

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	<ul style="list-style-type: none">• 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.	<ul style="list-style-type: none">• Initial set up costs of HTS technology could be significant.• May not be as specific as other techniques (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	<ul style="list-style-type: none">• Provides detailed insights into the molecular mechanisms and pathways through which compounds exert their effects.• Insights of mechanism of actions can help in	<ul style="list-style-type: none">• 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



	<p>predicting and understanding drug resistance, adverse effects and could reduce the failures rates in later stages of drug development.</p> <ul style="list-style-type: none"> • Allows rational design and can lead to a more targeted and potentially more effective therapy. • Enable to answer theoretical and fundamental questions with simulation. 	<p>tools and models from researchers.</p> <ul style="list-style-type: none"> • Compared to other methods has a higher chance of failure.
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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?

High-Throughput Screening (HTS): Pivotal in the early stage of drug discovery for identifying quickly among a very large set of potential compounds a few candidate molecules. This method can lead to a breakthrough discovery, especially when we know a lot about how a disease behaves and we really need to find a new treatment like an antimalarial drug.

Mechanistic Insight: Requires a fundamental understanding of the disease but informs the rational design of therapeutics and helps predict efficacy and potential resistance mechanisms. For instance, with antibiotics, since we haven't discovered new ones and resistance to current ones is rising, it's crucial to figure out exactly how existing antibiotics work so we can make new ones that work better.

Combination therapy: increases efficacy and prevent pathogen escape for disease with different signaling pathways or time-dependent phenotypes like early and later stages of the disease. For example, to prevent HIV from spreading or causing infection, there are different kind of inhibitors that can help: protease inhibitors, co-receptor antagonist, fusion inhibitors, and reverse transcription inhibitors. Using a combination of these treatments is often the best approach.

New Tools: looking at large combinatorial potential candidates both gene, molecules and can generate molecules with enhanced properties (e.g., binding affinity, specificity, efficacy).

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

High-Throughput Screening (HTS)

Advantage: Enables the rapid screening of vast compound libraries to identify active molecules against a target.

Disadvantages: could identify candidate compounds with poor chemical properties and may require further chemical modifications to increase solubility, potency, or lower dosage. Another



major is as the throughput of the assay increases, its complexity is reduced and lose its physiological relevance, at the same time its toxicity and cost are increased.

Mechanistic Insight

Advantage: specific therapeutic choice based underlying physiology of the disease and additionally, allows to test a variety of hypothesis in silico before validating them in in vitro models.

Disadvantages: time-consuming, may require more resources that do not always lead to successful therapeutic candidates.

Combination therapy

Advantage

Increased potency than relying only on one disease's mechanisms and can reuse multiple existing therapies.

Disadvantages

Complexity in optimizing combination dosages, increased risk of drug-drug interactions, potential for cumulative toxicity and consequently may face more regulatory hurdles.

New Tools

Advantage

Can increase throughput and provide new insights

Disadvantages

May require significant expertise or the creation of new tools which may produce unpredictable outcomes.

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

These four strategies require an understanding of the disease phenotype and disease's biological mechanisms. A common initial step is the identification and validation of targets that are critical for the disease's development or progression (this could be for ex. a protein, a gene, or a biological pathway). Whether large scale screening, combinatorial approaches, or directed evolution, they require an efficient system for evaluating numerous compounds or combinations quickly and accurately. This involves developing assays that, are reproducible, and scalable. Across all approaches, the therapeutic candidates need to demonstrate both safety and efficacy with minimal off-target effects, low toxicity, and they must be selective in reaching the target site. The initial step is the establishment of in vitro models to validate the understanding of what needs to be treated. "The in vitro models should return to healthy phenotypes upon successful treatment and discriminate between potential therapies, the main objective being to prioritize essential compounds before more complex in vivo models" (from the lecture). Lastly, due to the high versatility of some the infectious diseases (for ex. gene mutation in case of Influenzas) these approaches should be flexible and quickly modifiable.

Engineered Cells

Engineered Microbes & Viruses

Engineered Proteins – Cytokines & Antibodies

Engineered Genetic Material

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 efficacy 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

