

Module 8 Assignment

585.751.81 Immunoengineering

1. Your boss wants to develop a new food allergy diagnostic and thinks that a new biomarker will work better than looking at IgE levels and skin prick test. What can you tell your boss about what the diagnostic development process will look like? (this can look like an informal email – and can use bullet points) Include in your answer: (30 points)
 - Who needs to be involved in ideation
 - How it needs to be tested and validated
 - Target sensitivity and specificity
 - Available technologies, market, and cost
 - Source of sample and basic biology of a few potential targets
 - Total time required

Dear Boss,

Following our recent discussion regarding a new food allergy diagnostic test using a more performing biomarker than IgE levels and skin prick test, I have outlined the key considerations for the development process of this test:

Ideation Team

We will need to put together a cross-functional team with bioinformaticians, biologists, immunologists, clinical nutritionists, and doctors. They will help us to identify potential biomarkers to detect food allergies beyond just IgE levels. We'll also need bioengineers to design the testing device. Plus, we may engage academic research institutions to assist us in this research.

Sample Source and Basic Biology of Potential Targets

Sample source will be saliva, blood, or other non-invasive obtained biological samples are preferred for user comfort and compliance. Potential targets will be cytokines, chemokines or other markers which play a role in the allergic response in the context of food allergy beyond IgE.

Testing and Validation

We will first evaluate the identified biomarkers 'relevance using specific cell lines in-vitro and through animal models. We will then conduct a pre-clinical validation with a small group of participants selected based in criteria set by our clinical team. These initial trials will be followed by progressively larger cohorts as we move through phase I, II and III. Understanding the regulatory framework is crucial; we may explore alternative regulatory pathways that could expedite our diagnostic test's time to the market.



Target Sensitivity and Specificity

We need to aim for high sensitivity to minimize false negatives and high specificity to reduce false positives. Our benchmarks should initially be at par and then exceed, as we improve the device, current standards set by IgE level assessments and skin prick tests.

Available technologies, market, and cost

Our goal is to develop a diagnostic test that addresses the limitations of current methods like the skin prick test by:

- Being more representative of the immune system's response where the reaction occurs.
- Accurately representing the antigens of targeted allergies.
- Being applicable for people with skin disease.
- Reducing the amount of sample needed and speeding up the detection time.
- Offering a cost-effective solution with minimal infrastructure needed to make the test.
- Allowing for the simultaneous testing of multiple antigens.

Current technologies

- **Microfluidic Chips:** Capable of screening thousands of antigens at once, these chips are expensive and require significant infrastructure. Researchers have developed paper microfluidic arrays to decrease assay time to less than 10 minutes, but their detection levels are less than gold standard, and require signal amplification,
- **Nanostructure sensors:** These sensors need smaller serum samples (25 μ l) and can diagnose in less than 1 min. They are low-throughput technique, limited to detecting one allergen at a time, and scaling up production could require large capital.
- **Synthetic biology or Cell-based Assays:** to report relevant levels of histamine. They can test multiple allergens (multiplexing), and multiple levels of concentration. These assays have very high sensitivity, and correlate with skin test, and lastly, there is less discomfort like the skin test. The main challenge to address includes enhancing cell line stability and reducing the current 36-hour processing time.
- **Dissolving Film or Orally Dissolvable Patches:** it is a promising technology aligned with our objectives. This film requires smaller samples, it has improved allergen delivery and efficacy. It also lowers the risk of systemic side effects and simplifies standardization by eliminating the need for measuring allergen doses at the doctor's office. Additionally, the throughput can be scaled with controlled antigen loading.

The total time from ideation to marker launch can range from 5 to 10 years, depending on a variety of factors including the complexity of the device and its validation, regulatory approvals, and production scale-up.

I look forward to discussing these points further or let me know if you have any questions.

2. How might the microbiota be used both as a diagnostic and therapeutic agent in autoimmunity? Why is this an attractive approach? What are limitations with using the microbiota as a diagnostic or therapeutic agent? (15 points)

Bacteria can function as diagnostics, serving as either one-component or two-component biosensors, for the detection and reporting on human diseases, whether in the body or ex vivo on collected samples. Their primary advantage is to detect transient molecules that may be degraded, modified, or absorbed before exiting the gut. Additionally, they can function as

memory circuits, continuously monitoring inflammation over extended periods. One particularly promising application of bacterial diagnostics is their capacity to detect tumors, including those of very small sizes, thus having immense potential for the early detection and treatment of cancer. Another compelling aspect of bacterial functionality is quorum sensing, enabling them to detect bacteria and act as sense-and-response systems. This feature is useful in regulating the expression of products like cytotoxic drugs at specific body sites mitigating off-target side effects.

Engineered bacteria can transport DNA, RNA, or drugs to specific places within the human body with low systemic exposure, such as gut inflammation, that would have otherwise been rapidly degraded in the bloodstream or during transit of the upper gastrointestinal tract. These sites are usually hard to reach through oral routes or injection methods, like the colon or inside the tumors.

The most used bacterial function in clinical settings, is to activate both innate and adaptive immune response, such as stimulating dendritic cells, and expressing tumor-associated antigens. Conversely, they can be engineered to secrete anti-inflammatory cytokines, anti-tumor necrosis factor (TNF), or induce expression of genes for the delivery of toxins.

Major limitations of using microbiota as diagnostic or therapeutic agent are prevention of transfers between individuals, controlling growth and off-target toxicity, ensuring genetic stability, addressing potential loss of function, and lack of realistic in vitro testing environments.

3. Compare and contrast cell engineering bacteria versus mammalian cell lines for therapeutic approaches. Also, give specific examples where you would use one versus the other.

Finally, describe challenges for entry to market for these technologies. (15 points)

Mammalian cell engineering offers several advantages for therapeutic applications:

- Precision in Disease Management:
Dynamic adjustment of the dose of therapy, timing, and duration, as well targeted delivery.
- Integration of Complex Functionalities:
This approach can implement advanced functionalities, such as the creation of a feedback regulation loops. Starting from disease detection, these loops can activate genetic circuits that adjust therapeutic output in real-time, thus modulating the disease phenotype, and self-regulating the production of therapeutic agents based on disease state.
- Enhanced Control Compared to Bacterial Engineering:
Mammalian cell engineering provides greater control and flexibility by allowing for modular and abstract integration of simpler functions. This is achieved by focusing on the specific inputs and outputs to interconnect, thus facilitating precise regulation of gene expression, the conversion, or loss of specific DNA, RNA, or protein. In contrast, bacteria cells may be unstable and are less suitable for more complex biologic therapies.
For instance, bioengineers designed a mammalian cell-based logical two-input AND gate to treat psoriasis response to inflammatory signaling $\text{TNF-}\alpha$ and IL-22. The engineered cell produced anti-inflammatory cytokines, IL-4 and IL-10. This was full controlled process, as the production of IL-4 and IL-10 ceased following the withdrawal of $\text{TNF-}\alpha$ and IL-22 signals.
- Allow systemic administration.

However, 1) the incomplete understanding of the biological sensing mechanisms and genetic circuits makes the design of these technologies an empirical process, requiring iterative research cycles, 2) obtaining and maintaining the necessary cells for engineering due to patient sickness, can be difficult, 3) the immune system rejects these engineered foreign cells posing a significant barrier for adoption, and 4) the advanced nature of mammalian cell technologies leads to high costs and complex manufacturing processes.

In contrast, bacterial cell engineering presents a more straightforward approach due to its inherent simplicity: there are fewer endogenous genes. They could be designed to be easily ingestible by the body and are shielded from the immune system. Unlike mammalian cell-based therapies, bacteria-based cell engineering does not require cells from patients and are generally easier and faster to grow.

However, several challenges must be addressed: 1) engineered bacteria are typically restricted to certain sites, 2) safety concerns: bacteria are after all pathogenic and could be virulent leading to infection, 3) validation challenges: there is a lack of robust pre-clinical models or testing screen, and 4) genetic stability issues: like low growth rates, or loss of function due to genetic mutations. These factors can pose significant hurdles to regulatory approval due to the novelty of the approach and the lack of precedence.

A notable example of successful engineered bacterium is the genetic modification of *Lactococcus lactis*, a common food-grade bacterium that is non-pathogenic and non-colonizing, to secrete proinsulin autoantigen (Ag) alone or in combination with the tolerance-promoting cytokine IL-10. This therapy could be used for treating Type 1 Diabetes (T1D).

4. a) Design a combination therapy that uses **both** biomaterials **and** a biologic-based therapeutics to treat one of the following allergic or autoimmune diseases: (40 points)
- Asthma
 - Seasonal allergies
 - Multiple sclerosis
 - Rheumatoid arthritis
 - Chron's disease

In consideration of your design, specify your design constraints used such as:

1. Cost
2. Manufacturing
3. FDA regulations
4. Biophysical properties – such as size, shape, stiffness, etc.
5. Definition of success, such as a comparison to gold standard or acceptable side effects
6. Whether it is inspired by nature or biological mechanisms

b) Finally, compare and contrast the biomaterial and biologic design processes with the experience of this and last week's exercises.