC INTEGRITY STATEMENT AND SUBMIT TOGETHER WITH THIS ASSESMENT.**
2. **THERE ARE FIVE COMPULSORY QUESTIONS AND ONE BONUS QUESTION.**
3. **SUBMIT THE ASSESSMENT ONLINE VIA BLACKBOARD.**
4. **WHERE QUESTIONS REQUIRE THE INCLUSION OF REFERENCES, THIS HAS BEEN INDICATED.**
5. **LABEL YOUR FILE AS FOLLOWS: FINAL ASSESSMENT_STUDENT NUMBER.**
6. **SUBMIT A TURN IT IN PLAGIARISM REPORT WITH LESS THAN 20% SIMILARITY. THIS SHOULD ONLY INCLUDE THE QUESTIONS REQUIRING REFERENCES.**
7. **GOOD LUCK!**

Use the information provided in the table below to guide your review of relevant literature on COVID-19.

COVID-19 Vaccine Developer/Manufacturer	Type of Candidate Vaccine	Number of Doses	Timing of Doses	Route of Administration
University of Oxford/AstraZeneca	ChAdOx1-S	1		IM
CanSino Biological Inc./Beijing Institute of Biotechnology	Adenovirus Type 5 vector	1		IM
Sinovac	Inactivated	2	0,14 days	IM
Moderna/NIAID	LNP-encapsulated mRNA	2	0, 28 days	IM

Q1. Provide a referenced description differentiating between the two vaccine strategies being used by University of Oxford and Moderna. (10 marks)

University of Oxford - uses a non-replicating chimpanzee adenovirus (ChAdOx1) derived from isolate Y25 that lacks the E1 region (encoding viral transactivator protein essential for viral growth) and E3 region (encoding immumomodulatory proteins) as the viral vector. The vector carries a shuttle plasmid with the full, codon-optimised gene for SARS-CoV-2 spike glycoprotein (S) and a leader sequence for human tissue plasminogen activator gene (tPA) under the influence of a modified human cytomegalovirus major immediate early promoter (IECMV) (Doremalen *et al.,* 2020).

Moderna (mRNA-1273)– uses a nucleoside modified sequence-optimised mRNA that encodes the S2-P antigen made up of the spike glycoprotein of SARS-CoV-2 (S) with a transmembrane anchor and intact S1-S2 cleavage site. It is encapsulated in a lipid nanoparticle made up of four lipids.

Q2. Discuss in detail how each of the vaccines from Q1 above function to stimulate an appropriate adaptive immune response to COVID-19 infection. Make sure to explain the immune responses that will be generated. Use references to support your answer.

(25 marks)

ChAdOx1-S (13 marks):

(I) vector attaches to receptors such as Coxsackie adenovirus recerptor (CAR) or CD46 on host cells (muscle cells and APCs, respectively) and is internalized by clathrin-mediated endocytosis. The viral capsid begins to disassemble and the virion is transported to the nucleus where the capsid is completely disassembled and DNA released into the nucleus. Under the influence of the strong exogenous promoter, the spike glycoprotein is expressed in sufficient amounts to be processed via the MHC I pathway. The proteins are presented to naïve CD^{8+} T cells which are activated to release IL-2. The CD^{8+} T cells proliferate and differentiate into CTLs that lyse the cells bearing the spike glycoprotein. This will also result in the differentiation into memory T cells that will be able to lyse any cells infected with the virus in the future. Effector T cells spike are measurable around day 7, spike at day 14 and continue up to day 56 (Folegatti *et al.,* 2020)

(II) Vector is endocytosed by APCs (DCs), processed via MHC II and presented to naïve CD^{4+} T cells in the lymph nodes. The CD^{4+} T cells release IL-2, proliferate and differentiate into Th1/Th2 cells that promote CTL cytotoxicity and antibody production. Spike-specific antibody production is reported around 28 days after a primary vaccination and neutralizing antibodies are reported after a booster at 28 days (Folegatti *et al.,* 2020). Neutralizing antibodies are to a number of different epitopes.

(III) Antigen from other infected cells can be phagocytosed by DCs and processed via MHC II and presented to naïve CD^{4+} cells resulting in antibody production.

mRNA-1273 (Moderna) (12 marks):

(I) LNP-mRNA is taken into host cells (non-APCs and APCs especially DCs) at the site of injection via endocytosis. Within the endosome, the lipid membrane of the nanoparticle fuses to the endosomal membrane due to pH differences and the mRNA escapes the endosome into the cytosol. Once in the cytosol, mRNA is transcribed into the antigenic proteins which are then processed via a proteasome into smaller peptides that are transported to the ER and loaded onto MHC I. These antigens are then presented to CD^{8+} T cells, which are activated to release IL-2, proliferate and differentiate into CTLs. However, no CD^{8+} responses were evident against SARS-CoV-2 in Rhesus macaques (Corbett *et al.,* 2020) and only low responses were recorded in humans after the booster (Jackson *et al.,* 2020).

(II) Within DCs, exogenous antigenic protein (from for example, lysed cells, secreted protein) is taken up by endocytosis, broken down into antigenic peptides and loaded onto MHC II. This is then displayed to CD^{4+} T cells, activating them to release TNF>IL-2> IFNγ. They are able to proliferate and differentiate into a Th1-

biased response that promotes CTL activity. Evidence of a Tfh response was also evident by the secretion of IL-21 which further promoted antibody production. The stimulation of Th2 response is evident with binding antibodies converted to IgG within two weeks of the prime but neutralizing antibodies developed only after the booster at 29 days (Jackson *et al.*, 2020). Neutralising antibodies are produced against the receptor binding domain and the N-terminal domain of S1 (Corbett *et al.*, 2020, Jackson *et al.*, 2020).

Q3. Explain how using adenovirus type 5 vector could affect the efficacy of the CanSino Biological Inc. vaccine candidate. (5 marks)

Human adenovirus type 5 (HAdv-C5) causes a cold in humans and is limited in use as a vaccine vector due to seroprevalence. About 40-45% seroprevalence is reported in USA and 90% in sub-Saharan Africa. Seroprevalence refers to the presence of neutralizing antibodies in individuals before vaccination. This would mean that anyone with circulating neutralizing antibodies would mount an immune response against the vector before the expression of the target (in this case SARS-CoV-2 S protein) preventing an immune response to develop against SARS-CoV-2 and therefore rendering the vaccine ineffective. A rare adenovirus can be used so that there is no seroprevalence.

Q4. The attributes considered for selecting a vaccine candidate include: potential for efficacy, vaccine stability, administration of the vaccine, safety of the vaccine and vaccine availability. Describe each attribute and explain the criteria used to assess them. (15 marks)

Efficacy – the ability of the vaccine to optimally result in a stable immune response that completely protects an individual against a subsequent attack by the wild-type pathogen responsible for the infection/disease.

It is evaluated by the activation of APCs to initiate antigen processing and the production of cytokines, the activation of both B and T cell responses with the production of long-lasting memory cells, the development of neutralizing antibodies to several epitopes (not just a single epitope unless it is known to be immunodominant) and the appropriate activation of the innate immune response.

Stability – a vaccine must be able to retain its chemical, physical, microbiological and biological properties within specified limits throughout its shelf-life despite handling and prolonged storage conditions.

Effect of temperature is evaluated by thermostability over time. Vaccines should be stable for at least 2 weeks when stored between 2-8 ^{o}C, stability at other temperatures e.g. -20 ^{o}C is determined and expected shelf-life is about 12 months at temperatures <-60 ^{o}C. Potency is also evaluated ie the ability to maintain its immunogenicity and can be evakuated by determining the change in a vaccine property that is direct or indirect indicator of vaccine efficacy. Stress testing to extreme temperatures, light, oxidizing agents, freeze-thaw, pH changes that can results in degradation/hydrolysis.

Administration of vaccine – refers to the manner in which the vaccine is presented to the body.

It is evaluated based on the acceptable route of administration that results in the maximal effectiveness of the vaccine with limited adversity. It also depends on the type of adjuvants and their side effects. The dosage of the vaccine and whether there is a need for more than one dose. Single dose vaccines are preferred.

Safety – The vaccine itself should not cause any harm to the vaccinated individual (healthy or immunocompromised)

It is evaluated during development and during production. During development, initial evaluation is performed in animal models before moving to human trials. The presence of side effects, size of dose on the immune response, risk of enhancement of disease, type of population included in trials, developmental/reproductive studies are performed. Mortality rates are monitored and morbidity without long-lasting effects is established. After production each batch is tested for potency, purity and sterility.

Availability – refers to the ease of scaling up vaccine production when needed and how accessible it is to those who need it.

It is evaluated by cost, easily accessible supply of reagents and source of antigen, who the high-risk groups are, distribution conditions e.g. need for cold-storage.

Q5. Consider that you are a scientist evaluating a vaccine candidate. Use the table below to determine the maximum score that you would use to rate each attribute discussed in Q4 according to their importance/priority. The total score must add up to 100 points. Attributes can be scored higher, less or equally to each other. Write a brief reason for why you have assigned a higher/lower/equal score to an attribute. (10 marks)

Flexible marking within this context.

Vaccine attribute	**Maximum possible score**	**Reason for score**
Efficacy	25	This is one of the most important aspects because it evaluates whether a vaccine functions or not
Safety	25	Is equally important to efficacy because if a vaccine works but causes harm in humans it cannot be approved
Stability	10	Stability is rated the lowest because as long as a vaccine is effective, safe and available, the storage and handling conditions can be adapted for each vaccine based on specific requirements.

Administration	15	Rated lower than efficacy because using sub-optimal routes/doses may still result in the vaccine producing an immune response although it would not be at maximal efficacy
Availability	25	Rated the same as efficacy and safety because it is important that when the need arises e.g. in an epidemic/pandemic that the vaccine can be made available to those at risk in a timeous manner to minimize associated mortalities.
Total Score	**100**	

Bonus Question:

Discuss the possible risk(s) associated with the vaccine strategy used by Sinovac. (5 marks)

Sinovac uses an inactivated SARS-CoV-2 virus as their antigenic preparation. ADE (antibody dependent enhancement) results when non-neutralizing or sub-neutralizing antibodies developed against an antigen due to previous exposure or vaccination are able to bind to viral particles upon infection and enhance their uptake into host cells via complement-mediated responses and receptor-independent mechanisms leading to enhanced replication and pathogenicity. Another phenomenon is referred to as vaccine-associated enhanced respiratory disease (VAERD) which occurs when more binding antibodies vs neutralizing antibodies are produced likely due to the presence of conformationally incorrect antigens. This results in immume-complex deposits that trigger the activation of complement and resulting in inflammation and obstruction of airways. These two phenomena have been reported for alum-based inactivated vaccines and in this type of vaccines developed for SARS-CoV-1. Therefore, the potential for both exists in SARS-CoV-2.