Module 7 Assignment

585.751.81 Immunoengineering

1. Here are some examples of recent start-ups tackling challenges related to infectious diseases, and pick one and research further: (15 points)
   - Karius
   - Moderna
   - Day Zero Diagnostics
   - VIR Biotechnology
   - IDbyDNA
   - Vaxess
   - You may also choose a start-up not found on this list that you find interesting

VIR Biotechnology

As you research, answer briefly (1-2 sentences for each):

1. What does their technology do and what disease is targeted?

VIR Biotechnology has built a monoclonal antibody (mAB) discovery platform and a T Cell-Based Viral platform enabling to target a range of infectious diseases such as Chronic Hepatitis Delta (CHD), Hepatitis B (CHB), HIV, Respiratory Syncytial Virus (RSV), Influenza, COVID 19 and pre-cancerous human papillomavirus (HPV) lesions.

2. What problem does their technology solve?

VIR Technology leverages the human immune system to fight viruses, employing machine learning and artificial intelligence, to engineer effective medicine from human-derived antibodies.

3. What stage of development are they at?

The FDA has granted approval for the COVID 19 medicine, while the remainder of the drug pipeline is either in preclinical or Phase 2 clinical trials.

2. A new diagnostic test is being developed for tuberculosis. Tests with characterized patient samples have been performed on the new test. Values below correspond to the number of samples in the test positive for tuberculosis. (10 points)
   - Calculate the sensitivity and specificity of the test for each group and also for all groups combined.
   - Also, comment on the utility of this test based on these criteria.

HIV– groups (n = 222)
TB+ = 50/54
Latent TB = 8/62
Nontuberculosis mycobacteria = 6/64
Healthy controls = 0/42

	TB+, Latent TB	NonTb + Healthy
Positive Test Result	58	6
Negative Test Result	58	100
	116	106

Sensitivity	0.5
Specificity	0.943396226

For people without HIV, the test has a 50% probability of correctly detecting TB in those who have it, this means that for people without HIV who have TB, the test performs no better than random chance. However, within the same group, the test shows a very high probability, ~ 94% of correctly identifying individuals without TB as healthy. For individuals without HIV who do not have TB, the test is very accurate classifying them as TB-free.

HIV+ groups (n = 180)
Pulmonary TB+ = 70/80
Extrapulmonary TB+ = 36/42
TB- = 6/58

	TB+, Extra.TB+	TB-
Positive Test Result	106	52
Negative Test Result	16	6
	122	58

Sensitivity	0.868852459
Specificity	0.103448276

In the group of people with HIV, the test performs differently; It can significantly detect people with TB, achieving an accuracy of approximately 86%, which means that for people with HIV who have TB, the test effectively detects the infection in most cases. However, the test performs poorly for healthy individuals within the same group. It struggles to accurately classify healthy people as disease-free.

HIV- + HIV+

	TB+	TB-
Positive Test Result	164	58
Negative Test Result	74	106
	238	164

Sensitivity	0.68907563
Specificity	0.646341463

Assuming that the experiment is representative of the number of people with HIV and without, including both healthy individuals and those with TB, the test's overall ability to distinguish between TB+ and TB- individuals is subpar. Specifically, the test ability in detecting TB+ individuals and correctly identifying TB- people are approx. 68% and 64%. These performances could put on pause the approval for the test's deployment.

3. Compare and contrast *Large scale screening* to *Mechanistic insight* approaches to discover new therapeutic targets. Please focus on the advantages and disadvantages of each approach. (15 points)

	Advantages	**Disadvantages**
Large scale screening	• High throughput: due to automation allows the rapid screening of very large libraries of compounds against a specific target and can accelerate the identification of potential active compounds or hits. • Does not require prior knowledge for finding a novel drug.	• Initial set up costs of HTS technology could be significant. • May not be as specific as other technics (false positives/negatives). • May require the libraries to be established which could be time consuming and costly. • With an increase of throughput: loss of the physiological relevance, and impact on host toxicity. • Provides limited insights into the mechanistic action of the compounds.
Mechanistic insight	• Provides detailed insights into the molecular mechanisms and pathways through which compounds exert their effects. • Insights of mechanism of actions can help in	• Low throughput: compared to HTS, analyzes of fewer compounds or hypotheses at a time. • Requires understanding of the disease. • Requires more resources, time and potentially more sophisticated

	predicting and understanding drug resistance, adverse effects and could reduce the failures rates in later stages of drug development. • Allows rational design and can lead to a more targeted and potentially more effective therapy. • Enable to answer theoretical and fundamental questions with simulation.	tools and models from researchers. • Compared to other methods has a higher chance of failure.

4. Compare and contrast the four major different types of biologic therapeutic approaches. In your analysis answer: (20 points)

- When would you want to use each approach for infectious disease?

Engineered Cells
Engineered cells, such as chimeric antigen receptor (CAR) T-cells, have the potential in fighting infectious disease by targeting pathogens directly or by modulating the immune response. For instance, T-cells engineered cells can be tailored to target specific pathogens with high specificity.

Engineered Microbes and Viruses
Can help to fight virus infection by a) neutralizing their toxic effects on the host b) they can sense the bacteria and produce anti-microbial peptides c) they can release molecules to repress the virulence genes of the bacteria and d) they can secrete anti-bodies that inhibit invasion of bacteria.

Engineered Proteins
Engineered proteins, such as cytokines and antibodies can target infected cells, block the entry of pathogens into host cells, or modulate the immune system. For example, they are used in HIV to target infected CD4+ T cells and direct CD8+ T cells to kill these infected cells.

Engineered Genetic Material
These therapies can address many challenges of current vaccines:
- Can be rapidly scaled and manufactured with sufficient quality control.
- Can be quickly reprogrammed to address high dynamics of the virus (e.g. in Influenza) and be potent across all age group.
- DNA molecules can provide effective adjuvants that could be used to potentiate allergies.

- What is one of the major advantages and disadvantages to each approach?

Engineered Cells
Advantage: Engineered cells, such as chimeric antigen receptor (CAR) T-cells can provide a long-lasting immune response. For instance, in HIV patients, CCR5 genetically modified CD4+ T cells had an extended survival.

Disadvantages:
There are several challenges facing the engineered cell fields:
- Complex and synthetic therapies: autoimmunity could be a concern.
- Costly
- Cell source: difficulty to access to effective cells.
- Lack of genetic engineering efficiency or specificity.
- Expansion ex-vivo is not aways efficient: it can modify cell phenotype, it is not easy to keep it sterile, could be expensive.

Engineered Microbes and Viruses
Advantage: these therapies can specifically target, kill pathogens, and deliver therapeutic payloads directly to the infection site.
Disadvantages: one major concern is the safety of the therapy and off-target effects. Engineered virus-like particles mitigate to some extent these risks.

Engineered Proteins:
Advantage:
Antibodies can be engineered to recognize any number of proteins target and to provide multi-functions, such as ligand or receptor blockade, receptor downregulation, depletion and signaling induction. They can be easily linked to therapeutic payload and enhance biodistribution.
Disadvantages:
The shelf-life could be poor which causes an increase in costs, have a limited duration of action necessitating repeated dosing, and there is a potential for immune reactions against foreign proteins.

Engineered Genetic Material
Advantage: can be used to deliver specific proteins (e.g., mRNA) to cells, loaded into MHCs and act as a vaccination.
Disadvantages:
Delivery to the target cells or tissues remains a significant challenge, as does the potential for off-target effects and immune responses. Moreover, the control and durability of the response is not always completely understood and ethical considerations surrounding genetic modifications are important concerns.

- What design criteria are shared between approaches in creating a therapy?

The shared design criteria in creating a therapy, include:
- **Specificity and selectivity**: engineering the therapeutic agents to recognize specific molecular biomarkers, receptors, or genetic sequences associated with the disease.
- **Safety and minimization of off-target**: careful design to avoid immune responses, off-target genetic modification, or unintended side- effects.
- **Efficacy**: optimizing the therapeutic agent to maximize its therapeutic benefit.
- **Stability and solubility:** include protecting the drug from too fast degradation, ensuring stability in the bloodstream, and effective delivery to the target tissue or cells.
- **Controllability and reversibility**: controlling the rates the therapy is taken up, distributed, and cleared from the body: pharmacodynamics and pharmacokinetics.

- **Route and frequency of administration:** minimizing toxicity.
- **Immunogenicity and biocompatibility**: minimize the immune response against the therapy to prevent rejection, or adverse events.
- **Scalability and manufacturability:** involve optimizing the production process, yield, and optimizing the cost.
- **Business opportunities**: is there a market? Is the market share being not too crowded.
- **Patentable**

5. Design a biomaterial therapeutic to treat one of the following infectious diseases:
   - Tuberculosis (Bacteria)
   - Malaria (Parasite)
   - HIV (Virus)

   In your consideration of your design, please list specifically how the therapeutic was designed with design constraints such as:
   1. Manufacturing/Cost
   2. Safety
   3. Specificity
   4. Potency
   5. Biomaterial properties – e.g. stiffness, degradability, size, shape, etc.

Malaria Biomaterial Therapeutic

Malaria is a parasitic infection transmitted to humans by the Plasmodium species, with 5 species known to infect humans. Among them, *P.falciparum* and *P.vivax* are the most prevalent. The disease's lifecycle begins when Anopheles mosquitoes, having fed on the blood containing parasitized RBCs (pRBCs) of an infected individual, transmit the parasite to a new human host [1].

Historically, quinine (QN) has been the most effective in malaria treatment, though lacks efficacity against *P.falciparum* and has triggered resistance. In response, artemisin-based combination therapy (ACT) has shown the most promising results in treating malaria.

These therapies combine artemisinin-derivatives with chloroquine (CQ) and include artesunate (ATS) with amodiaquine, mefloquine, and proguanil [1].

We use PLGA as the base material for our nanoparticle delivery system. PLGA nanoparticles have been extensively studied for drug delivery, known for their safety and biocompatibility in clinical settings. They degrade into non-toxic byproducts that the body can easily eliminate. Producing these nanoparticles is cost-effective due to streamlined production processes.

To enhance treatment success, we might also use big data to identify biomarkers to classify individuals in group who could benefit most from a specific ACT treatment. We then encapsulate an ACT therapeutic within the PLGA nanoparticles.

One target for increasing drug delivery specificity is the Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1), found on the surface of pRBCs. We attach antibodies against PfEMP1 to the nanoparticles [1].

Given the acidic environment (pH 5.0-5.4) inside the Plasmodium's digestive vacuole, we incorporate a pH sensitive linker that releases the drug right at the infection site, boosting the treatment's effectiveness [1].

Research by Najer et al. [2], polymer-based RBC membrane mimicking RBC membrane, can not only block the parasite from entering new cells but showed high binding affinity with P.falciparum merozoite surface protein1. We design the nanoparticles with specific stiffness that mimics RBCs, to help them stay in circulation longer.

Developing this treatment will involve extensive lab and animal studies to refine its safety, efficacy, and precision. Preclinical trials will assess its performance against different strains of malaria and evaluate any potential side effects.

[1] L. N. Borgheti-Cardoso *et al.*, "Promising nanomaterials in the fight against malaria," *J. Mater. Chem. B*, vol. 8, no. 41, pp. 9428–9448, 2020, doi: 10.1039/d0tb01398f

[2] A. Najer *et al.*, "Nanomimics of Host Cell Membranes Block Invasion and Expose Invasive Malaria Parasites," *ACS Nano*, vol. 8, no. 12, pp. 12560–12571, 2014, doi: 10.1021/nn5054206