Technological & Financial Analysis of a Carbohydrate Vaccine for Tuberculosis

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Tuberculosis

Currently:

- Leading cause of death in developing world
- 2 billion infected
- 8 million/year - active TB
- 3 million/year die
- 10-15 million latent in U.S.

Projected:

- 37,800,000 of current HIV patients will become active and die in the U.S.
- New Cases: 1 billion 2020
- 36 million deaths from new infections

Vaccine Needed!

What is Tuberculosis?

- *Mycobacterium tuberculosis*
- Infection by inhalation
- Contagious when active
- Symptoms: weight loss, fever, appetite loss, cough, chest pain, bloody sputum
- Patients will die within weeks to months without treatment

New Drugs: Significance

- Drastic effect on population
  - Lower death rates
  - Extend life expectancy
  - Eradicate disease (small pox 1967-73)
- Financial gain
- Personal motivations

Romantic view!

New Drugs: Reality

- Average cost over $400 million from research to consumer
- Strictest protocols for drug approval in U.S.
  - Food & Drug Administration
  - Lengthy and tedious process
  - average = 15 years
  - Success rate: 5/5,000 potential drugs

A Researcher's Concerns

- Will my procedure work?
- How accurate is my theory?
- How can I increase the product yield?
- Can this process be scaled up?

An Investor's Concerns

- Amount to invest in research?
- How long will it take?
- Risk of losing money?
- How much can I lose?
- Expected profit?
- What timeframe?
- Failure at any FDA Phase?
- Product price?
- What is the market?
- Advertisement campaign?

We can provide simultaneous answers to these questions!

Project Overview

- Proposal

  Carbohydrate- based tuberculosis vaccine

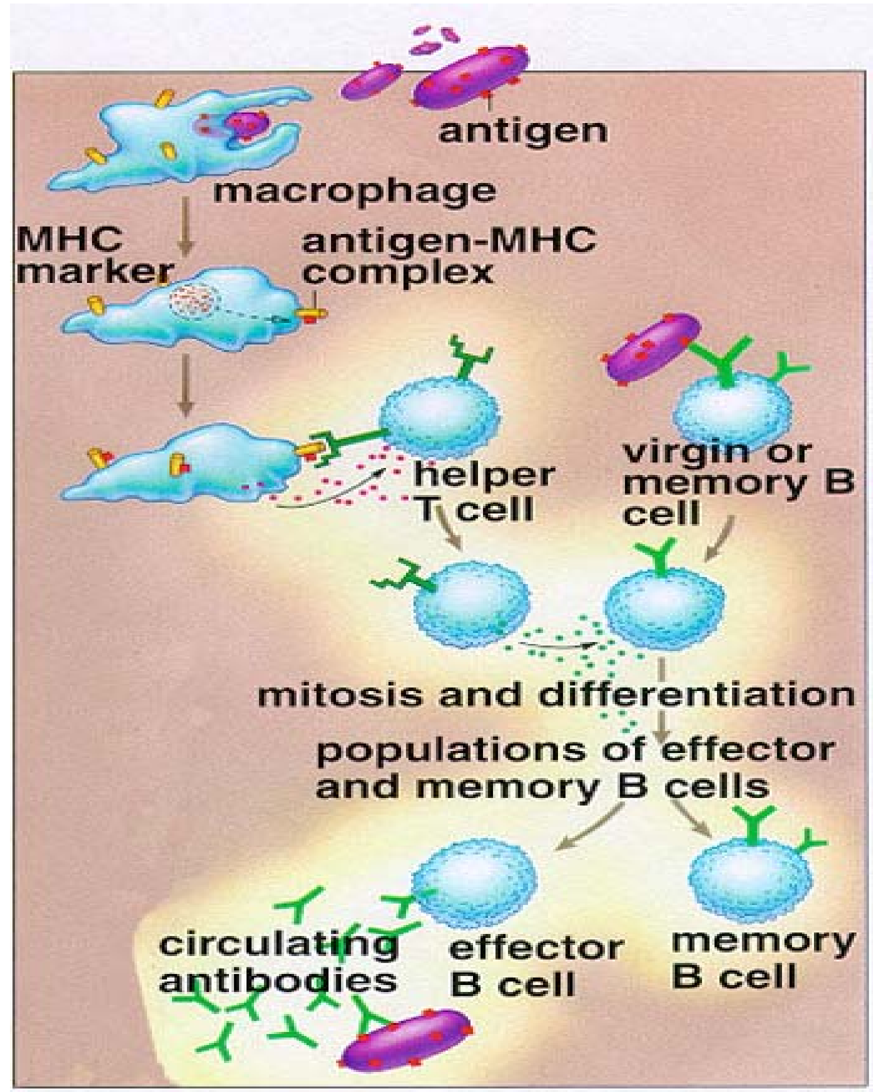
- Acknowledgment of technical uncertainties
- Success estimate
- Two directions
  - How to develop vaccine
  - Decisions to be made

Vaccines

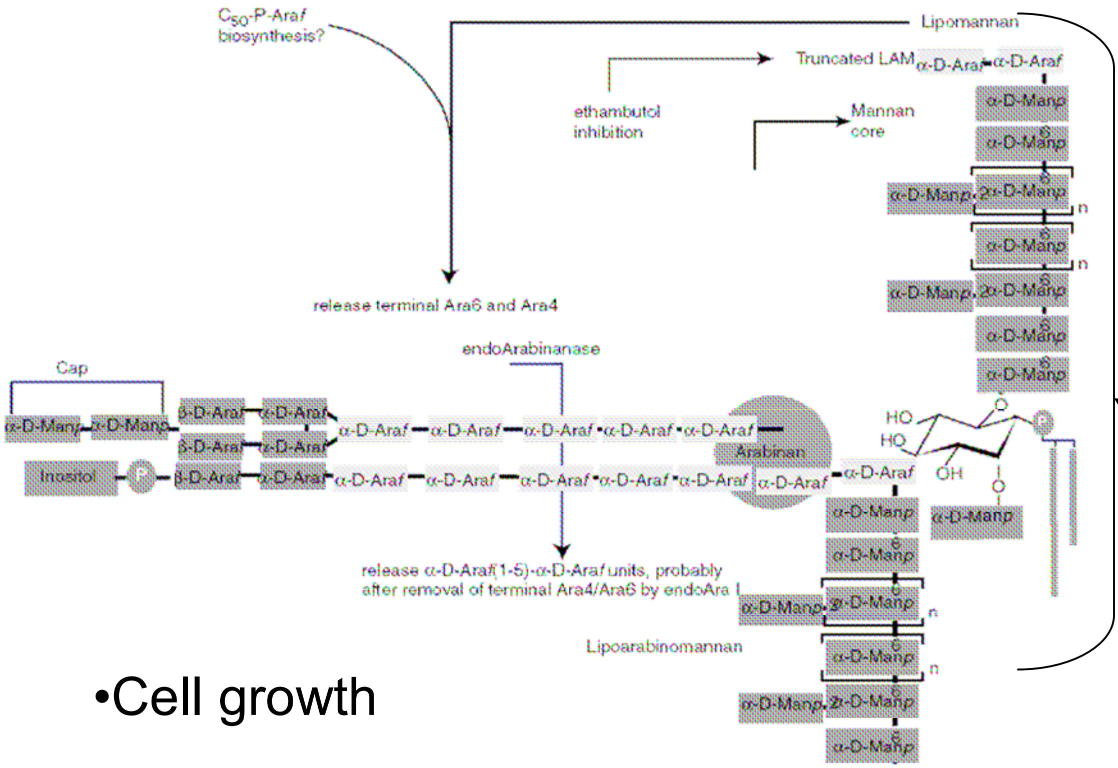
- Definition: weakened or killed pathogens or parts of polysaccharides and/or proteins that stimulate immune response
- Benefits of using parts of the organism
  - Will not cause infection with organism
  - Stimulates antibody production in body

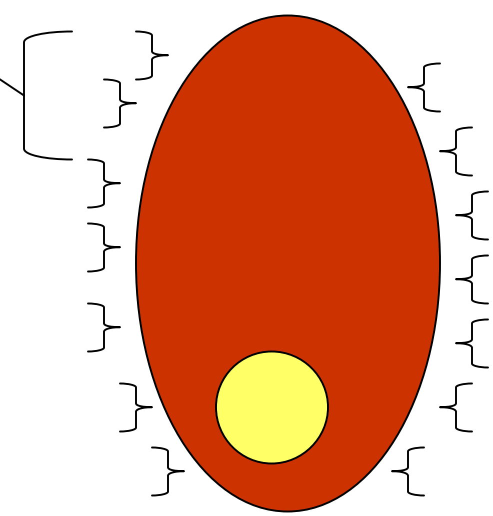
Antibody Stimulation Goal

- Antigen recognition
- Engulfing
- Cell death & degradation
- Fragments displayed on cell surface
- Proliferation and activation of T cells
- Antibody circulation



M. Tuberculosis Cell Wall

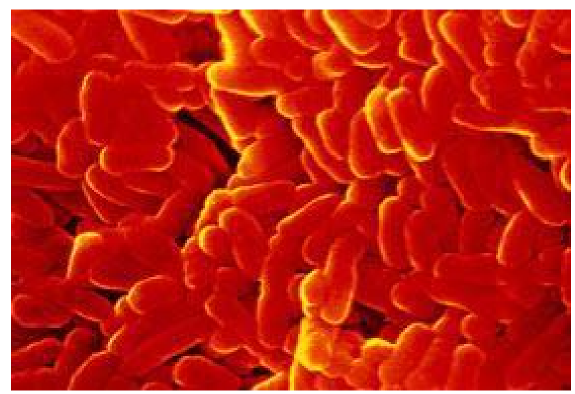




- Cell growth
- Polysaccharide recovery
- Cleaving
- Conjugation to carrier protein

Bacterial Growth

- *Mycobacterium tuberculosis* ATCC 25177
- Inoculated in Lowenstein-Jensen (LJ) plates
  - Generation time = 6 - 8 weeks
- Transfer to LJ liquid medium
  - Generation Time = 15 hours
- Deviation: growth time



Cell Membrane Separation

- Centrifugation
  - Pellets: 3,000 x g for 20 min
  - Wash in phosphate- buffered saline
  - Re-suspend in distilled water
- Sonication
  - Weakening of the cell wall with electrical pulses
  - Three cycles of 30 s pulses
  - Carbohydrate yield: 70-80%

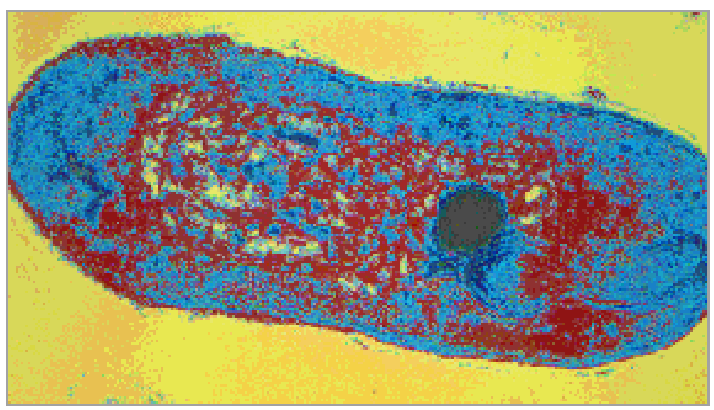
- Centrifugation
  - 3,000 x g for 20 min
- Supernatant filtration
  - Separation of the capsule
- Lyophilization (optional)

Deviation: sonication cycles

As presented by Stokes et al., 2004

Cell Membrane Cleaving

- Fragments between 2 and 10 kDa
  - Ensures no virulence activity
  - High titer response with less than 10 kDa



Acetolysis

- Step 1: Acetolysate
  - Acetic acid, acetic anhydride, and sulfuric acid
  - 8 hrs @ room temp. (RT)
  - Pour into 30 g ice water
- Step 2: pH stabilization
  - At RT
  - pH = 7.5 with NaOH
- Step 3: Sugar acetate extraction
  - Use chloroform
  - Yield = 96.3%
- Step 4: Evaporation
  - Dry over anhydrous sodium sulfate

Deacetylation

- Step 5:
  - Methanol, barium methoxide, & Dowex 50
- Step 6:
  - Sephadex G- 25, eluted at 10 mL/hr
- Step 7: Gel filtration (0.2 µm)

Deviations:

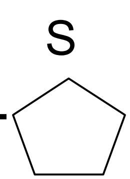
Cleaving, size, yield, and
reaction

Carbohydrate Attachment

- Step 1- Amination of polysaccharides (PS)
  - Deviation: insufficient amino substitution

$$\mathbf{PS}\text{-}CHO + NH_4Cl \xrightarrow{NaBH_3CN} \mathbf{PS}\text{-}NH_2$$

- Step 2- Thiolation of PS with 2-iminothiolane

$\mathbf{PS}\text{-}NH_2$ +  $\text{-}NH_2^+Cl^- \rightarrow \mathbf{PS}\text{-}NH\text{-}C(NH_2^+Cl^-)\text{-}(CH_2)_3\text{-}SH$

Carbohydrate Attachment

- Step 3- Bromoacetylation of tetanus toxoid (TT)
  - Deviation: contamination in tetanus sample

TT-NH_2 + NHS-CO-CH_2-Br → **TT**-NH-CO-CH_2-Br

As presented by Pawlowski, et al, 1999

Carbohydrate Attachment

- Step 4- Conjugation activated PS and TT
  - Deviation: incomplete conjugation

Step 2 product + Step 3 product →

TT-NH-CO-CH_2-S-$(CH_2)_3$-C($NH_2^+Cl^-$)-NH-**PS**

- Step 5- Separation:product from free reactants
  - Deviation: contaminants, pH variation

Research and Pre-FDA

- Laboratory research
  - Create the vaccine
  - Improve the product yield
  - Create the deliverable drug

- Animal testing
  - Test biological activity and safety

FDA Approval Process

- *Phase I*
  - Metabolic and pharmacologic effects in humans
  - Dosing effects
  - Effectiveness
- *Phase II*
  - Effectiveness of the drug
  - Short-term side effects
  - Health risks
- *Phase III*
  - Overall benefit-risk relationship
- *Applications and Committees*
- *Conditions of Failure*
  - Design failure in research
  - Clinical hold in FDA

What do technical deviations mean in

???

Goals

- Directed at risk taker, risk averter, or risk average
- Aid with critical decisions
  - Research and investment
  - Failed FDA phase
- Market strategies and demand models
- Risk assessment
- Success and profit estimation

Decision- Making

- First Stage or “Here and Now” Decisions
  - There will be consequences for “things that I do today”
  - Example: buying a house

- Second Stage or “Wait and See” Decisions
  - Made in response to the realization of uncertainty
  - Need to be addressed, not made
  - Example: opening an umbrella when it rains

Decision- Making

First Stage Decision	Second Stage Decisions
Focus processes in pre-FDA research ▪ Protein/ polysaccharide conjugation ▪ Capsule cleaving/ recovery ▪ Bacterial growth	▪ Time to begin plant construction ▪ Time to begin marketing campaign ▪ Additional research after failed FDA stage

Financial Definitions

Market – brings together buyers and sellers

Demand – schedule with various amounts of a product consumers are willing/able to purchase at a price

Risk - uncertainty of project and associated financial loss or gain

Net Present Value (NPV) – how much the project is worth at a point in time; indicative of favorable venture

Market

- Diverse target groups
  - Melanoma patients in the U.S.
  - Cancer world wide
- Tuberculosis
  - 12 million hospital personnel
  - 1.4 million military personnel
- Depends on resources of investors

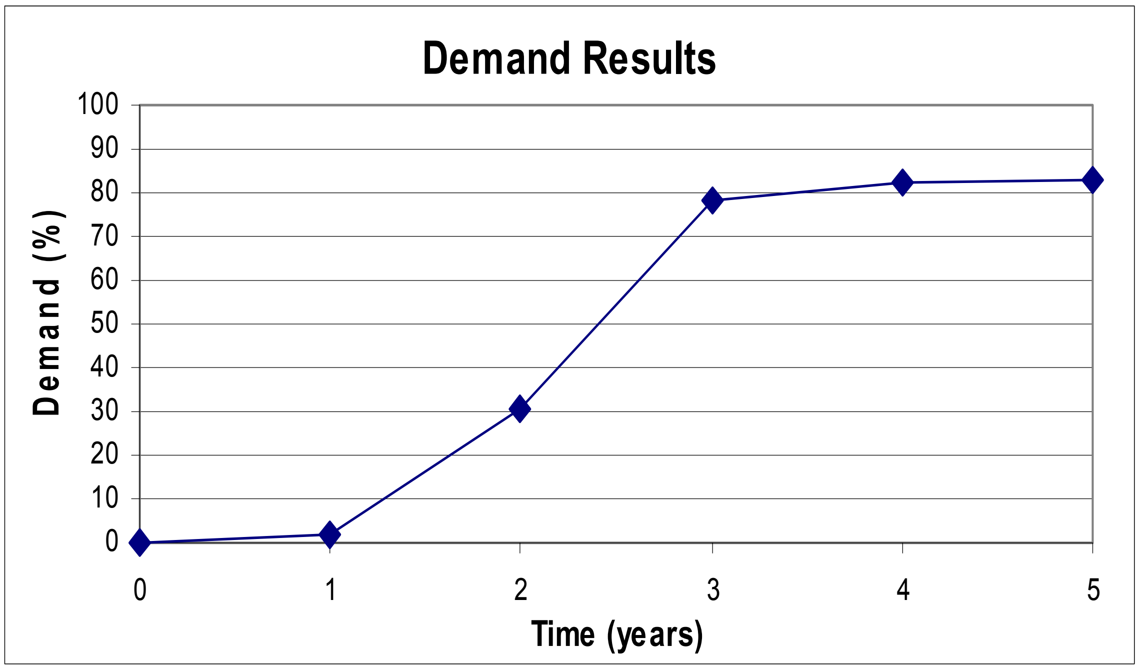
Economics

- Definitions:
  - **α** = Measurement of customer knowledge of product
  - **β** = Measurement of customer preference
  - $\mathbf{d_1}$ = Amounts of a product at a price that consumers are willing to purchase
- Purpose
  - Price
  - Return on Investment (ROI)
  - Production schedule

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

Demand Model

Iterative Calculation – 82% Market



d1 = New drug

α = varied

β = 0.29

P2 = $115.09

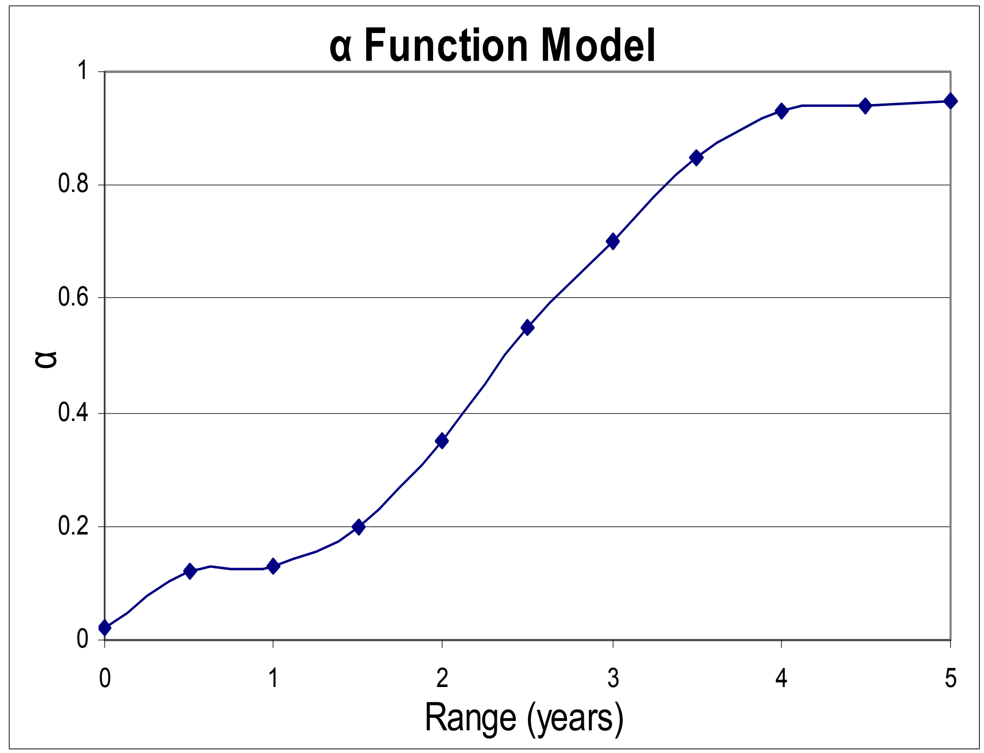
P1 = $140.00

D = 13.4 million units

New Product 3.5 times better

α Function Model

Year	Market Target	Installations Visited
0	2 %	170
0 to 1	10 – 15 %	760
1 to 2	35 – 40 %	1,940
2 to 3	70 – 75 %	2,690
3 to 4	90 – 95 %	1,520
4 to 5	95 – 100 %	170
	Total	**7,250**

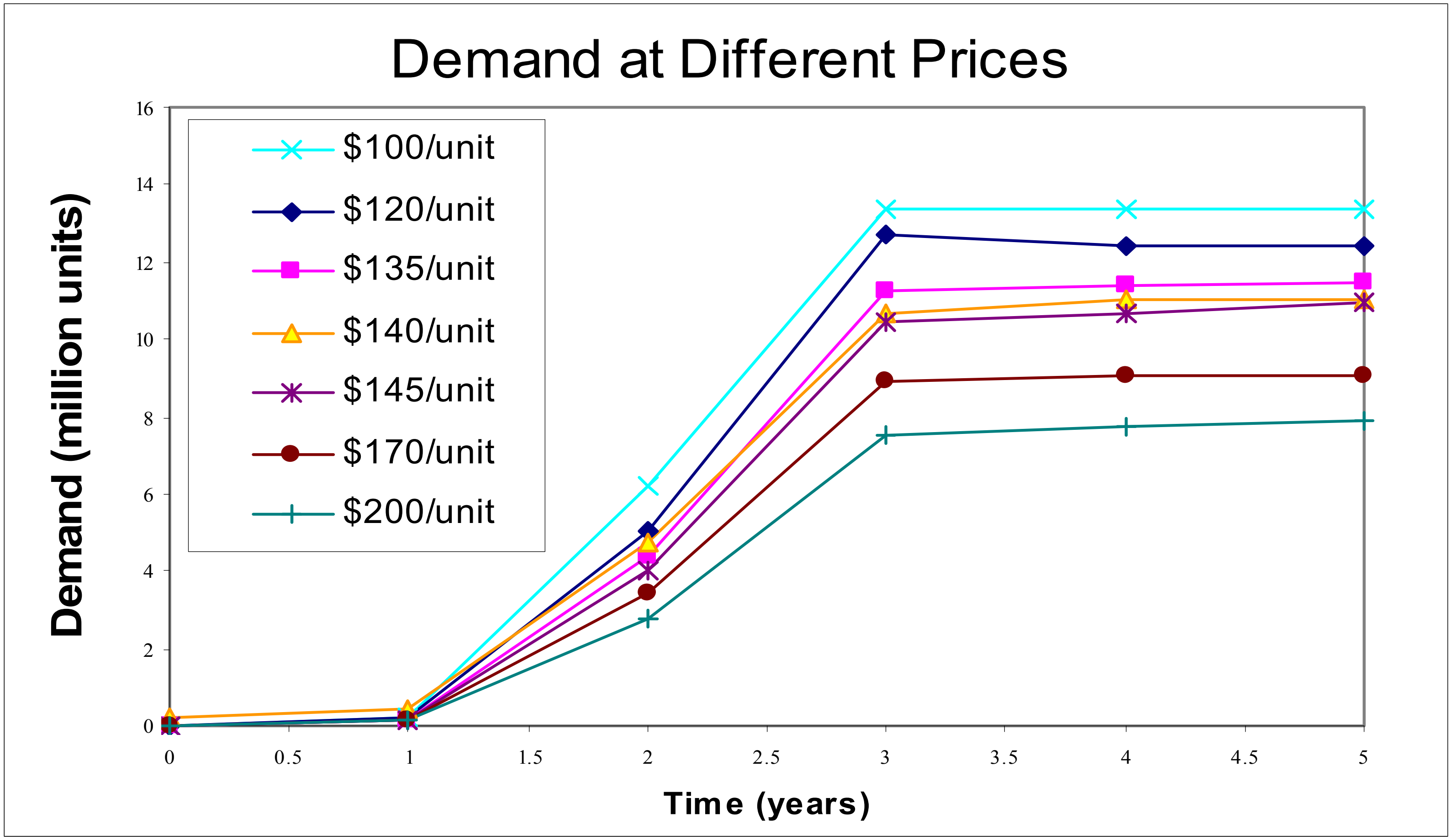


- Increased with advertisement
- Aggressiveness of marketing campaign

β Parameter

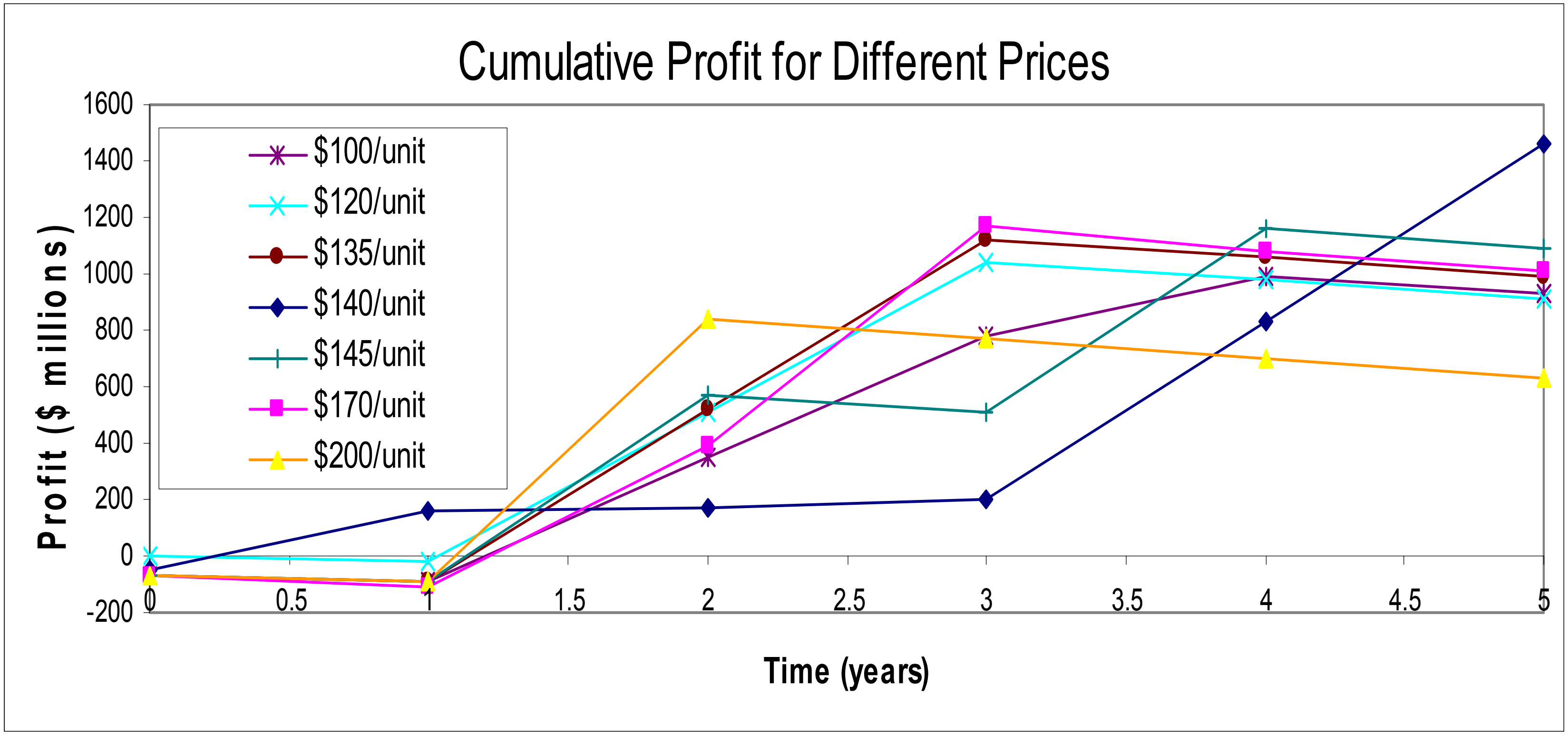
Property	***Description***	***Weight (w)***	***New Product (y_1)***	***Existing Product (y_2)***
Efficacy	New product more effective than existent one	0.7	0.8	0.2
Side Effects	New product has less side effects than existent one	0.3	0.7	0.3
Delivery Method	Currently, only available via injection	0	N/A	N/A
Availability	Target institutions, not public	0.05	0	0
Brand	No similar product	0.05	0	0
β= measurement customer preference		$H = \Sigma(w^*y_i)$	0.77	0.23
		$\beta = H_2/H_1$	**β = 0.29**	

Price & Demand Relation



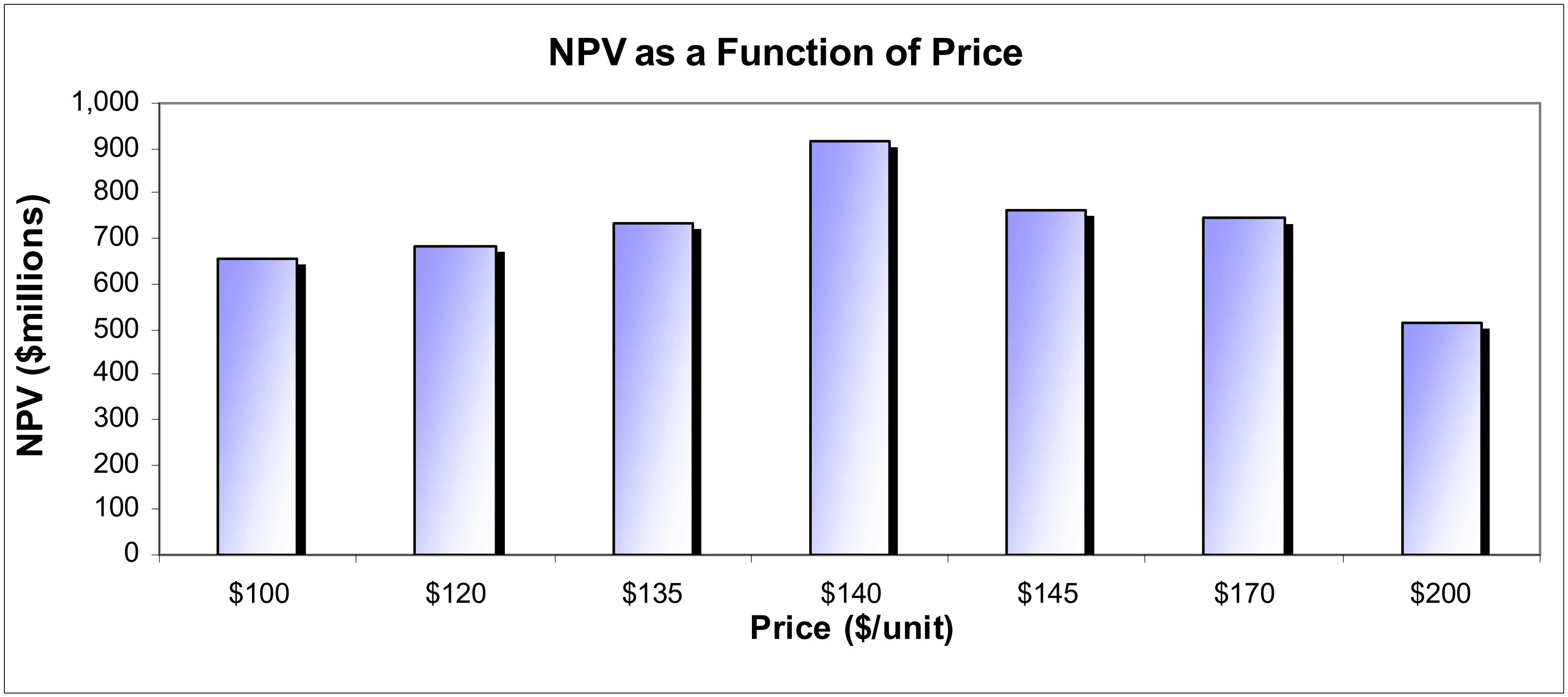
- Higher demand for lower priced product

Profit Results



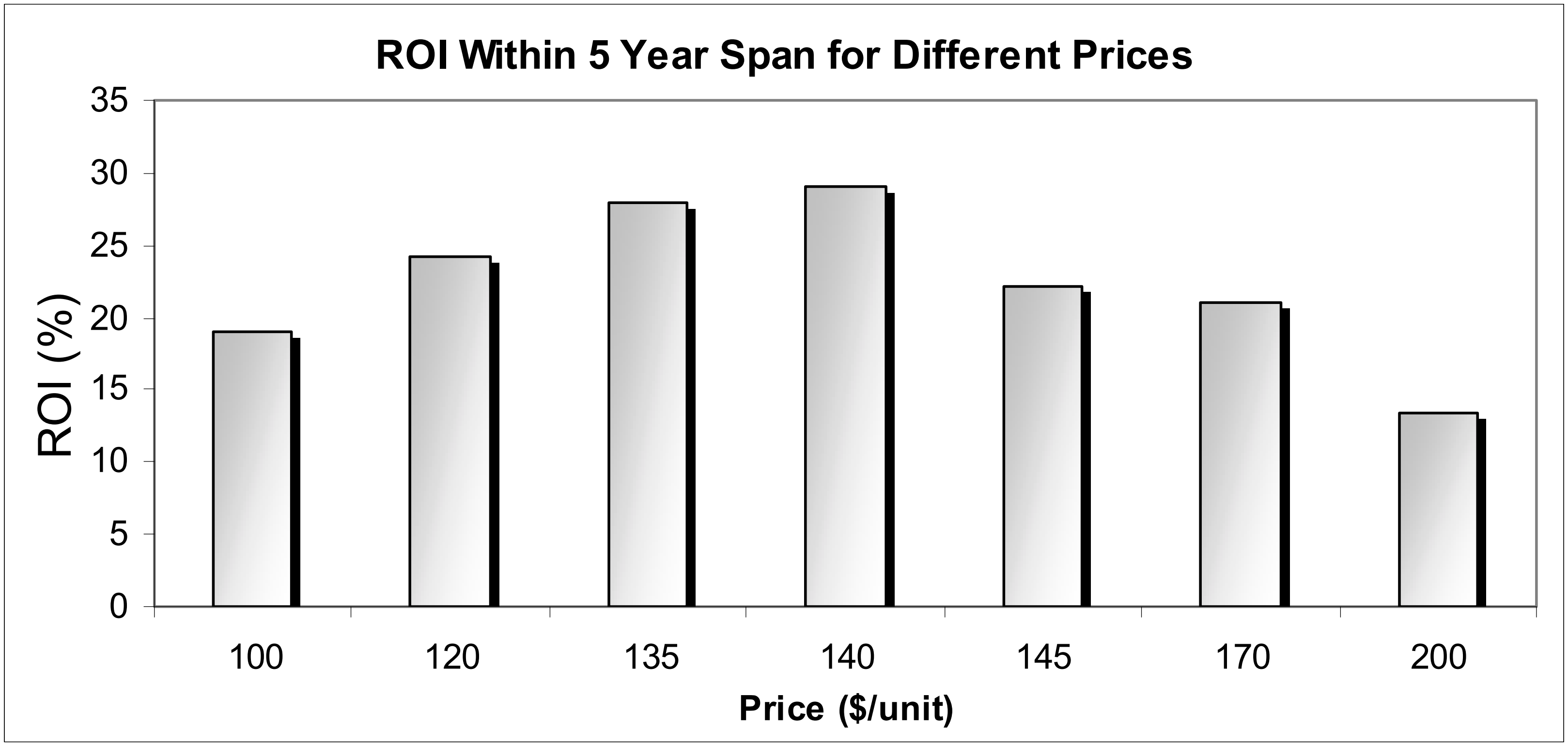
- Different profits
- Depending on α and d_1

Price Optimization



- Higher NPV preferred
- Discounted rates, etc. (later)

Return on Investment



- ROI = Profit/FCI; approx. 3 years
- Maximum ROI = 29.08%

Demand and Risk Relation

- Selected values of α, β, price, and demand
  - α varies with time
  - $\beta = 0.29$
  - $P_{opt} = \$140.00$
  - Demand = 13.4 million units
- Risk calculated accordingly

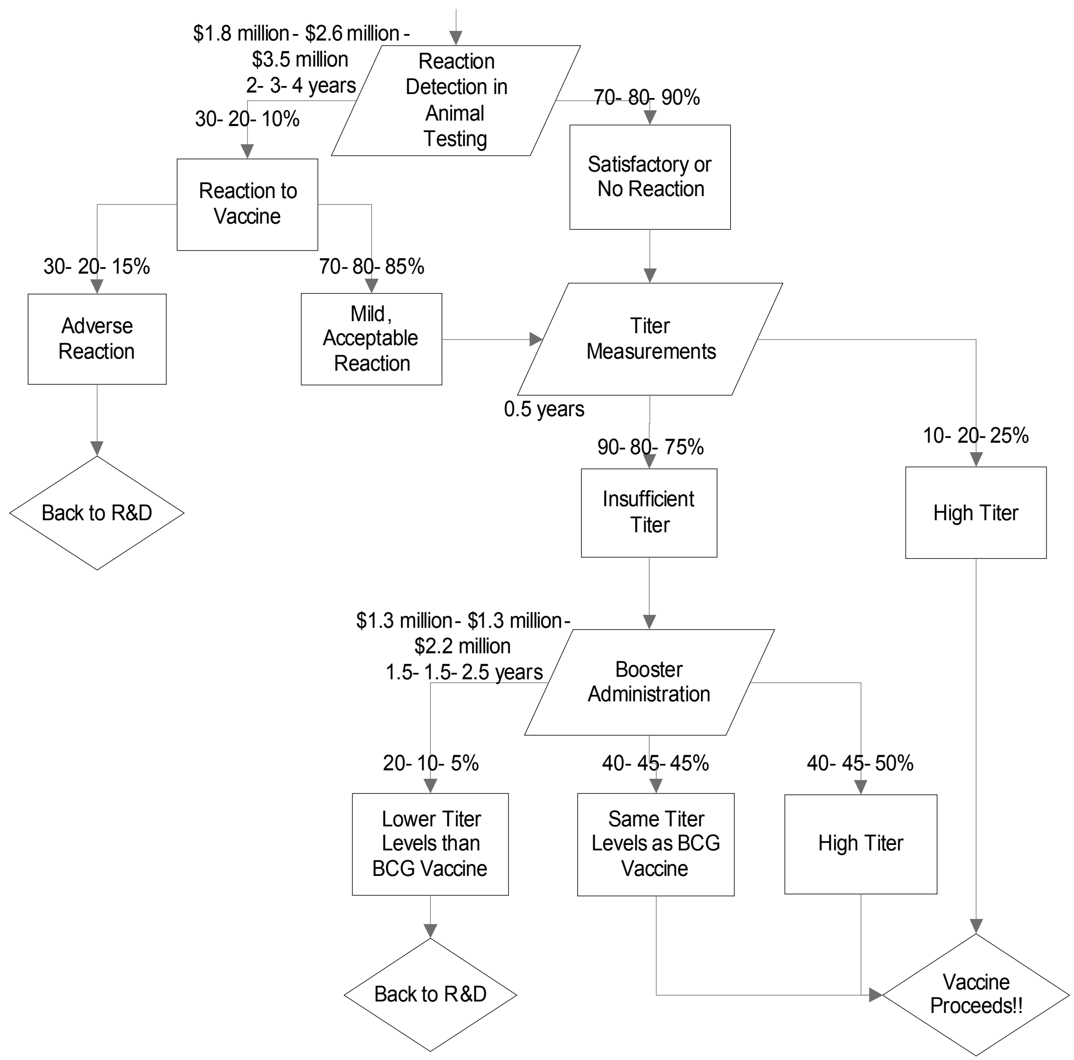
What is Risk?

- Predictor of the product's success
- A collection of paths that vary with first stage decision
  - Research time invested: 6, 8, or 10 years

Cumulative Probabilities and Costs

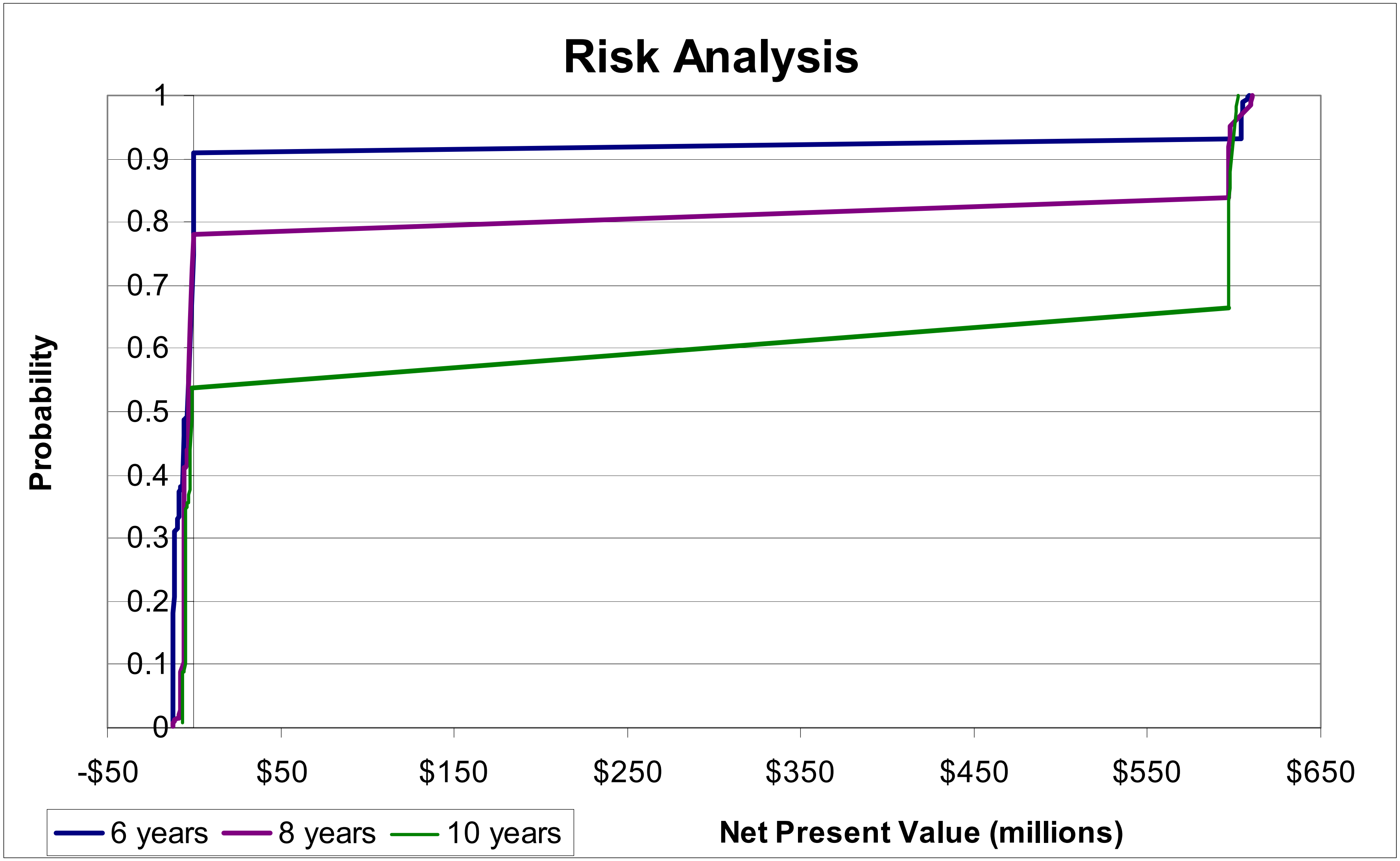
- Pathways
  - All possibilities considered
  - Realistic probability assigned
- Probabilities compounded and costs summed over a particular path
- Risk and net present values calculated

Sample Pathway



Sample Pathway

Characteristics of Example Path	Values
Overall Possibility of Occurrence	0.259%
Cost of Pathway (millions)	$49.0
Time in Research (years)	6
Time in FDA (years)	10
Net Present Value (millions)	$605



Risk Analysis
Probability
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-$50
$50
$150
$250
$350
$450
$550
$650
6 years
8 years
10 years
Net Present Value (millions)

This is a very risky project!

What can we do?

FDA: Attempt #2

- Where drug was abandoned before, return to research for one year
- Continue in FDA at same phase
- Expect higher probability of success

Comparison of Risk Analyses

Summary of Pathways in Risk Analysis		
	Initial	*"Second Chance"*
Number of Pathways	71	443
Successful Pathways	12	96
Percent Success (6 year path)	9.2%	23.3%

Risk Summary: TB Vaccine

First Stage Decision- Time Invested in Research				
		6 Years	*8 Years*	*10 Years*
Single trip Through FDA	Risk	90.8%	78.1%	53.7%
	Expected Worth (millions)	$555.43	$494.43	$362.56
Recycle Through FDA	Risk	76.7%	65.3%	47.1%
	Expected Worth (millions)	$485.97	$428.16	$323.74

Conclusions

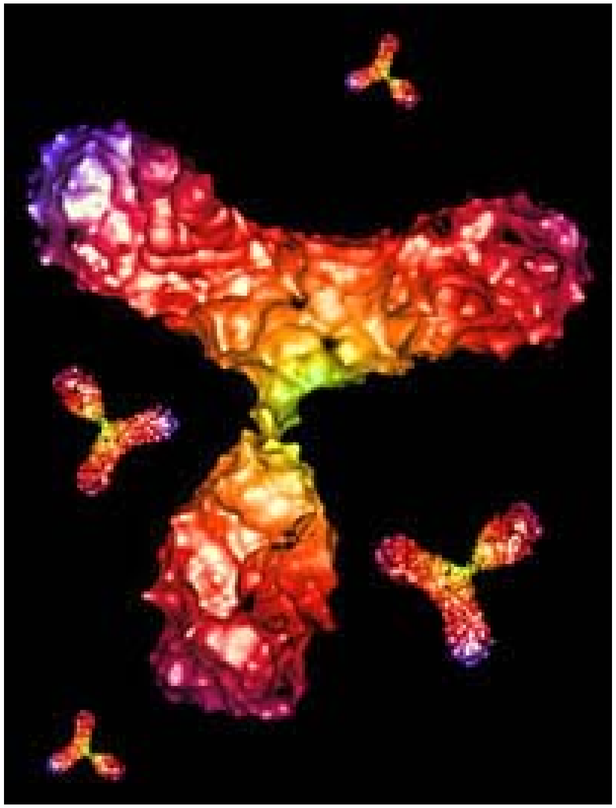
- 1st stage decision
  - 6, 8, or 10 years pre-FDA research
- Price optimization
  - $140/unit
  - ROI = 29% (5 years)
- FDA risks based on 1st stage decision
  - From 54% to 91%
- Risks based on 2nd stage decision to re-cycle drug
  - Decreased to 47% to 77%
  - Decreased Expected Worth
- Pre- FDA research
  - Significantly increases success probability
  - Decreases Expected Worth

General Conclusions

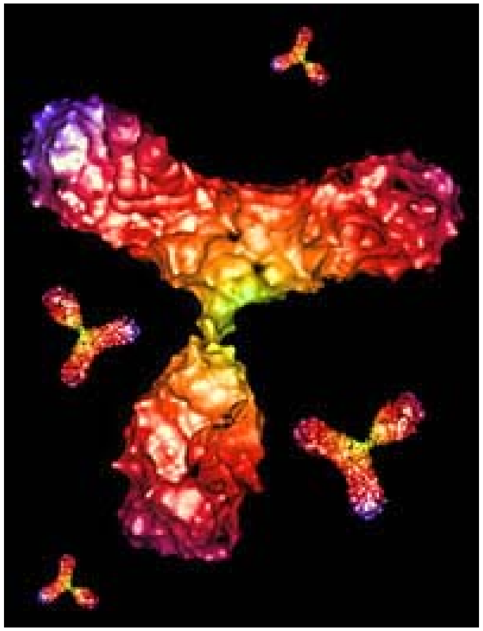
- New drug analysis outcome
  - General form of pre-FDA research & FDA approval process
  - Market analysis
  - Demand and pricing models
  - Risk analysis
  - Expected worth estimation
  - Assistance in critical decision- making

Tuberculosis Vaccine

Questions?

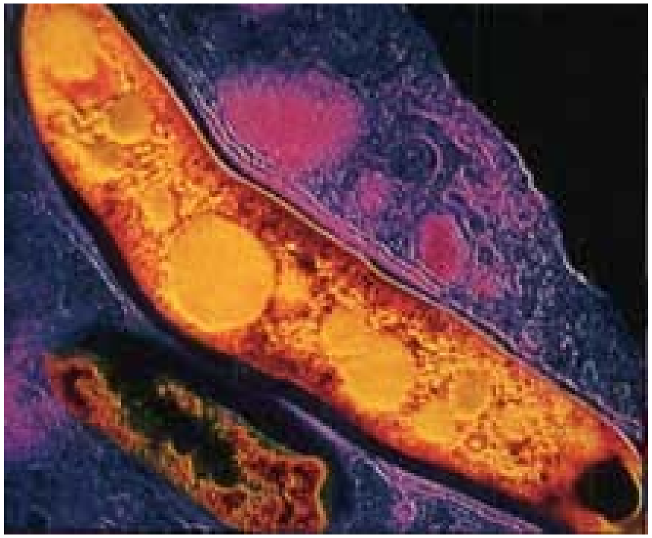


Appendix



Antibody Stimulation: Goal

- Use parts of *Mycobacterium tuberculosis* capsule and force the body to create antibodies against it
- Replicate ‘natural’ antibody production process
- Stimulate response for future and current infection



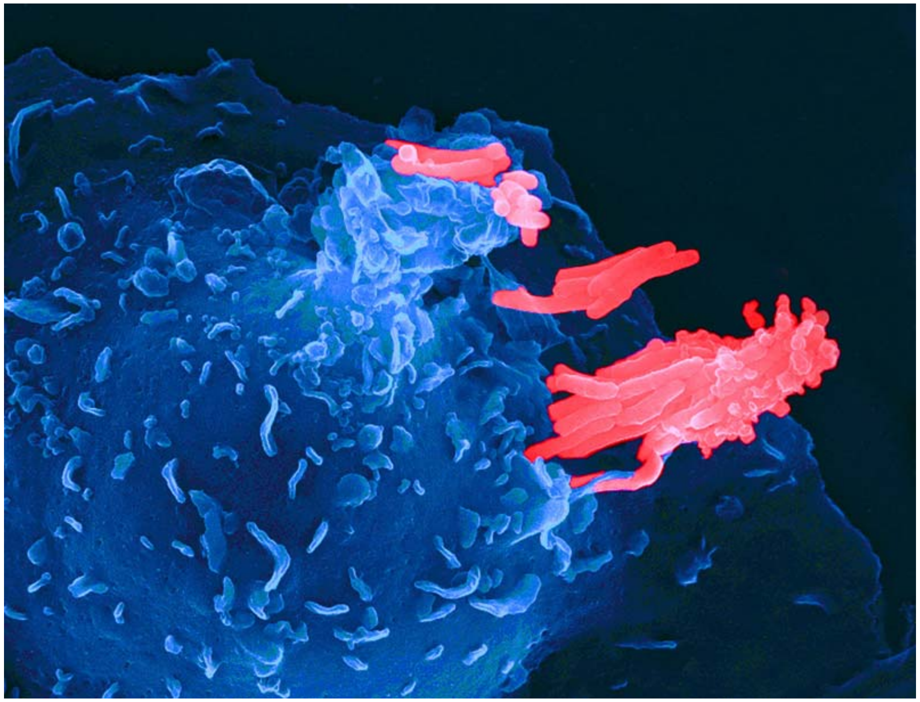
Method Benefits

- Has the highest yield and the most documentation
- Modeled for all saccharide -protein conjugate vaccines
- Researched with Tetanus Toxoid

Antibody Suppression

TB multiplies inside macrophages

- Binds to macrophage surface protein (C3b) - receptor for complement cascade
  - No guidance to site of infection
- Prevents formation of phagolysosome



Carbohydrate Attachment

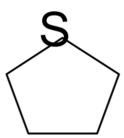
- Step 1- Amination of polysaccharides (PS)
  - Deviation: insufficient amino substitution

$$\textbf{PS}\text{-CHO} + NH_4Cl \xrightarrow{NaBH_3CN} \textbf{PS}\text{-}NH_2$$

$$\text{PS + solid ammonium chloride} \xrightarrow{\text{solid sodium cyanoborohydride}} \text{aminated PS}$$

Carbohydrate Attachment

- Step 2- Thiolation of aminated polysaccharides with 2-iminothiolane

PS-NH_2 + 

-$NH_2^+Cl^-$ → **PS**-NH-C($NH_2^+Cl^-$)-$(CH_2)_3$-SH

Aminated PS + 2-iminothiolane → thiolated PS

Carbohydrate Attachment

- Step 3- Bromoacetylation of tetanus toxoid (TT)
  - Deviation: contamination in tetanus sample

TT-NH_2 + NHS-CO-CH_2-Br → **TT**-NH-CO-CH_2-Br

Stock TT + solid N-hydroxysuccinimide ester of bromoacetic acid → bromoacetylated TT

Carbohydrate Attachment

- Step 4- Conjugation of thiolated polysaccharides with bromoacetylated tetanus toxoid
  - Deviation: incomplete conjugation

$$\mathbf{PS}\text{-NH-C}(NH_2^+Cl^-)\text{-}(CH_2)_3\text{-SH} + \mathbf{TT}\text{-NH-CO-}CH_2\text{-Br} \rightarrow$$
$$\mathbf{TT}\text{-NH-CO-}CH_2\text{-S-}(CH_2)_3\text{-C}(NH_2^+Cl^-)\text{-NH-}\mathbf{PS}$$

- Step 5- Separation of conjugates from free reactants
  - Deviation: contaminants, pH variation

General Technological Risks

- Experimental failure
- Unexpected outcomes
- Lack of chemical reactivity
- Deviations in product recovery
  - Methodology or instrumentation
- Unavailability of resources or test subjects
- Risk to human health

TB Vaccine Risks & Deviations

- Growth on plates not determined
- Non- effective sonication
- Yield of cell membrane (pellets) uncertain
- Carbohydrate cleaving inaccurate
  - Over 10 kDa or less than 2 kDa capsule fragments
  - Deacetilation not achieved – lower yield

TB Vaccine Risks & Deviations (cont'd)

- Insufficient amino substitution of polysaccharide
- No sulfhydryl groups after reduction of disulfides
- Residual salts and impurities in tetanus toxoid
- Incomplete bromoacetylation reaction- no activated amino group
- Incomplete conjugation of polysaccharide and tetanus toxoid
- pH variance at any step
- Contamination by free reactants

Vaccine Components

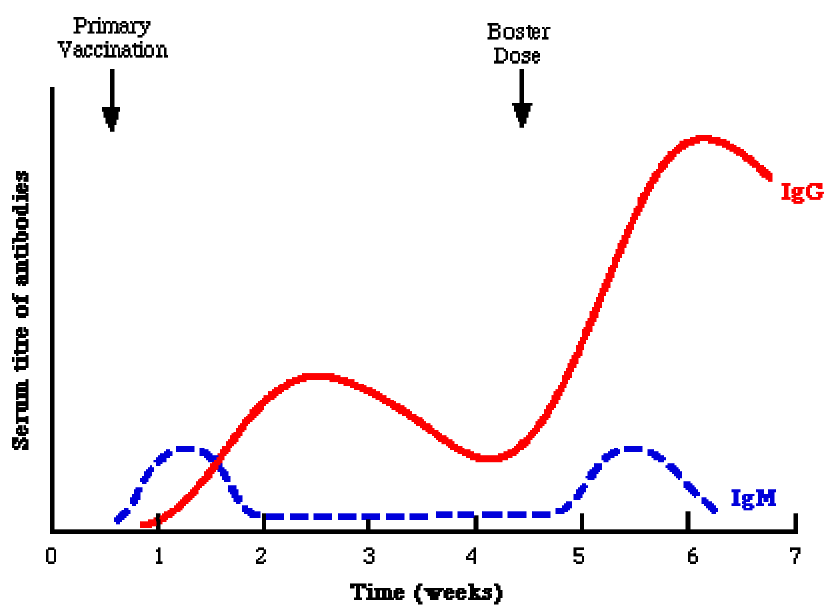
- 25ug polysaccharide- tetanus toxoid conjugate
- Sodium Phosphate Buffer
- 0.9% Sodium Chloride Saline

Animal Testing Procedures

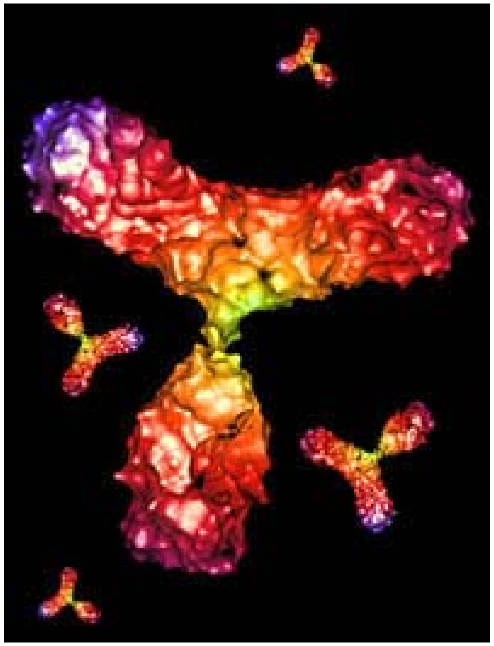
- 80 male BALB/c inbred mice injected with complex
- 10 mice injected with saline, 10 with carbohydrates as controls
- Mice immunized subcutaneously at 0 and 28 days with 1 ug of conjugates in 0.25 mL phosphate- buffered physiological saline (PBS)
- Blood samples collected every 7 days for 120-day period

Animal Testing Procedures

- Serum titers measured when samples are sent to testing facility
- IgG levels monitored periodically



Business Plan



Competition

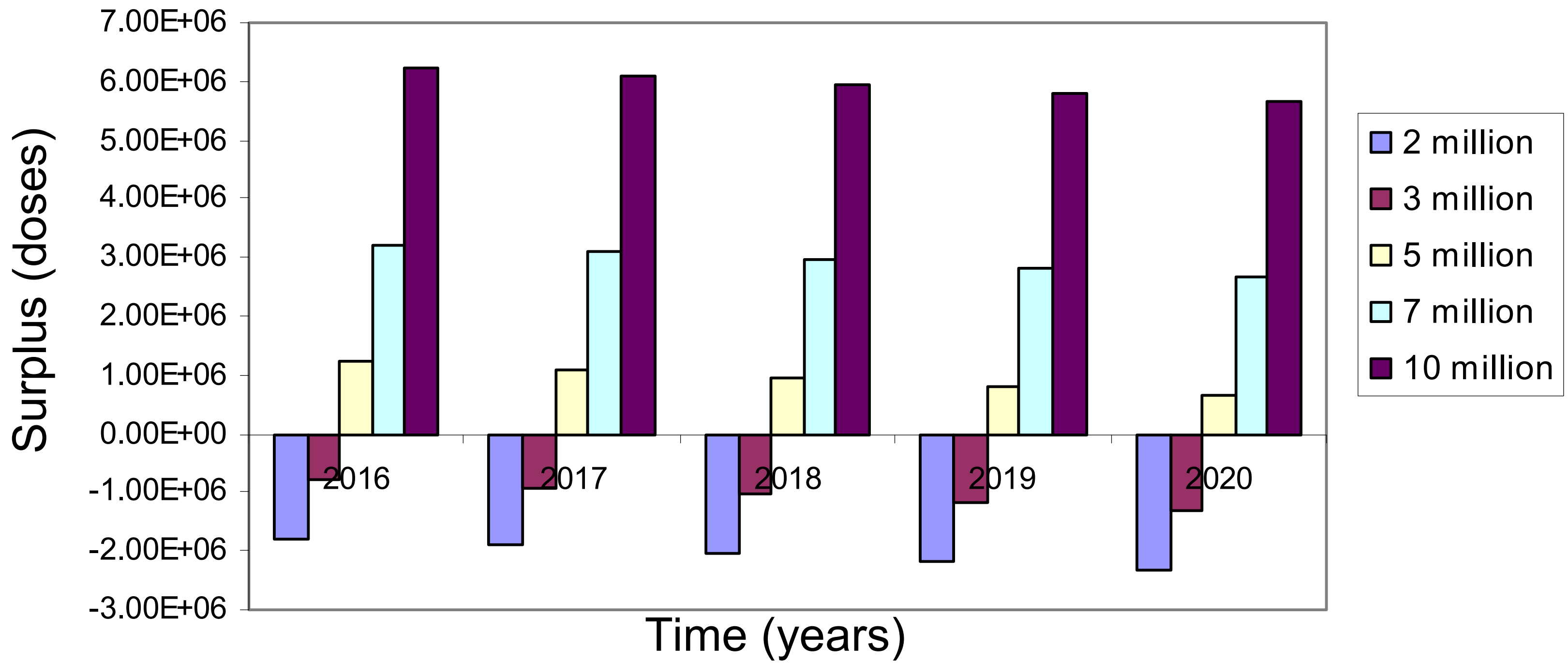
Crucell & Aeras Global TB Vaccine Foundation

- Bill & Melissa Gates Foundation
  $82.9 million to Aeras
- $2.9 million to Crucell
- Improve on BCG vaccine
- Phase I clinical trials in 5 years + 8 yrs FDA
- Earliest distribution

Year 2019!

Production Capacity

- Current Market = 2.68 million/year
- 3.5% Market Growth - linked to hospitals



α Function Model

Range (Years)	Advertisement Method	Expenses
0	Word of mouth, Presentations, FDA Results, Journals, Visits (2 sales reps)	$121,584.00
0 to 1	Visits + Website + Television (9 sales reps)	$1,361,768.00
1 to 2	Visits + Website + Television (23 sales reps)	$2,212,865.00
2 to 3	Visits + Website + Television (32 sales reps)	$2,759,984.00
3 to 4	Visits (18 sales reps)	$1,094,256.00
4 to 5	Visits (2 sales reps)	$121,584.00
	Total	**$7,672,032.00**

- First 3 years – most costly

Advertisement: Methods & Costs

Method & Description	Cost ($/year)
Sales Representative (84 installations) • Salary • Transportation, car, plane tickets • Misc., meals, reimbursements, other	 45,000.00 13,440.00 2,352.00
World Wide Web • Web Page • Web Master salary • Fee, other	 2,400.00 32,000.00 240.00
Television • 30 second commercial 3 times/day	 780,000.00

Demand Equation

$$\beta p_1 d_1 = \alpha p_2 d_2 \left(\frac{d_1^{\alpha}}{d_2^{\beta}} \right)$$

$$\beta = \frac{S_2}{S_1}$$

$$\alpha = awareness \quad of \quad product$$

$$p_1 = our \quad price$$

$$p_2 = competitor\ 's \ price$$

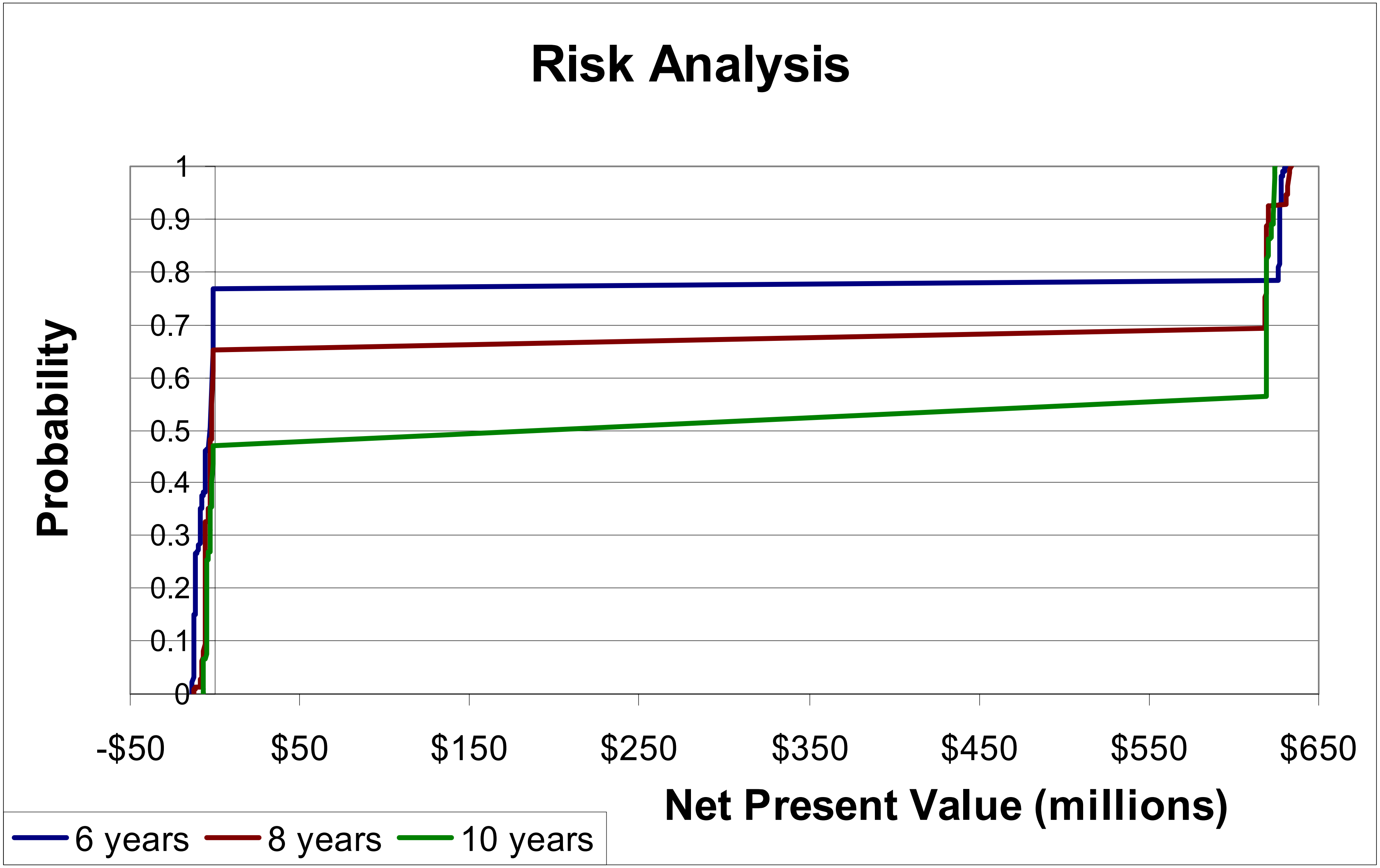
$$d_1 = our \quad demand$$

$$d_2 = competitor\ 's \ demand$$

$$Y = p_1 d_1 + p_2 d_2$$

$$\Rightarrow d_2 = \frac{Y - p_1 d_1}{p_2}$$

$$\therefore d_1 = \left(\frac{\alpha p_2}{\beta p_1} \right) \left(\frac{Y - p_1 d_1}{p_2} \right)^{1-\beta} \cdot d_1^{\alpha}$$



Risk Analysis
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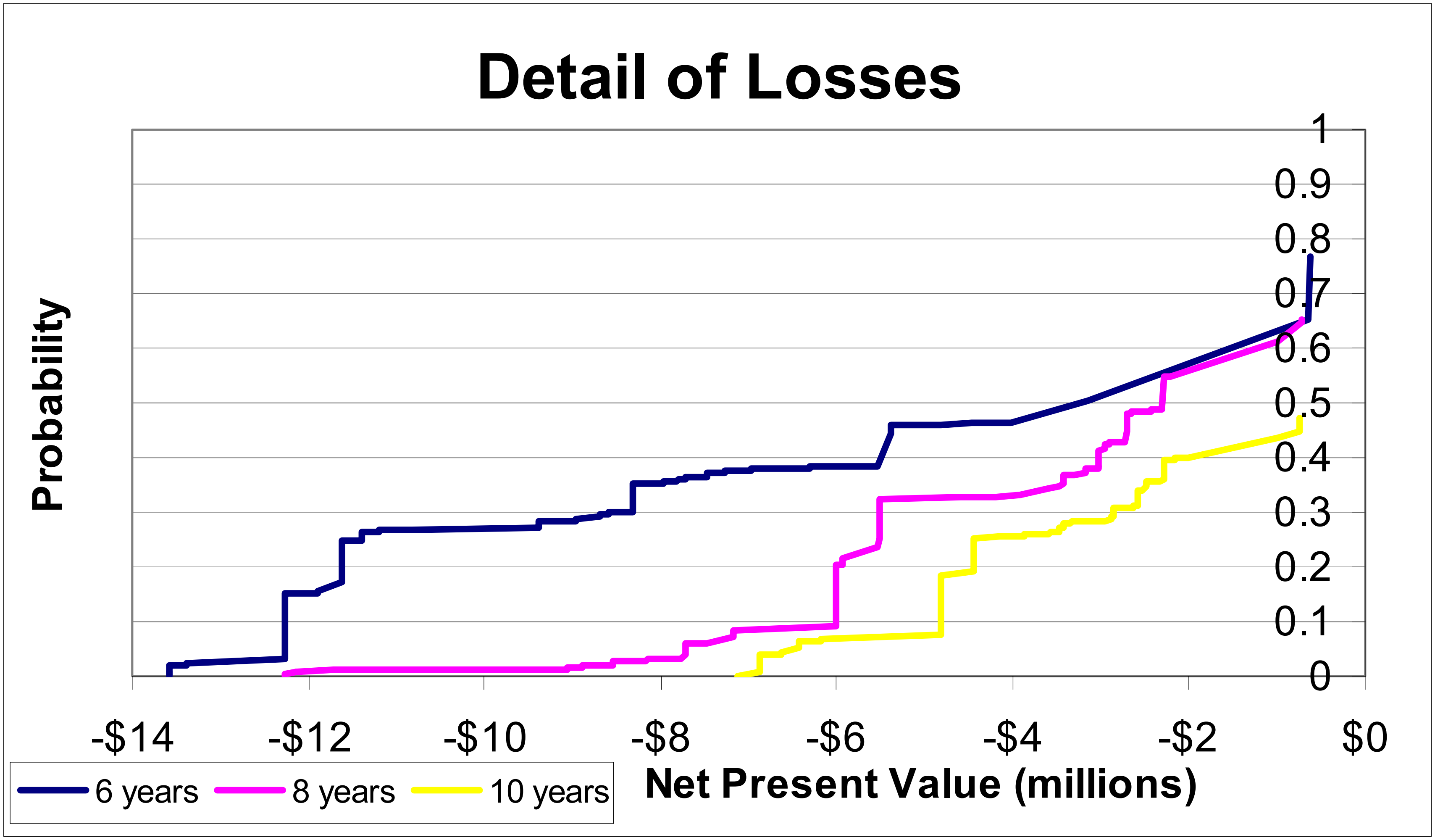
0.5
0.4
0.3
0.2
0.1
0
-$50
$50
$150
$250
$350
$450
$550
$650
Net Present Value (millions)
6 years
8 years
10 years



Detail of Losses
Probability
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-$14
-$12
-$10
-$8
-$6
-$4
-$2
$0
Net Present Value (millions)
6 years
8 years
10 years

Timeline: 2nd Stage Decisions

Year	Research	Animal Testing	FDA: Phase I	FDA: Phase II	FDA: Phase III	FDA: Applications	Plant Construction	Marketing	Production
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
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