Financial and Technological Risk Analysis for the Development of New Drugs

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Abstract

A feasibility study regarding the risk and viability of a new drug was performed. All aspects of pre-FDA research and testing and the FDA approval process have been considered and a probability study constructed. Although the model is specific for a carbohydrate-based tuberculosis vaccine, many of the analysis procedures and considerations apply to any new drug. Market strategies and demand models were developed. A set of first stage decisions concerning the degree to which the theory is researched and tested was established, and the risk and expected worth of each scenario were determined. The focus on first and second stage decisions helps determine the time period of research that would best suit the needs of the investor. The investor can use the model and have a clear view of what decisions need to be made beforehand with the results of a general financial analysis. The financial study will aid the investor – whether he be a risk averter or a risk taker- in the decision of the pre-FDA research length and the intensity.

Although the development of a new drug is of high risk and uncertainty, as shown in this paper, it is certain that a successful drug would have great benefit to humans due to its wide-spread effect. There are many diseases- malaria, HIV, cancer, leishmaniasis, and tuberculosis, to name a few- that are currently responsible for the deaths of millions of people per year around the world, and steps are being taken to counter their negative effects. Using the risk modeled in this paper, the investor is responsible for weighing the technical and financial risks of the project against the potential for revolutionary change world-wide.
I. Introduction

New drugs are at the vanguard of the medical research community at all times. The discovery and development of a new drug may have drastic effects on the population of the world. It may lower death rates, extend life expectancies, or even eradicate a disease as in 1967-73 with the small pox epidemic. With these great expectations, it is easy to forget the realities of the development process of a new drug.

The system of new drug approval in the United States is one of the most rigorous in the world. On average, it costs a company over $400 million to get a new drug from the research laboratory to the consumer. On average, only five in 5,000 potential drugs that enter preclinical testing make it to human testing. Furthermore, only one in five of those will make it through the clinical trials and the approval process.

In addition to the low success probability of a new drug, the FDA approval process is an extensive one, consuming an average of 15 years from beginning to end. It proves to be very helpful to have a model that can provide probabilities of success in a generalized form. With prior knowledge of the potential for each of the possible outcomes, which depend on the primary decisions made, it is easier to make those decisions with regard to financial investment and time in research. In accordance with each of these potential decisions, the risk and potential profit may be assessed to aid in this first critical decision. In short, the initial risk assessment affects the investment in research for a project, which in turn affects the likelihood of success and profit.
The initial investment and degree of research greatly affect the risk associated with the project because of the associated financial and technological uncertainties. No amount of research can accurately cover all uncertainty and prepare a drug for FDA testing. However, part of optimizing the drug research and approval stages is deciding, within a theoretical study, when to conduct thorough, up-front testing and when to move on to the FDA approval process. When considering up-front testing, it is necessary to make an initial decision of how long to conduct research in light of the expected earnings and predicted time and financial costs.

The first topic to be covered will be background information on the research and testing protocols established by regulatory agencies in order to take a new drug from its developing stages to its distribution, along with the technological risks involved. Following is a presentation of first stage decisions that need to be made at the beginning of the venture. The possible outcomes are evaluated and presented based on the first stage decisions. The market, advertising, and demand model is presented based on a new carbohydrate-based vaccine but is explained in a generalized form for any new drug. Finally, a risk assessment of the entire process is presented.

II. Research and Testing

Research and testing will determine a new drug’s future. The FDA (Food & Drug Administration) is the regulatory agency that determines if the new product is suitable for use in humans. Several steps must be followed and results presented in order to initiate the distribution of a drug to the general public. Even before the process begins, several decisions must be made regarding the pre-FDA stages of the product. Figure 1 shows
the generalized process for all new drugs. It is important to note that the research and pre-FDA stage of the process will be the step upon which different outcomes are obtained. The process in the diagram must be followed from left to right.

![Diagram of the conventional track of new drugs](image.png)

**Figure 1. Conventional Track of New Drugs**

### A. Research & Pre- FDA

A drug’s path through the Federal Drug Administration’s approval process is lengthy, detailed, unpredictable, and affected significantly by the degree of research performed before the FDA process. In the stages before the approval process, laboratory research and animal studies must be conducted. These studies will focus on effectively creating the vaccine in high yield. Within animal testing, the biological activity of the new drug against the targeted disease is evaluated in light of the safety for human consumption. Although the time period will vary from drug to drug, on average, the research and initial tests take six to ten years before completion. Animal studies for any new drug are standard. For a new drug, a group of animals is administered the drug, and the response, side effects, and efficacy are studied and documented.
A product’s success through FDA is largely dependent on its success in the research, development, and testing stages of formulation. The extent to which process steps are researched is chosen by the first stage decision and affects the amount of time spent in pre-FDA research and, thus, the initial investment in that research, as described further in the section on Risk Analysis. In order to appropriately select the process steps to be studied, it is necessary to consider the technological risks of a new drug.

**B. Technological Risks**

For any new drug, many risks and deviations accompany the technological aspect. Risks associated with a new product will be specific and will require multiple tests and studies. Some of the general risks found in the development and production of a new drug are:

- failure of experimental plan or unexpected outcomes
- chemical reactivity - or lack thereof – of reactants
- deviations in product recovery – methodology or instrumentation
- unavailability of resources- equipment, expertise, etc
- unavailability of test subjects
- risk to human health

These risks and technical uncertainties will be minimized by research and testing in the pre-FDA stage. The degree of research, as determined by the first stage decision as explained in the Risk Analysis section, will indicate the degree of confidence over the potential problems outlined above. Once the technological risks have been realized, the FDA phase and marketing of the new product are pursued.
C. Decision Pathways

An efficient method to present possible outcomes of different stages in pre-FDA testing or the FDA process is to consider all possibilities and realistically assign a probability. This should be done for all of the possibilities in every step of the development and testing of the new drug. The tracing of these decisions from the beginning to the end of the process is known as a pathway. It is important to note that the end of the process might be somewhere early in the process due to a clinical hold imposed by the FDA; or, it might be a successful pathway in which the drug passes all FDA requirements and testing.

D. Pre-FDA: Carbohydrate – Based Tuberculosis Vaccine

In order to relate the analysis to a particular example, a carbohydrate-based tuberculosis vaccine is presented. A flowchart predicting various pathways through the development stages of pre-FDA research may be constructed, as in Figure 2 for the carbohydrate-based tuberculosis vaccine, to assess the potential for success scenarios to move on to FDA testing. For a given pathway, the probability at each step—considering that the drug will act in a particular way—is multiplied by each of the probabilities at the other steps. The resulting probability, when normalized against that of all possible pathways, reveals the overall likelihood of the drug following that path. It should be noted that the variability in investment required at each stage with the number of years spent in research primarily depends on research labor salaries and the amount of materials required at each step.
By considering multiple pathways or possible scenarios for pre-FDA testing, an estimation of the probability of success, associated costs, and lengths of time may be evaluated. In addition, the amount of money to be invested in the experimental phase of pre-FDA trials will be an important factor in the overall outcome of the drug. Initial research investment in focus, time, and finances is a first stage decision, and depending upon the choice made, other factors such as timelines and success in the FDA approval process can be determined.
Vaccine Pre-FDA Testing

$650,000-$1 million-$1 million
0.5-1-1 years

20-10-5%

Unsuccessful
Back to R&D

Unsuccessful

Tuberculosis Capsule Formation

80-90-95%

Successful
Conjugate Formation

$1.2 million-$1.5 million-$1.5 million
1.5-2-2 years

20-10-5%

Unsuccessful
Back to R&D

Successful

$1.8 million-$2.6 million-$3.5 million
2-3-4 years

30-20-10%

Unsuccessful
Back to R&D

Reaction Detection in Animal Testing

70-80-90%

Satisfactory or No Reaction

Reaction to Vaccine

30-20-15%

Adverse Reaction
Back to R&D

70-80-85%

Mild, Acceptable Reaction

Titer Measurements

0.5 years

90-80-75%

Insufficient Titer

10-20-25%

High Titer

$1.3 million-$1.3 million-$2.2 million
1.5-1.5-2.5 years

20-10-5%

Lower Titer Levels than BCG Vaccine
Back to R&D

40-45-45%

Same Titer Levels as BCG Vaccine

40-45-50%

High Titer

Vaccine Proceeds!!

Costs, time per stage, and percentages are valid for 6-8-10 year pre-FDA periods

Figure 2. Pre-FDA Process Probabilities Flowchart
III. FDA Approval Process

The FDA approval process for a new drug, in general, requires three stages of clinical trials and a number of applications and inspections. Although the number of subjects used in each of the different phases may vary according to the severity or complexity of the target disease, the numbers used are accepted as standard. The levels of interest (such as enzyme levels or titers, for example) will be greatly dependent on the drug. The methodology, however, is similar for all drugs. An investigational new drug application is submitted as the FDA reviews data from animal testing. The drug is then administered to a group of subjects – from which there is a control group – and the levels are monitored in a period of time. The applications and licenses at the end of the process may vary slightly, again, according to the drug’s function and complexity.

A. Phase I

Phase I introduces the new drug into human studies. These studies are closely monitored and may be conducted in healthy volunteers. The main focus is to determine the metabolic and pharmacologic actions of the drug in humans, dosing effects, and effectiveness. The number of subjects range from 20 to 100.

If, at any point, the new drug causes adverse side effects or fails to provide adequate antibody stimulation, the FDA may put a clinical hold on the trial. The drug would then go back to research and development in the effort to increase the efficiency or reduce the side effects. Researchers may also present data that the side effects are mild or acceptable. In either case, within 30 days from reapplication, the FDA may
approve the data that the researchers have provided as evidence that improvements have been made to the vaccine, and trials may continue at the same evaluation stage.

B. Phase II

Once the drug has been considered safe at Phase I, whether undergoing a clinical hold or not, it may proceed to Phase II. The purpose of the Phase II of clinical trials is to obtain preliminary data on the effectiveness of the drug based on serum titers. In addition, it is of interest to determine the short-term side effects and health risks associated with the new drug. A relatively small number of patients are used in the studies, usually 100 to several hundred people. The estimated time period is at least three years.

A clinical hold may be instated based on severe adverse side effects, minor adverse side effects, or insufficient titers. As in Phase I, the testing of the drug pauses until the necessary data for modification is provided to continue the trial. The drug must be considered safe and reasonably effective to proceed on to the next phase.

C. Phase III

Once the effectiveness of the new drug has been established, the overall benefit-risk relationship of the drug is established. This is accomplished with studies that include anywhere from several hundred to several thousand people.
D. Applications and Inspections

If the drug is considered safe and effective, it continues to a level of applications and inspections which are estimated to take one year. Clinical testing results must contain sufficient evidence of the drug’s safety and effectiveness so that the drug can be submitted in a biologics license application. Simultaneously, the product goes before a committee, which contains scientists, physicians, biostatisticians, and a consumer representative and advises the FDA on the safety and efficacy of the drug. At the same time, the production plant is subjected to an intense inspection to ensure good manufacturing practice within the facility and to confirm adequate labeling for the marketed product. Providing all three phases of the FDA approval process and the related reviews are passed successfully, the drug may continue on to full production.

E. Cumulative Probabilities and Costs

As described in relation to pre-FDA, the probability that a drug will take a particular path in the FDA flowchart is found by multiplying the probabilities assigned to each stage along the way. For scenarios that link each pathway through pre-FDA to one through FDA, the overall probabilities are again multiplied for an overall evaluation of the likelihood of that successful or unsuccessful case. In a given pathway, the number of years and the costs associated with each stage are summed, and the present value of such an investment is calculated.

The time at which plant construction and incoming revenue begin is reliant on the drug’s success. In the model presented, the discounted fixed capital investment
depends on the time at which plant construction begins, based on both the first stage
decision of time invested in research and the time the drug spends in FDA testing.
The discounted fixed capital investment and the revenue generated from a success
scenario are incorporated into an overall net present value of the project. These
values, along with the probability of each scenario, may be used to evaluate the risk
of the project, as explained in the Risk Analysis section.

**F. FDA: Carbohydrate – Based Tuberculosis Vaccine**

The probabilities of the successful scenarios from pre-FDA testing passing the FDA
approval process have been calculated for the estimated probabilities of the vaccine
following each pathway. The entire process is expected to take a minimum of ten
years and approximately $14 million, providing the vaccine experiences a minimum
number of delays and clinical holds. According to the first stage decision of years
spent in pre-FDA testing, the probability of success at each FDA stage will vary, but
the overall expected FDA process time and investment will not. The FDA approval
process is outlined in the flowchart in Figure 3.
Vaccine begins FDA Approval Process

Phase I Clinical Testing
- No Antibody Stimulation
- Adverse Side Effects
- Drug Considered Safe

Clinical Hold-Back to R&D
- Severe Adverse Side Effects

Phase II Clinical Testing
- Minor Adverse Side Effects
- Vaccine Proven Ineffective - Does Not Meet Titer
- Vaccine Considered Safe - Meets Titer

Clinical Hold
- $6.4 million
- 3 years
- 200 test subjects

Phase III Clinical Testing
- $14.4 million
- 5 years
- 500 test subjects

- Booster to Increase Titer
- 85%
- Vaccine Considered Safe and Effective - Meets Titer

Clinical Hold
- 1 year

Pre-Approval Facility Inspection
- Biologics License Application

Vaccine Products Advisory Committee

Drug is Not Approved - Vaccine Fails
- 10-5-2%

Drug is Approved - Production Proceeds
- 90-95-98%

Percentages correspond to 6-8-10 years of pre-FDA testing

Figure 3. FDA Approval Process Flowchart
G. Conditions of Failure

As with any endeavor of any uncertainty, there is a potential for project failure. As noted in the Figure 3 for the sample tuberculosis vaccine, for the sake of analysis, an unsuccessful outcome at any step returns the project to further research and development, which is considered a failure.

In a second analysis, however, the probabilities and risk have been calculated in light of the potential for a drug to return to the FDA approval process after an additional year of research and testing. For instance, if a drug elicits adverse side effects in Phase I of FDA testing, it is presumed to return to research for one year and then begin Phase I again at the discretion of the FDA. Within this second analysis, it is assumed that the drug will continue on in the FDA process with an increased probability for success due to the additional research and problem-solving. Ultimately, however, if the FDA does not approve the drug following all three phases and the applications and inspections, the drug fails. The revised probabilities for the sample, carbohydrate-based tuberculosis vaccine are displayed in Figure 4.

Thus, two sets of analysis on the pre-FDA and FDA processes have been provided—considering a clinical hold as a failure and that recycling the drug for more research and a better chance at the FDA process. With the provision of these two sets of analysis, it is up to the investors to decide whether allowing the drug a second chance is worth the increased time and investment associated, based on the market considerations and risk assessment.
Figure 4. FDA Approval Flowchart for "Second Chance" Tuberculosis Vaccines
IV. Market Selection

The market for a new drug greatly depends on the resources of the investors. The targeted ailment may also play an important role in this decision. For example, the new drug may target a group of people – like melanoma patients in the United States, for instance – or a world-wide population for the eradication of a disease, like leishmaniasis. In most cases, there is a range of consumers related to the disease of interest that may be the target market. For a tuberculosis vaccine, the market can be selected to be a few hundred or a few million people. It may include anywhere from a portion of the high risk population in the United States to a vaccination for every person in the world to eradicate the disease.

The chosen market for a tuberculosis vaccine in this analysis encompasses hospital and military personnel in the United States. Hospital personnel are currently required to be screened for tuberculosis annually because of their higher potential of being exposed to the disease through the patients. Because the disease is a concern in the medical community, as well as for medical employees, a tuberculosis vaccine would make the screen unnecessary.

In addition, the military is currently being deployed to countries all over the world, in which they are most likely to become exposed to tuberculosis. A vaccine would also be of interest to the military, given the combat-ready status that they require of their people. The military currently has 1,427,000 members, and is also expected to grow in the next
few years. As such, the capacity of the plant will be geared towards providing the 13.4 million doses required in five years.

The possibilities of targeting associations such as the World Health Organization (WHO) and UNICEF (The United Nations Children’s Fund) are also feasible. Currently, these organizations would require the purchase of over 200 million doses of tuberculosis vaccines- an available option for future ventures and expansion. In the case for any new drug, the market may be determined by considering several different factors, including – but not limited to – resource and financial availability, viability of distributing the product, efficacy, competition, and demand.

**V. Market Analysis**

A market analysis will be crucial to the production, distribution, and financial gain for a new drug. The market takes into consideration competition, marketing strategy, advertisement, and demand model among others. Although these factors will vary greatly from drug to drug, it will be the mainframe of the market analysis.

**A. Advertising**

After advertisement of any new drug is started, word-of-mouth by patients to other patients or from doctors to patients will be important. In addition, developments of a drug may also be made public through journals and other citations published in the research and medical fields. The location of the plant often serves as a means to proliferate the development of the new drug; by keeping fellow researchers up-to-date
on the new advances, an interest may culminate in the success of the marketing of the new product.

Four general methods of advertising, which may be applied to multiple products, have been selected: conferences, sales representatives, television, and the World Wide Web. Conferences refer to presentations, interviews, meetings, and any other interaction with a potential customer that may result in an interest of purchasing the new drug. An itemized estimation of costs is shown in Table 1.

<table>
<thead>
<tr>
<th>Method &amp; Description</th>
<th>Cost ($/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sales Representative</td>
<td></td>
</tr>
<tr>
<td>• Salary</td>
<td>45,000.00</td>
</tr>
<tr>
<td>• Transportation, car, plane tickets</td>
<td>13,440.00</td>
</tr>
<tr>
<td>• Misc., meals, reimbursements, other</td>
<td>2,352.00</td>
</tr>
<tr>
<td>World Wide Web</td>
<td></td>
</tr>
<tr>
<td>• Web Page</td>
<td>2,400.00</td>
</tr>
<tr>
<td>• Web Master salary</td>
<td>32,000.00</td>
</tr>
<tr>
<td>• Fee, other</td>
<td>240.00</td>
</tr>
<tr>
<td>Television</td>
<td></td>
</tr>
<tr>
<td>• 30 second commercial</td>
<td>780,000.00</td>
</tr>
</tbody>
</table>

The market may further be divided into sections, whether it is by geographic location, state, or installation, in order to facilitate the advertisement of the new drug. The market for the carbohydrate- based tuberculosis vaccine has been divided by the number of hospital and military facilities nation-wide, estimated at 7,565 installations. Considering the cost of advertisement, a distribution of installations
visited in the next five years was planned in order to cover 96% of the total market within that time period. Year 0 in Table 2 represents pre-FDA testing and the FDA approval process in vaccine development. The 2% corresponds to the number of customers who would be interested in the product as soon as it is released. These customers would be acquired through the journals and reviews published on the carbohydrate-based vaccine. Table 2 shows the number of installations to be visited in a particular year in order to advertise to a particular fraction of the market.

<table>
<thead>
<tr>
<th>Year</th>
<th>Market Target</th>
<th>Installations Visited</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2 %</td>
<td>170</td>
</tr>
<tr>
<td>0 to 1</td>
<td>10 – 15 %</td>
<td>760</td>
</tr>
<tr>
<td>1 to 2</td>
<td>35 – 40 %</td>
<td>1,940</td>
</tr>
<tr>
<td>2 to 3</td>
<td>70 – 75 %</td>
<td>2,690</td>
</tr>
<tr>
<td>3 to 4</td>
<td>90 – 95 %</td>
<td>1,520</td>
</tr>
<tr>
<td>4 to 5</td>
<td>95 – 100 %</td>
<td>170</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>7,250</strong></td>
</tr>
</tbody>
</table>

Costs and methods of advertisement are itemized in Table 3 and apply to all new drug products on the market. The variables in the estimation are the aggressiveness of the advertisement (for example, number of sales representatives may be increased) and the size of the market. In the latter case, to have a generalized model for any new drug, a factor must be considered that accounts for how much larger or smaller the market of interest is in comparison to the one presented.
The first three years of production and manufacturing are the most costly for most of the new drugs on the market. The number of representatives needed to cover the set number of target locations is also included. It is estimated that a representative can visit each hospital a total of three or four times in order to make a sale. Including the travel time that it would take the sales representative to travel to these locations in addition to two weeks of vacation per year, each representative is able to visit 84 locations in a year. Routes will be planned according to the location of the sales representative at the time, so hospitals or military bases in proximity will be visited in order to minimize travel time and increase installations covered.

<table>
<thead>
<tr>
<th>Range (Years)</th>
<th>Advertisement Method</th>
<th>Expenses</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Word of mouth, Presentations, FDA Results, Journals, Visits (2 sales reps)</td>
<td>$121,584.00</td>
</tr>
<tr>
<td>0 to 1</td>
<td>Visits + Website + Television (9 sales reps)</td>
<td>$1,361,768.00</td>
</tr>
<tr>
<td>1 to 2</td>
<td>Visits + Website + Television (23 sales reps)</td>
<td>$2,212,865.00</td>
</tr>
<tr>
<td>2 to 3</td>
<td>Visits + Website + Television (32 sales reps)</td>
<td>$2,759,984.00</td>
</tr>
<tr>
<td>3 to 4</td>
<td>Visits (18 sales reps)</td>
<td>$1,094,256.00</td>
</tr>
<tr>
<td>4 to 5</td>
<td>Visits (2 sales reps)</td>
<td>$121,584.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>$7,672,032.00</strong></td>
</tr>
</tbody>
</table>
B. Beta Parameter

Beta is a parameter that describes how much the customer will prefer one product over another. Many considerations will affect $\beta$. For a new drug, some of these factors may include effectiveness, side effects, form (liquid, pill, etc.), method of administration (oral, injection, etc.), brand name, convenience, and availability of the new drug, to name a few. Each of the considerations is assigned a weighted average and the sum of the averages becomes the value of $\beta$.

For a carbohydrate-based vaccine for tuberculosis, it is anticipated that the customer will prefer a product that is more effective and has fewer side effects. Currently, vaccines, as a standard, are administered as liquid injections and this is the only product of its kind, so the other options mentioned above are not included. Weights were assigned to these considerations as seen on Table 4, and $\beta$ was calculated on the basis that the carbohydrate-based vaccine would be 3.5 times better than the BCG vaccine. Effectiveness has a higher weight than side effects; the effectiveness was estimated to be higher than that of BCG, as the side effects were estimated to be lower. Considering the extremely low efficacy of BCG versus the highly scrutinized FDA procedures (and personal expectations), this scenario is a conservative one. Because attenuated organisms are not injected into a patient, minimal side effects are expected in most cases. As a result, $\beta$ was estimated at 0.29. It is important to note that $\alpha$ and $\beta$ will affect the demand model. The parameter $\beta$, $\alpha$ function, and the demand model are also related because, providing this vaccine truly does have fewer
side effects and greater effectiveness, the more doctors and the public hear about the vaccine, the more they will prefer it over the competition.

Table 4. β Parameter Calculation with Different Preferred Properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
<th>Weight (w)</th>
<th>New Product (y₁)</th>
<th>Existing Product (y₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy</td>
<td>New product more effective than existent one</td>
<td>0.7</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Side Effects</td>
<td>New product has less side effects than existent one</td>
<td>0.3</td>
<td>0.7</td>
<td>0.3</td>
</tr>
</tbody>
</table>

\[ H = \sum(w*y₁) \]
\[ \beta = H²/H₁ \]
\[ \beta = 0.29 \]

C. Alpha Function

Alpha is a measurement of how well the customer knows the product. In most cases the value of \( \alpha \) will change with time for a new product. Initially, the value of \( \alpha \) will be very low, but once the customers see the product in commercials, are visited by the sales representatives, or hear the benefits from people who have tried it, \( \alpha \) will increase rapidly.

The values of \( \alpha \) can be determined by considering the marketing strategy and the aggressiveness of the campaign. It is important to note that the value of \( \beta \) (reflecting the consumer’s product preference) should be a determining factor in the marketing campaign. In addition, the exposure and advertisement of the new drug is tightly related to \( \alpha \). In the case of a carbohydrate-based vaccine, the value of \( \alpha \) was related
to the number of installations visited and the cost of advertising (refer to Table 2 in the Advertising section and Figure 5 below). The different values of \( \alpha \) were determined by considering the target market percent at a particular point in time. Although Figure 5 was constructed for the tuberculosis vaccine, the shape of the curve applies to most new drugs.

The \( \alpha \) function model is also a reflection of the aggressiveness of the marketing campaign. A less aggressive campaign will have a curve that is shifted closer to the \( x \)-axis; a more aggressive campaign will have a curves shifted above the one presented in Figure 5. The factors that will affect the demand curve the most are the availability of liquid funds and how fast the investor wishes to promote the new drug.

![Figure 5. Alpha Function Model](image)
**D. Demand Model**

The importance of a demand model lies in its inclusion of the demand and price of the existing and new drugs. In addition, a demand model can provide a price optimization and an estimated return on investment for the new product. It contains the $\alpha$ function, or the degree to which the public recognizes the product, and value of $\beta$, or the degree to which the new drug surpasses the existing drugs on different consumer preferences. The general equation for a demand model is shown in Equation 1, where $P_1$ and $P_2$ are the prices of the new drug and the existing drug, respectively. The demands of the new and existing products are represented by $d_1$ and $d_2$, respectively.

$$\alpha P_1 d_1 = \beta P_2 d_2$$  \hspace{1cm} (Equation. 1)

A more detailed and accurate calculation of the demand for a new drug is presented in Equation 2 below. By having the demand be dependent on a previous demand calculation, it is possible to find the value of the demand that satisfies all of the parameters ($\alpha$, $\beta$, $P_1$, $P_2$, and $Y$) with the use of iterations.

$$d_1 = \left[ \frac{\alpha}{\beta} \frac{P_2}{P_1} \left( \frac{Y}{P_2} - \frac{P_1}{P_2} d_1 \right) \right]^{1/\left(1-\alpha\right)}$$ \hspace{1cm} (Equation. 2)

Where:

- $P_1 =$ Price for the new drug
- $P_2 =$ Price for the existing drug
- $d_1 =$ Demand for the new drug
- $Y =$ Total budget delimited by the market selected
As observed, a problem may occur when the value of $\alpha$ is equal to 1. In most cases, the value of $\alpha$ – although it may approach 1 – will never be 1. Advertisement may have most of the market informed on the availability of the new drug, yet, there are subjects that may not be reached by advertisement. For example, if a person does not watch television or if they are a usual consumer of an existing drug, they will usually purchase whichever product is available (for the first case) or just purchase the usual drug in the case of repeat users. It is very likely that these people will not be affected by advertisement. Although this is not the case for all new drugs, it is a good estimate for most. There may be drugs that are directed, for instance, at a small group of people and where advertisement is limited to the prescribing physician. In this case, the value of $\alpha$ will be one and a modified version of Equation 2 is required.

By taking the values of $\beta = 0.29$ and an $\alpha$ that changes with time for the carbohydrate-based tuberculosis vaccine, and solving Equation 2 iteratively, the graph presented in Figure 6 is obtained. The values for $P_1$ and $P_2$ were $140.00$ and $115.09$ (current BCG price), respectively. The construction of the demand model for any new drug will provide how much of the market is attainable in a set amount of time.
Figure 6. Demand Model for Tuberculosis Carbohydrate-Based Vaccine

Figure 6 is representative of most demand curves for a new drug. An increase in demand is observed that behaves nearly linearly up to the second year of distribution. After the second year, the slope of the demand model is steeper, which indicates a rapid increase in demand in that period of time. Around the third year of distribution, the slope is less steep, signaling a plateau in the demand for the carbohydrate-based vaccine. At the end of the five years, it is estimated that the new carbohydrate-based vaccine can take over almost 82% of the market. The limitation below 100% of the market is a realistic one due to price and preference; although the new product will be preferred over the competitor’s, the higher price of the new product results in a cap on the total demand of the product sales.
E. Price Optimization

The price of a new drug will prove to be crucial in the success of the product, especially when the drug is not exclusive and there is a competitive market for it. The demand model, as explained previously, can be constructed with different prices, keeping all other parameters constant. Once solved, a plot of demand as a function of time can be graphed for different prices. The plot will most likely show that there will be a higher demand for the less expensive drug.

![Demand at Different Prices](image)

**Figure 7. Demand with Time for Different Prices of the Carbohydrate- Based Tuberculosis Vaccine**

Similar plots can be constructed for revenue as a function of time and profit as a function of time. Both graphs give a similar outcome. Figure 8 below is specific for the pricing, competition, market, and demands of a carbohydrate- based tuberculosis vaccine. Similar analysis can be performed for any new drug, and similar outcomes are expected. Figure 8 points out that one of the prices, although lower ($100/unit),
does not make as much profit as higher priced units. The irregular pattern of the
different curves reflects the effect of the \( \alpha \) function and the demand in the given time
period. For some of the set prices, although there is a demand higher than what can
be supplied due to the value of \( \alpha \), the production plant cannot supply the amount and
the profit reflects the limitation.

In addition, there is another behavior that does not prove to be efficient, as shown by
the curve for the $200/\text{unit}$ (see Figure 8). Although there is a high demand for the
production in the early stages of distribution, the total demand in the market has been
satisfied, and the last three years of production show that there is not a market to sell
the product. Of course, the market at this point could be re-evaluated and expanded,
but that is a second stage decision that is not covered in the scope of the project.
Profits are different for all prices investigated. It can be observed that for the
$140/\text{unit}$ price, there is a higher profit than any of the other options by the end of
five years.
Once the profit as a function of time is obtained, the net present value (NPV) of the product can also be found. The net present value indicates how much the plant is worth at a particular point in time. The NPV is useful when comparisons of investments are of interest. If the net present value is positive, then the product provides a satisfactory rate of return, and vice versa for a negative NPV. In addition, if the NPV is zero, the new product would provide a return that is equal to the discount rate. A positive NPV is indicative of the project being a favorable venture.

Equation 3 was used to obtain the NPV for each of the prices explored.

\[
NPV = \sum_{K=1}^{n-1} \left[ \frac{CF_K}{(1 + i)^K} \right] + \frac{CF_n + V_s + I_w}{(1 + i)^n} - TCI
\]  

(Equation 3)

Where:  
- \( CF \) = cash flow, which include the gross earnings and taxes  
- \( i \) = interest rate  
- \( V_s \) = salvage value of the company  
- \( I_w \) = working capital  
- \( TCI \) = total capital investment
For this analysis, a comparison of each NPV for the five different prices evaluated was plotted. Please refer to Figure 9. The plot results in an optimization method for pricing, where the highest NPV indicates the optimum price. Similar plots can be constructed for any new drug on the market. In the case of a carbohydrate-based tuberculosis vaccine, the optimum price was determined to be $140/ unit. The evaluated range of pricing, from $100/ unit to $200/ unit, encompassed the competitor’s price of $115.09/ unit.

Return on investment, or ROI, is another feature of interest to investors. ROI is the ratio of the estimated profits and the fixed capital investment over a given period of time. The calculation can also reflect when the investor should expect to recover his initial investment. A graphical comparison of the ROI with varying prices may be completed, as seen in Figure 10 below for the carbohydrate-based tuberculosis vaccine. The ROI is a maximum for the $140/ unit option and is indicative of the optimum price at which the vaccine should be sold. Similar graphs can be plotted for any new drug using the ratio of profit and fixed capital investment. The time at
which the initial investment is to be recovered can be calculated by setting the ROI ratio to zero and solving the equation for the time. The time estimated for the tuberculosis vaccine was three years.

![ROI Within 5 Year Span for Different Prices](image)

**Figure 10. ROI for New Carbohydrate-Based Tuberculosis Vaccine**

**VI. Risk Analysis**

In analyzing the risk of investing in a new vaccine that follows a similar production, testing, and approval process to that of the carbohydrate-based tuberculosis vaccine, the probabilities of success and non-success should be estimated using flowcharts like those shown in Figures 2 and 3 above. Additionally, several first and second stage decisions may be selected to assess the risk associated with each.

A first stage decision entails choices that are ‘here and now’ and are the decisions that need to be made before the uncertainty is revealed. For the carbohydrate-based tuberculosis vaccine, the first stage decision involving the degree to which the vaccine production process is researched, as outlined in Table 5, affects the degree of assurance
of the project’s success in FDA trials before it proceeds to the costly process. It should be noted that for each of the varying degrees of research, the entire production of the antibody will be researched, but, depending on the first stage decision, certain steps will be researched extensively. For the sake of net present value calculations and risk comparison, it is estimated that researching the conjugation between the tetanus toxoid protein and capsule polysaccharides will take six years. Continuing research to include the capsule cleaving and recovery process will require a total of approximately eight years. If the first stage decision chosen includes the aforementioned processes plus a focus on bacterial growth, the research is expected to take ten years. The number of years devoted to pre-FDA research dictates the initial investment and the overall probability that the project will continue to the next steps and succeed overall. The process for any new drug is very similar, depending on certain first stage decisions made regarding the initial research investment. The ultimate goal is to have a process with less risk in order to increase the probabilities of FDA approval.

Table 5. Typical First and Second Stage Decisions

<table>
<thead>
<tr>
<th>First Stage Decision</th>
<th>Second Stage Decisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focus process(es) in pre-FDA research</td>
<td>Time to begin plant construction</td>
</tr>
<tr>
<td>• Protein/polysaccharide conjugation</td>
<td>Time to begin marketing campaign</td>
</tr>
<tr>
<td>• Capsule cleaving/recovery</td>
<td>Additional research after failed FDA stage</td>
</tr>
<tr>
<td>• Bacterial growth</td>
<td></td>
</tr>
</tbody>
</table>

Conversely, a second stage decision may involve a process issue. These decisions—direct results of a particular situation as research and development continue—are made in order to adapt to the realization of an uncertainty parameter, such as length of time spent in
FDA or success of the drug. As mentioned before, a second analysis has been completed for the case in which a drug that suffers a clinical hold is returned to research for one year before continuing in the FDA process. This second stage decision may not arise unless the scenario actually occurs. In addition, second stage decisions for new drugs may be involve the production capacity of the plant or a modification to the production methodology.

The time in which to start plant construction is one second stage decision that may be chosen for risk analysis; for the given example, the plant will begin construction three years before the vaccine is expected to exit the FDA approval process. For any new drug, it is ideal to be able to commence product production as soon as the FDA approval process is completed. The vaccine’s success or delays in the FDA process, however, may change this initial decision. Furthermore, it was decided that active marketing of the potential product will begin one year after plant construction begins. Again, this second stage decision may vary according to the vaccine’s time and success in FDA testing, which are affected by the first stage decision. The investors may decide to change these first or second stage decisions, but the resulting risk will differ from that presented. The analysis presented above is specific for the pathways of a carbohydrate-based vaccine; each analysis will be very specific for the type of new drug produced.

For any similar drug passing through FDA testing, each successful scenario through the pre-FDA research may be paired with the varying successful or failed pathways through the FDA flowchart, and the probabilities should be compounded. Based on the
equipment, labor, and materials needed for the carbohydrate-based tuberculosis vaccine at each stage in the pre-FDA and FDA processes, the required investment is calculated. As expected, scenarios that are not successful in any pre-FDA or FDA stage and do not yield a marketable product return a negative net present worth.

The product price and expected market used in calculations for the tuberculosis vaccine were based on the demand, \( \alpha \), and \( \beta \) values presented in the Market Analysis section. Net present value, one condition that assesses the profitability of a project, may be calculated at each step in the pre-FDA and FDA testing processes to monitor losses or profits associated with an unsuccessful or successful drug. As for any drug risk analysis, the cumulative probabilities of each scenario may be plotted against the associated net present worth to create the risk curves. They represent a collection of paths derived from the variation in the first stage, or “here and now,” decision. Figure 11 is the culmination of scenarios under the second stage decision that drug fails if it receives a clinical hold.
As shown in Figure 11, the risk varies according to the first stage decision of topics researched (expressed in terms of the associated years in research) and initial investment supplied for pre-FDA testing. Risk ranges from 9% success for six years in research to 46% success for ten years in research. A detail of the potential losses for the evaluated scenarios is displayed in Figure 12.
Figure 12. Detail of Losses for Carbohydrate- Based Tuberculosis Vaccine

The close-up for the losses indicated by the risk curves shows that a six year first stage decision couples a higher risk with a greater potential for loss.

If the drug were allowed to cycle back to research and then to FDA for a single failure in the FDA process, the risk decreases but the potential net present value for gain does as well, as deduced from Figure 13. The probability of success now ranges from 23% to 53%. 
Figure 13. Risk Analysis for “Second Chance” Tuberculosis Vaccines

Figure 14 shows that the six year curve still has the greatest potential for risk and for losses. In comparison with Figure 12, all curves in Figure 14 have a greater chance of greater loss overall.
When the expected worth of the project is calculated for each of the first stage decisions over the range of probabilities of success and failure (those displayed in the risk curves above), it is clear that a decrease in risk accompanies a decrease in expected worth, as shown in Table 6.

Table 6. Risk and Expected Worth for Tuberculosis Vaccine

<table>
<thead>
<tr>
<th>First Stage Decision- Time Invested in Research</th>
<th>6 Years</th>
<th>8 Years</th>
<th>10 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single trip through FDA</td>
<td>Risk</td>
<td>90.8%</td>
<td>78.1%</td>
</tr>
<tr>
<td></td>
<td>Expected Worth (millions)</td>
<td>$555.43</td>
<td>$494.43</td>
</tr>
<tr>
<td>Recycle through FDA</td>
<td>Risk</td>
<td>76.7%</td>
<td>65.3%</td>
</tr>
<tr>
<td></td>
<td>Expected Worth (millions)</td>
<td>$485.97</td>
<td>$428.16</td>
</tr>
</tbody>
</table>

Figure 14. Detail of Losses for “Second Chance” Tuberculosis Vaccines
The high amount of risk associated with each of the cases presented above, as in any related endeavor, is expected because of the volatility of the biotechnology domain and the novelty of this type of vaccine production. It is important to note that although the risk is extremely high, in the case of a successful venture, there is potential for a high expected worth for the example of the tuberculosis vaccine. Depending on the span of the market and the cost of production for any new drug, a similarly high expected worth may be anticipated.

**VII. Conclusions**

After analyzing the critical decisions associated to the risk of a new drug in the market and the feasibility of a carbohydrate-based tuberculosis vaccine in particular, several conclusions were drawn.

- The first-stage decision for initial investment depends on a period of six, eight, or ten years spent in pre-FDA research.
- A product price of $140 was determined to be the optimum, as dictated by the demand and pricing models, and was applied in the calculation of risk and expected worth.
- Due to the volatility of the biotechnology field and the uncertain success of the vaccine through FDA approval process, the risk involved in this enterprise ranges from 54% to 91% and is influenced by which vaccine production steps are researched extensively (first-stage decision).
- In a second analysis that allows a failed FDA case to return to research and development, the risk decreases to range from 47% to 77% and the expected worth decreases for each case of varying first stage decisions.
• Overall, a ten year pre-FDA research period yields a significantly greater potential for success and affects the potential net present worth almost negligibly.

• The model presented above may be used for any new drug and includes:

  1. First and second stage decisions
  2. Generalization of pre-FDA research and the FDA approval process
  3. Market analysis
  4. Demand and pricing models
  5. Risk analysis
  6. Expected worth determination
VIII. References


Appendix I- Overview of Tuberculosis

Tuberculosis is the leading cause of death in the developing world, which encompasses 76% of the total world population. It is estimated that 2 billion people are infected. In addition, statistics show that 8 million people per year develop the active form of tuberculosis and 3 million of these die. In the United States, it is estimated that 10 to 15 million people have been exposed to the organism and have the latent form of the disease. Projections for the United States estimate that 90% of all HIV patients will become infected with tuberculosis and die: roughly 37.8 million people. Furthermore, in the year 2020, 1 billion new cases of exposure are expected. From the new infections, a total of 36 million deaths will occur. According to some scientists, it is very possible for tuberculosis to be the next world pandemic.4

A. Vaccines & New Technology

Vaccines are weakened or killed pathogens or parts of pathogens. The goal of a vaccine is to induce antibody production in the body. When a vaccine is administered, it is recognized by antibodies as a foreign body and will provoke the same immune response that exposure to the pathogen would. Vaccines for a number of infections are available. The main problem with many current vaccines is the side effects of injecting humans with the actual organism: they may have traces of live pathogens and cause the disease.

New research shows that the manufacturing of a carbohydrate- based vaccine is possible. Some of the benefits of a carbohydrate- based vaccine are that the
carbohydrates will include only part of the organisms or can be synthesized in a laboratory. There is no risk of infection with the organisms and side effects are reduced. If the carbohydrates are synthesized in a laboratory, the consistency and purity of each dose can be monitored and guaranteed. Some of the disadvantages include the possibility of the carbohydrates not producing a satisfactory immune response.

The first synthetic carbohydrate vaccine was successfully produced in Cuba but is not currently available for the United States market.¹ This vaccine for *Haemophilus influenza* type B averages in price at $3.00 per dose to the manufacturer and has a 99.7% effectiveness. On the basis that carbohydrate vaccines have been successfully developed, a tuberculosis vaccine will be explored and used as a general model for risk and financial analysis of a new drug.

Malaria, leishmaniasis, HIV, leprosy, and cancer have different routes of infection, incubation periods, and effects on the human body; what makes them alike is the targeted response of the immune system. The diseases provoke an antibody response that is not sufficient to rid the body of the organism. In addition, some of the disease-causing organisms use the cells of the bodies as protection against the immune response. In the case of cancer, it is not an organism causing the degenerate reproduction of cells. An efficient vaccine would have to give the body a recognition system that targets the invading organism (or abnormal growth for cancer) to make it an effective vaccine.
**B. Tuberculosis Mechanism**

*Mycobacterium tuberculosis* is an acid-fast bacillus that has a waxy capsule composed of sugars—mainly arabinose and mannose. *M. tuberculosis*, a pathogen that infects the cells in the lungs, has developed a mechanism to prevent cell destruction and to ensure survival. When a foreign body known as an antigen enters the body, macrophages (cells that engulf antigens) recognize, find, and attach to the antigen. *M. tuberculosis* binds to the receptors of the macrophage, and, in a sense, forces it to consume the organism. Once inside the macrophage, *M. tuberculosis* is able to prevent the formation of a lysosome (an acidic vacuole that kills bacteria) and, consequently, prevents the macrophage from killing the organism, stopping the immune response. T cells are not signaled to attack the antigen, and the bacterium is not displayed in the surface of the macrophage. As a result, there is no cell proliferation or activation, and the organism is safely kept in the macrophage where it can reproduce. The reasons for the prevention of lysosome formation are not clear. It is certain, however, that this method of reproduction and survival has proved to be extremely efficient for *M. tuberculosis*.

The evaluation of existing methodologies, procedures, and survival mechanism of the bacterium, suggest that when the carbohydrates on the waxy capsule of *M. tuberculosis* are administered in conjugation to a protein, the body will recognize it as foreign, attack, and degrade the vaccine component, initiating the body’s immune response. T and B lymphocytes will respond by producing antibodies and memory
cells, respectively. In a sense, the body will learn to recognize the antigen and produce its own antibodies for subsequent exposure to the disease.

**C. Competition**

Available alternatives to the vaccine being researched and produced affect the market demand and, thus, the financial success of the project. Currently, there are three available treatments specifically designed for tuberculosis—a vaccine, Bacille Calmette-Guerin (BCG), and two antibiotics, INH and Rifampin. The antibiotics are to be taken for an extended period of time, usually six to nine months and may affect several different organs, such as the liver and the kidneys. The length of time that these antibiotics are prescribed proves to be a hassle for the patients and usually results in an incomplete treatment. Either the patients discontinue the intake of the daily pill(s), or there are missed pills in that time frame. This drastically reduces the chances of an effective treatment.

Conversely, the vaccine is a one-time dose that the patient needs to be administered. Although it addresses and solves the extended period of time needed for treatment, it also proves to be inefficient. The BCG vaccine is made from a similar organism, *Mycobacterium bovis*, and not the actual organism, *Mycobacterium tuberculosis*. For public safety reasons, *M. tuberculosis* could not be used, and it entails the reason for its reduced number of treatment and cure capabilities. The BCG vaccine has less than 5% success. Another downfall of the BCG vaccine is that once the patient is inoculated, the TB skin test yields a false positive result. This makes it very difficult to determine if the patient has had a previous exposure to tuberculosis or if it is just
positive because of the vaccine administration. For the most part, the body does not produce antibodies that would protect it from tuberculosis, and most of the patients still contract the disease when exposed to it.

**Appendix II- Tuberculosis Vaccine Design and Production**

The novelty of the proposed methodology is in the approach of the formulation of a carbohydrate-based vaccine for tuberculosis. The method enlists previous works to isolate and separate the capsule, as well as to conjugate the recovered carbohydrates to the tetanus toxoid. The use of carbohydrates for antibody stimulation is a young field. It is valid to note that although the methodologies for conjugation have been used before with the intent of forming a tuberculosis vaccine, the product has not been marketed or been the subject of analysis such as that presented here. It is the intent of this project to replace the existing product with a safer and more efficient alternative. The novelty, then, is in combining the existing sources to form a product in a realm of limited research.

The resulting basic design for the carbohydrate-based tuberculosis vaccine is to obtain the fragments of the polysaccharide capsule and conjugate them to an adjuvant protein.

**A. Carbohydrates**

It has been documented that carbohydrates alone injected into humans does not induce an antibody response. Cells have carbohydrates in their structure, and so the body does not recognize them as foreign. Conversely, when carbohydrates are
conjugated to a protein, a stimulus in the immune system is observed by measuring antibody concentration (titers).¹

**B. Adjuvant Protein**

Tetanus toxoid is used as the adjuvant protein. Most of the population has had an initial tetanus vaccine, so the response with the same protein attached to carbohydrate chains is expected to be immediate and with high enough titers as to not require a booster. In the case where the patients have not been immunized against tetanus, an antibody response is expected in most cases since the tetanus toxoid is an acknowledged antigen.

**C. Methods**

1. **Bacterial Growth**

   The strain that will be used is ATCC 25177. Bacterial growth will be accomplished in Lowenstein-Jensen plates which provide the nutrient requirements for the growth of *M. tuberculosis*. The generation time on the plates is estimated to be 6 to 8 weeks. Once the organism has grown, it will be transferred to Lowenstein-Jensen liquid media, commercially sold in 1-ounce bottles. The plates and bottles will be incubated at 37°C. The generation time for the organism in the liquid media, is estimated to be 15 hours.²

2. **Capsule Recovery**

   Centrifugation is performed to obtain a cell pellet; it will result in a concentrated amount of cells from the medium. Centrifugation should be performed at 3,000 g

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for 20 minutes. The pellet is then re-suspended and washed in 20 mM sodium phosphate buffer and 0.05% Tween 80. The use of a buffer increases the viability and integrity of *M. tuberculosis*. A second centrifugation is required to remove the cellular debris. The pellet is re-suspended in distilled water, and then submitted to cycles of sonication, which sends pulses through the sample, and therefore, through the cells. The process results in the weakening of the cell wall, but not destruction of organisms. Fifteen cycles of 15 second pulses at 4 °C is recommended. Although there are recommended cycles with times, it is also suggested that they be monitored and modified as necessary to yield a higher capsule recovery. Centrifugation is then performed to separate the capsule (supernatant) from the proteins (cellular debris) at 25,400 g for 20 minutes, then at 148,000 g for 60 minutes.\(^9\) It is important to note that the cell capsule is what is extracted from the process. The weights of the proteins in the cellular cytoplasm are heavier than the carbohydrates on the surface. In addition, since the cell had been weakened, the separation of the capsule from the components of the cellular cytoplasm will be more effective.

Studies performed with *M. tuberculosis* show that the technique is an efficient one to obtain capsular separation and therefore, carbohydrate recovery.\(^9\) The supernatant of the sample is submitted to filtration through a 0.2 µm filter. Only carbohydrates of the cell wall are expected to be recovered. The size of proteins will be the limiting factor that will aid in separating them from the desired product.
Many chemical analyses exist for verification of the carbohydrate purity. Although these tests are expensive and very time consuming, there are reference laboratories that perform them. This may be good practice on a periodic basis – perhaps one analysis for each batch. The design of the vaccine, however, has allowed for the accommodation of small impurities. It is important to note that the probability of contamination with cellular protein is minimal. In the case that protein contaminations were to occur, it would not affect the engineered vaccine. The purpose of the vaccine is to present the human immune system with fragments of *M. tuberculosis* so that it can recognize it as a foreign body and attack it. Protein remnants would be recognized as foreign as well.

3. **Carbohydrate Cleaving**

The polysaccharides of the capsule will then be cleaved to obtain fragments between 2 and 10 kDa. The cleaving of the capsule is not required to be specific in molecular structure. Because the capsule of *M. tuberculosis* is used, the desired outcome is an immune response to any and all parts of the capsule. The cleaving is random (to a degree) and is limited only by the size of the fragment. The size chosen has several benefits. With the size range, the possibility of having a complete *M. tuberculosis* cell is brought to zero. This ensures that the patient will not be infected with the actual organism and therefore, cannot acquire the disease. In addition, it has been shown that a satisfactory immune response can be achieved with molecules in this range. Anything over 10 kDa would result in wasted materials, as the immune response is not enhanced with added antigen.
Acetolysis is the method chosen for the cleaving of the carbohydrates. This method has two separate phases: (1) acetylation and hydrolysis and (2) deacetylation. For acetylation and hydrolysis, the sample is treated with acetic acid, acetic anhydride, and sulfuric acid and let stand for 8 hours at room temperature. An additional incubation period of 10 hours at 5 °C follows. Cells are submitted to a shock treatment with ice water. The mixture is then brought to room temperature while monitoring and stabilizing the pH to 7.5 with sodium hydroxide. This process will result in the cleaving of carbohydrates at different sites, with an acetyl group added to the ends of the fragments. The time and temperature of the procedure has been changed so that instead of cleaving the carbohydrates repeated times until monosaccharides are obtained, the fragments obtained will be larger. The sugar acetates are then extracted with chloroform and allowed to dry and evaporate. Deacetylation is accomplished with methanol and barium methoxide. Dowex 50 is used to remove extra particulate from the solution. The sample is submitted to gel filtration and the fragments are recovered. The procedure results in fragments that are ready to be submitted to conjugation with the adjuvant protein.

4. **Carbohydrate – Protein Complex Synthesis**

Following the growth of the *M. tuberculosis* cells and the cleaving of the capsular polysaccharides, it is necessary to combine the sugars and the carrier protein, tetanus toxoid. In general, the separate components must be activated, so that the protein and polysaccharides, when combined, readily attach and form the desired complex. A method similar to that presented by Pawlowski, Kallenius, and
Svenson\textsuperscript{[4,5]} is proposed. This previous research will be highlighted because of its high yields at each step and its particular work with tetanus toxoid.

The polysaccharide samples (PS) are first aminated by the following reaction:

\[
\text{PS-CHO} + \text{NH}_4\text{Cl} \rightarrow \text{PS-NH}_2. 
\]

They are dissolved in distilled water and 200 fold molar excess solid ammonium chloride. Solid sodium cyanoborohydride is added, at which the reaction mixture is stirred for six days, producing an approximate substitution yield of 90\%. Upon sufficient completion of amino substitution, the polysaccharides are thiolated by the following reaction:

\[
\text{PS-NH}_2 + \text{S}-\text{NH}_2^+\text{Cl}^- \rightarrow \text{PS-NH-C(NH}_2^+\text{Cl}^\text{-})(\text{CH}_2)_3\text{-SH}. 
\]

They are dissolved in EDTA buffer and supplemented with 20 fold molar excess of 2-iminothiolane. The reaction mixture is stirred over approximately 1.5 hours, and the expected yield is about 70\%.

At the same time, tetanus toxoid (TT) must be purified and activated by bromoacetylation for conjugation as follows:

\[
\text{TT-NH}_2 + \text{NHS-CO-CH}_2\text{-Br} \rightarrow \text{TT-NH-CO-CH}_2\text{-Br}. 
\]

A stock solution of tetanus toxoid is purified from salts and residual impurities using a desalting column and eluted with bicarbonate buffer. Solid N-hydroxysuccinimide ester of bromoacetic acid is added and stirred for about 1.5
hours. The solution is then desalted again on a desalting column and concentrated in a 30kDa ultrafiltration cell, yielding activation of about 70%.\textsuperscript{5}

The prepared capsular polysaccharides and tetanus toxoid samples are ready to be combined and the resulting complex purified as shown below:

\[
\text{PS-NH-C(NH}_2\text{Cl')-(CH}_2\text{)}_3\text{-SH} + \text{TT-NH-CO-CH}_2\text{-Br} \rightarrow \\
\text{TT-NH-CO-CH}_2\text{-S-(CH}_2\text{)}_3\text{-C(NH}_2\text{Cl')-NH-PS}
\]

The thiolated polysaccharides (the product of the second reaction) are dissolved in bicarbonate-EDTA buffer, and tributyl phosphine in isopropanol is added in five molar excess. The bromoacetylated tetanus toxoid (the product of the third reaction) is added in a polysaccharide/tetanus toxoid ratio of 50. The unreacted bromoacetylated tetanus toxoid is inactivated by 2-mercaptoethanol, and the reaction mixture is concentrated by a 30kDa ultrafiltration cell. The excess polysaccharides are then removed by hydrophobic interaction chromatography with ammonium sulfate, and the conjugates are desorbed with phosphate buffer followed by isopropanol. The final product is concentrated once again using a 30kDa ultrafiltration cell, giving an estimated yield of 90% and an overall yield of 40%.\textsuperscript{5}

Further work provided by Pawlowski, Kallenius, and Svenson confirms the success of a carbohydrate-based tuberculosis vaccine with respect to the production of a substantial immune response.\textsuperscript{6} When arabinomannan oligosaccharides were coupled to tetanus toxoid by the methods presented
above, the resulting vaccine provided prevention of infection as well as the BCG vaccine. The research article also provides alternative carrier proteins and adjuvants for a tuberculosis vaccine, the consideration of which is beyond the scope of this paper.

Due to a deficiency in personal laboratory research and data with capsular polysaccharides and tetanus toxoid, further research should be performed to determine the exact reaction concentrations, reaction times, product yields, and immune responses. The risk associated with the technical aspects of the engineering of the carbohydrate-based tuberculosis vaccine were assessed and presented as part of the report. Please refer to the Research and Testing and Risk Analysis sections.

**D. Technological Risks**

Many risks and deviations accompany the methodology.

1. In the growth phase of the organisms, it is possible that there be a marked deviation from the expected growth time in the Lowenstein-Jensen plates.

2. Recovery of capsule as estimated for *M. tuberculosis* has been approximated to 1% of the total cell weight. The weight and capsule recovery will need to be determined experimentally, and even then, it is possible to have deviation from batch to batch.

3. A modification to the sonication procedure of the capsule separation phase may be required due to low yields of carbohydrate recovery.
4. Acetolysis may need to be modified depending on the size of the recovered fragments. It is possible that fragments larger then 10 kDa or smaller than 2 kDa may be obtained.

5. The capacity of the equipment may have been over or underestimated depending on the above deviations.

6. In the first step of conjugation, the polysaccharide may not undergo sufficient activation by amino substitution.

7. In the thiolation step, it is possible that insufficient sulfhydryl groups were activated after the reduction of the disulfides.

8. Residual salts and impurities that are not properly removed from the tetanus toxoid would contaminate the product and might denature the protein.

9. An incomplete bromoacetylation reaction with the tetanus toxoid would result in a lack of activated amino groups.

10. For undefined reasons, conjugation between the polysaccharides and tetanus toxoid may remain incomplete.

11. At any step in the conjugation process, a variance in pH may denature the protein and render the complex ineffective.

12. As in any sterile biotechnological process, the presence of contaminants and free reactants is highly unfavorable and may inactivate the complex or contaminate the final product.
**E. Vaccine Components**

The conjugate must be prepared in a vaccine before its injection into test animals and clinical trial patients. In a 0.5 mL dose, it is estimated that 25 µg of the polysaccharide-tetanus toxoid complex will be sufficient to illicit the desired immune response and antibody stimulation. This will be supplemented with sodium phosphate buffer to maintain the pH at 7.2 to avoid denaturing the protein and thus inactivating the complex. Finally, it is standard to dilute these components to the 0.5 mL volume with 0.9% sodium chloride saline solution. Because the vaccine contains inactive parts of the tuberculosis capsule and not the bacteria itself, it is not necessary to include a preservative. The vaccine should, however, be stored under refrigeration. In addition to being the carrier protein, tetanus toxoid serves as an adjuvant to stimulate the immune response, so an additional adjuvant is not necessary.

INH is a commonly prescribed antibiotic for the treatment of tuberculosis. INH must be taken daily by the patient for six to nine months, depending on the dosage of the pills. Although the price for INH is low at $25.00, the extended period of time results in a hassle for the patient or the patient may just decide not to finish the antibiotic regime because of lack of symptoms. Similarly, Rifampin is another antibiotic that can treat tuberculosis. The price of the full treatment is $360.00 and consists of taking a pill daily for four months. Like INH, Rifampin treatment proves to be tedious, having the same undesired results as INH.²
Appendix III- Research and Testing

A. Research Labor

The cost of labor for the research and testing stages and for the manufacturing process was estimated. At the research level, a research specialist will be hired to assist with the execution of experiments, the compilation of results, and the development of the cell growth, carbohydrate cleaving, and complex conjugation processes. A research laboratory manager is needed to manage the projects and staff in the lab. Eight research assistant seniors will be hired to assist in experimental design, data collection, data analysis, and the training of research assistants. These research assistants will perform routine procedures in the experiments clerical duties in the lab. The estimated yearly research labor cost is $560,000.

In additional, medical and laboratory personnel are required to conduct animal and FDA testing and to analyze the results. The medical director will implement and monitor clinical studies and prepare parts of the FDA applications. A clinical research manager will write the protocols for each clinical trial and organize informed consent forms from the test subjects. A senior clinical research associate ensures that good clinical practices, as set by the FDA, are being followed. The senior biostatistician is needed to monitor the statistical integrity, adequacy, and accuracy of the clinical trials. The clinical data manager oversees the collection of data obtained from testing. To assist with the preparation and quality control of regulatory documents, a senior medical writer will be hired. A regulatory affairs associate will ensure that the products meet FDA policies and worldwide regulatory requirements.
by way of the compilation of files and reports for submission. Finally, a medical affairs director will oversee the above personnel by tracking updates on standard operation procedures, maintaining quality and financial standards, and medically monitoring the ongoing clinical trials. The staff required for testing and clinical trials will cost approximately $910,000 per year.

**B. Animal Testing Subjects**

For the testing of a carbohydrate-based vaccine, it is recommended that animal testing consist of one hundred male BALB/c mice is suggested- 80 will be injected with the complex, 10 will be injected with saline solution as a control, and 10 will be injected with unconjugated carbohydrates as an additional control. They will be injected subcutaneously with 1 $\mu$g of conjugates in 0.25 mL phosphate-buffered physiological saline. The potential for adverse reactions in the mice will be observed. If the mice have adverse reactions to the vaccine, the product returns to research and development in an attempt to make the appropriate modifications. If there is no reaction or the reaction is considered mild and acceptable, the vaccine continues in the testing. Blood samples will then be collected from each mouse over a 120 day period, in which the IgG serum titer levels will be monitored. If the titer levels are not sufficient, the mice may be reinjected with the conjugate at day 28. The titer levels will continue to be monitored for the remainder of the 120 day period. If the titer is high enough without the booster or the same or higher than the current BCG vaccine after the booster injection, the vaccine will move on to FDA clinical trials. Similar success criteria would be applied to other injectable drugs, such as a notable resistance to a disease or the significant reduction of cancer cells.
The bacterial growth and capsular polysaccharide recovery of *M. tuberculosis* is expected to take six months to one year based on the initial investment supplied for research. Similarly, formulation of the conjugate formation step is expected to take 18 months to two years. If either of these steps is unsuccessful, the vaccine will return to research and development in that area. Once a satisfactory polysaccharide-protein complex has been produced, the animal testing stage can begin.

**Appendix IV- Plant Specifications**

**A. Timeline**

The timeline in Figure 11 was constructed considering product research stages, the FDA approval process, plant construction, and advertising campaigns. It is important to note that the timeline is a rough, conservative estimate of process development. The initial investment in research will drastically alter the timeline projections. Figure 15 was constructed for a carbohydrate-based tuberculosis vaccine under the first stage decision of six years in research, but the headings are representative of the different steps that all new drugs would have to advance through to deliver a final product. The time allotted for research and testing are highly dependent on the progressive success of the vaccine; further research may or may not produce an effective conjugate, and the vaccine may or may not be delayed by clinical trials. Plant construction should begin three years before the expected completion of the FDA approval process. Ideally, the plant facilities would be completed at the same time the vaccine passes the final stages of FDA approval, allowing immediate
production. The official marketing campaign will begin approximately one year after plant construction is initiated. The accuracy of the timeline is difficult and dependent on the success of each of the phase of the product, which is dependent on the first stage decision of the amount of time invested in research. Most importantly, this rough timeline and the number of years associated with different research and FDA process scenarios influence the year at which the fixed capital investment for plant construction is incorporated into cost calculations and the number of years over which the project value is discounted.

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<th>FDA: Phase I</th>
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**Figure 15. Projected Timeline for Research and Production of Vaccine**

**B. Production Capacity**

For a new drug, the capacity of the plant must be carefully and strategically chosen depending on marketing and demands. In the case of the tuberculosis vaccine, the production capacity of the plant was set at 5 million doses per year to cover the
demand discussed in the Market Analysis section in five years. Several capacities were evaluated and market growth was taken into consideration. The current market was estimated at 2.68 million vaccines per year. Because the medical field is the bulk of the clientele (12 million approximately), the market growth in this sector was taken to be equal to the overall market growth. The medical field is the second fastest growing sector in the economy with an average growth rate of 3.5% per year. As shown in Figure 16, the most efficient production capacity was at 5 million doses per year. It is valid to mention that the target market has been delimited, but other potential customers are also available, such as the WHO and UNICEF; they would be prime candidates to purchase the surplus of vaccines.

![Capacity of Plant](image)

**Figure 16. Plant Capacity**
C. Scaled-Up Equipment

The scaled-up equipment is relatively small-scale compared to typical processing plant equipment. As such, the equipment does not differ much from that used in laboratory research. For the bacterial growth of *M. tuberculosis*, multiple incubators are needed. Two centrifuges are required for various stages of separation. A sonicator will be used to debilitate the polysaccharide capsule and a microfiltration cell will further separate and purify the polysaccharide samples.

For the conjugation steps, three mixing tanks are required. Two desalting columns will be used to purify the samples from salts and other impurities. Two hydrophobic interaction chromatography columns will be used to remove excess reactants and two 30 kDa ultrafiltration cells will be used to concentrate the reacted solutions and to obtain the desired portions of those solutions in the retentate. pH regulators will monitor the pH throughout the process to avoid protein denaturation and ensure product quality.