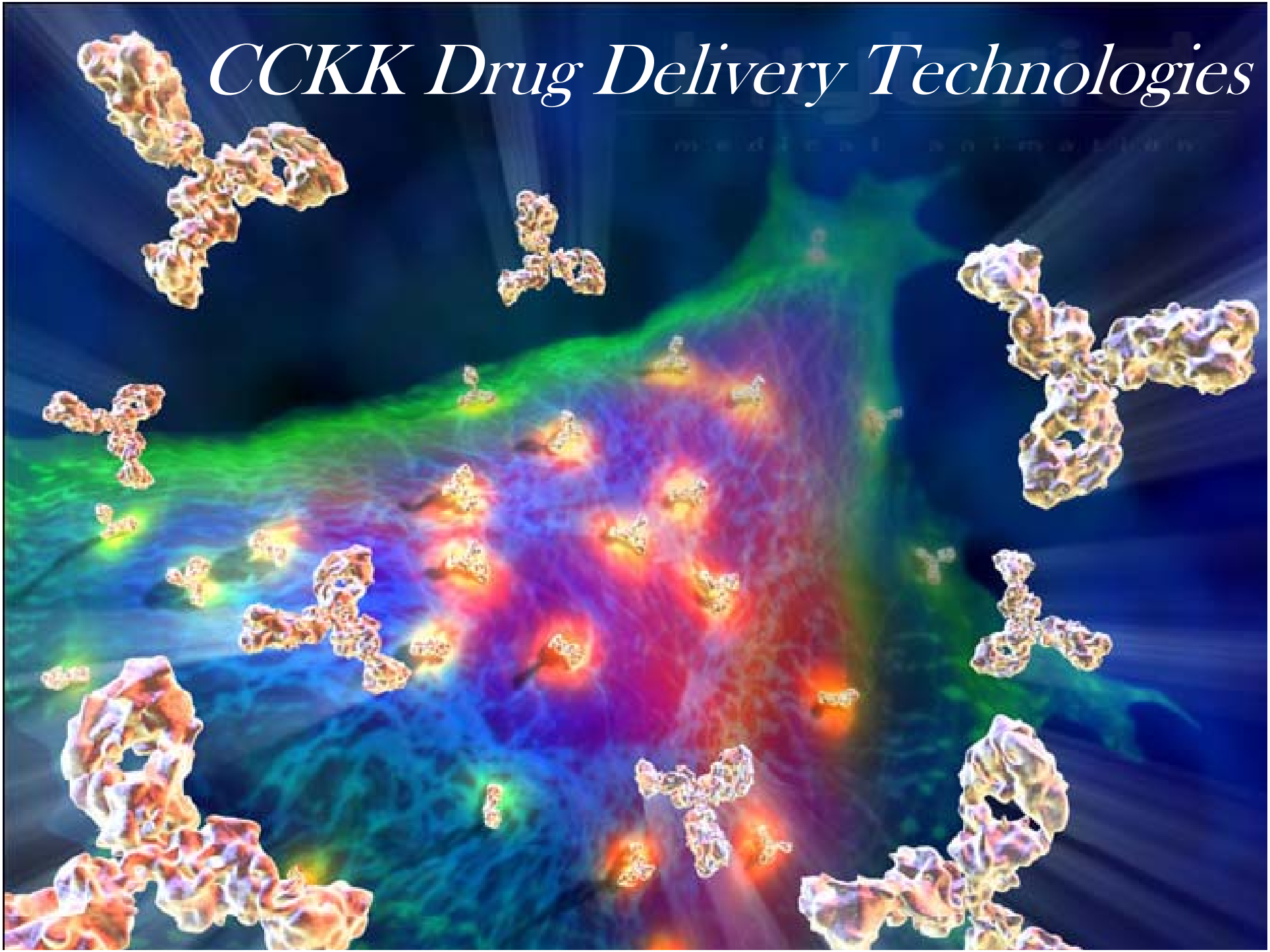
CCKK Drug Delivery Technologies



CCKK Drug Delivery Technologies:

Monoclonal Antibodies for Delivery of Chemotherapy Drugs for Cancer Therapy

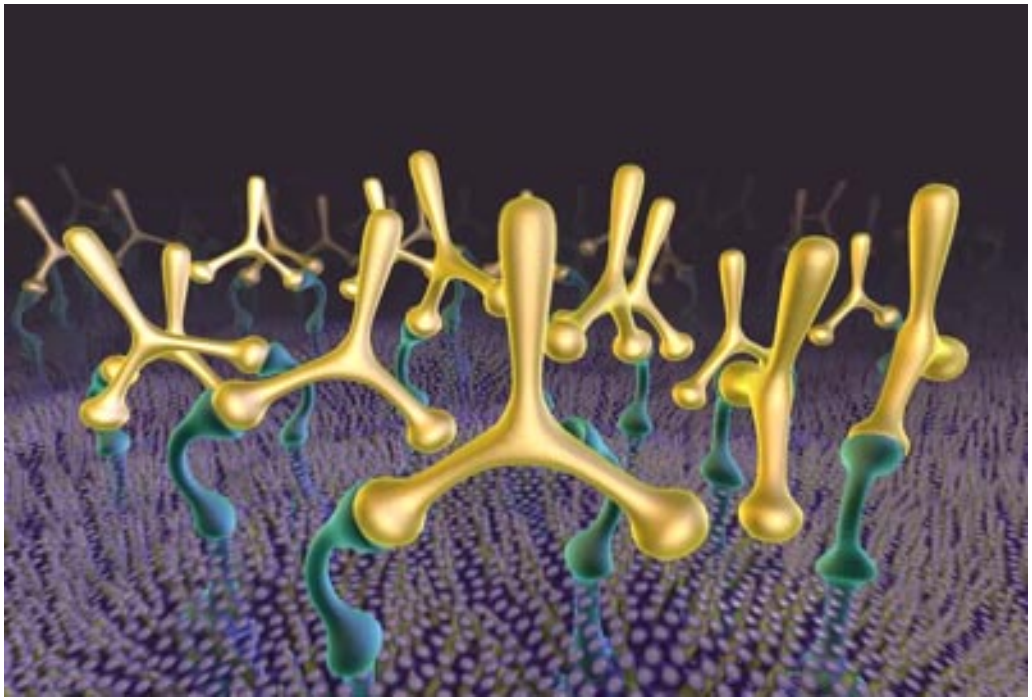
Sarah Clawson
Paul Cole
Sarah Katen
Nell Keith

Introduction

- Project Objective: Improve chemotherapy drug delivery for maximum efficacy with minimal side effects
- Disease Considered: Gliomas – common brain tumor
- Method of Delivery: Micelle and monoclonal antibody complex

What is a Monoclonal Antibody?

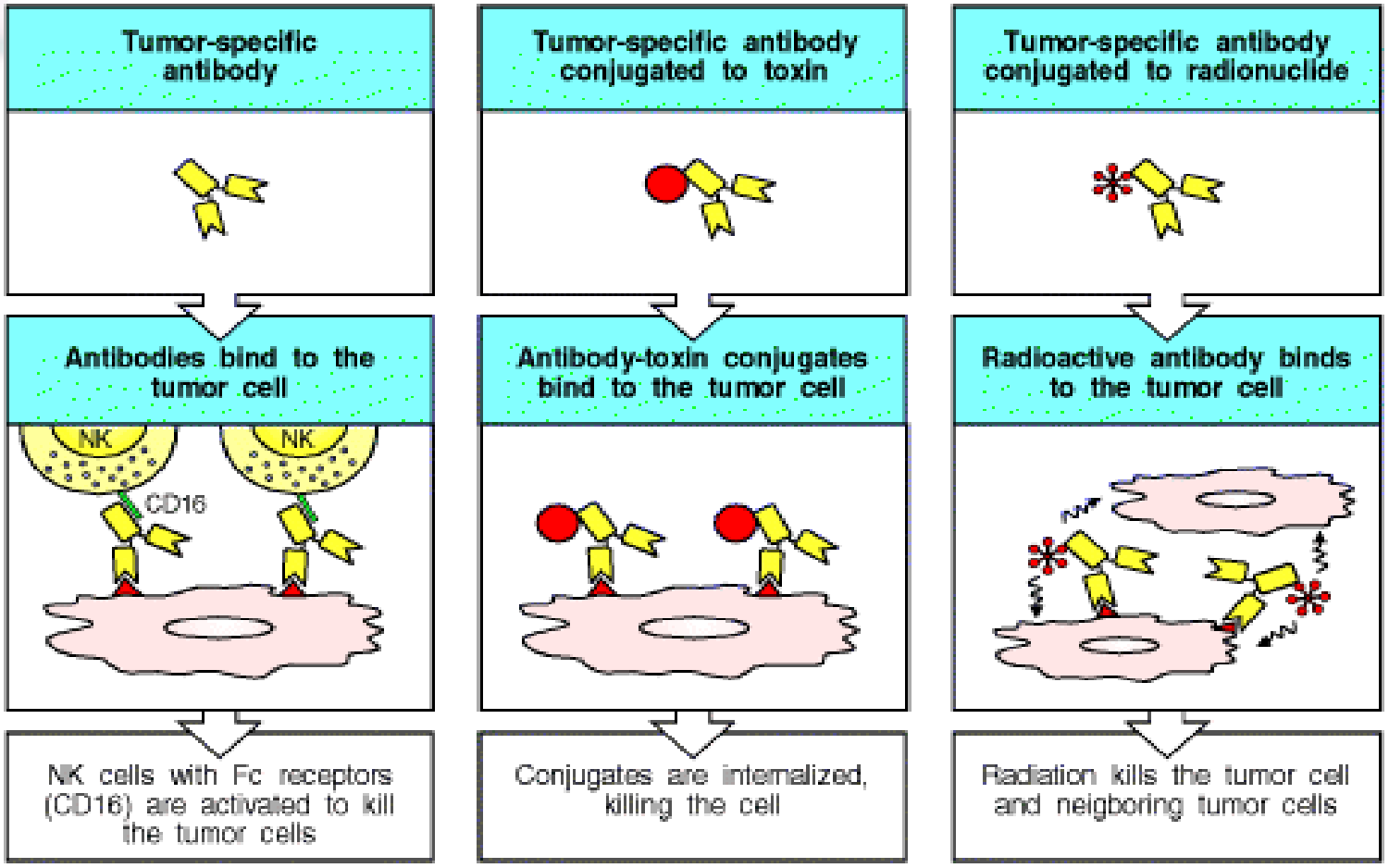
- MAb - an antibody that binds to a single epitope
- In tumor cells the epitope is often a protein on the cell surface



Stevens, Glen. Brain Tumors: Meningiomas and Gliomas. April 22, 2003. http://www.clevelandclinicmeded.com/diseasemanagement/neurology/braintumor/braintumors.htm

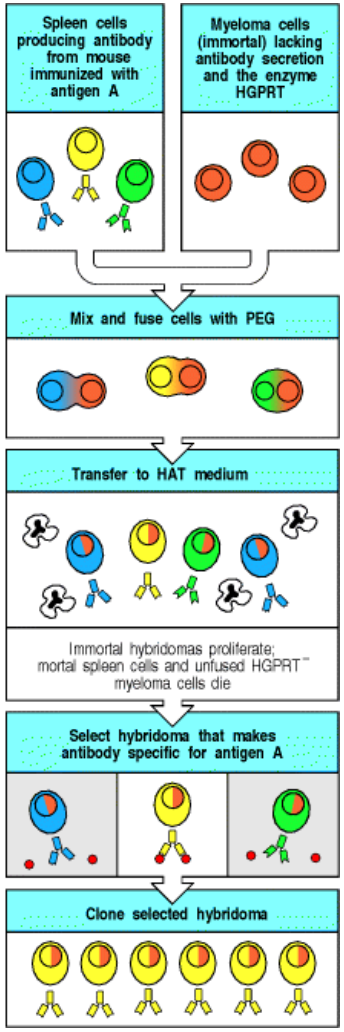
Image Courtesy of: http://www.strayvr.com/FullyHuman.jpg

Treatment Options Using MAbs



Janeway, Charles A., et al. Immunobiology. 5th ed. New York: Garland Publishing, 2001.

Production of Monoclonal Antibodies



Janeway, Charles A., et al. Immunobiology. 5th ed. New York: Garland Publishing, 2001.

Immunogenicity in Humans

- Most MAbs are produced using murine (mouse) cells

- The human body may recognize the MAbs as being foreign. This can result in:
  - Allergic reaction
  - Failure of treatment

Janeway, Charles A., et al. Immunobiology. 5th ed. New York: Garland Publishing, 2001.

Engineering MAbs to Reduce Immunogenicity

- Graft the antigen binding loop of the mouse antibody to the framework of a human antibody
- MAb still has the same antigen/antibody binding specificity

Janeway, Charles A., et al. Immunobiology. 5th ed. New York: Garland Publishing, 2001.

Malignant Gliomas Tumors

- Gliomas are the most common type of primary brain tumors in adults
  - Anaplastic Astrocytoma (III)
  - Glioblastoma Multiforme (IV)
- Conventional therapy:
  - Surgery
  - External-beam radiation
  - Chemotherapy
- Median survival: 40-60 weeks

A Primer of Brain Tumors. American Brain Tumor Association. 1991. <http://neurosurgery.mgh.harvard.edu/abta/primer.htm#Section6>

81C6 MAb

- 81C6 is a monoclonal antibody that binds to tenascin, a tumor-associated extracellular matrix protein
- Tenascin is found in:
  - Gliomas
  - Connective tissue (trace amounts)
  - Developing organs (trace amounts)

Cokgor, Ilkcan, et al. “Phase I Trial Results of Iodine-131-Labeled Antitenascin Monoclonal Antibody 81C6 Treatment of Patients With Newly Diagnosed

Chiquet-Ehrismann, Ruth. *What Distinguishes Tenascin From Fibronectin*? The FASEB Journal. Vol. 4. June 1990.

Blood Brain Barrier

- Tight junctions in capillary endothelial cells that prevent molecules from entering into glial cells

- Entry is achieved by a molecule's solubility in lipids or by transporters

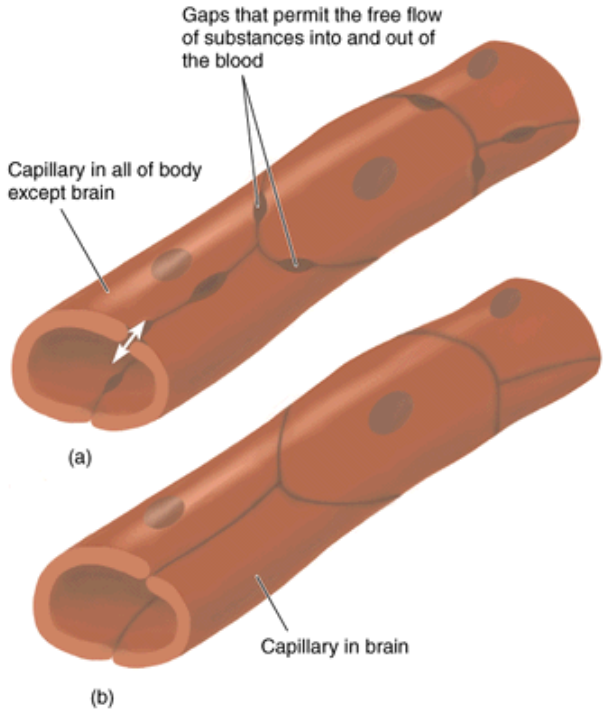


Image courtesy of http://homepage.psy.utexas.edu/homepage/class/Psy332/Salinas/Cells/BBB.gif

Purves, Dale, et al. Neuroscience. 2nd ed. Sinauer Associate, Inc. 2001.

How can the Blood Brain Barrier be overcome?

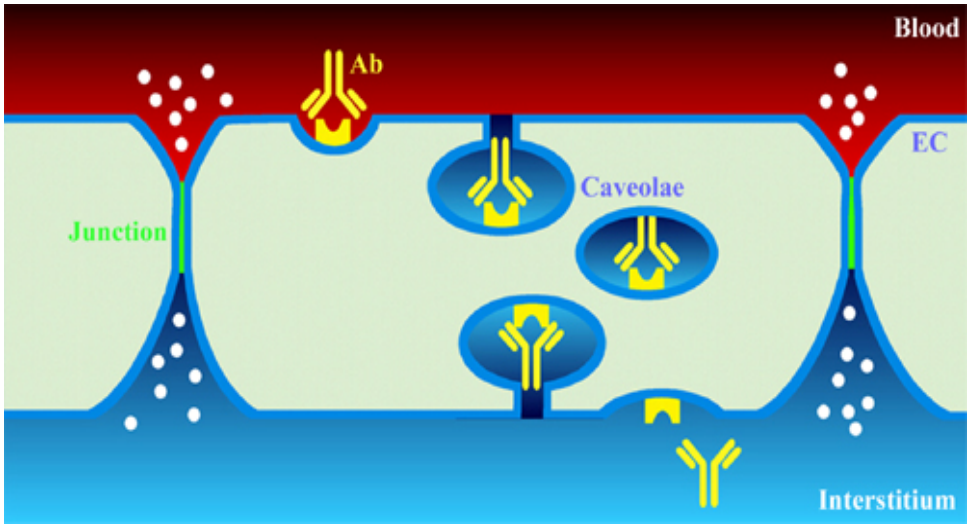
- Getting molecules past the blood brain barrier (BBB) can be achieved by attaching the molecule to a vector

- The vector can be a modified protein or monoclonal antibody that is normally transported through the blood brain barrier

Pardridge, W.M. "Vector-mediated drug delivery to the brain." Advanced Drug Delivery Revues. Vol. 36. April 1999.

Vector Mechanism

- Vector aids in transport across the BBB by transcytosis
  - Transcytosis – transport of substance across epithelium by uptake into and release from coated vesicles



http://images.google.com/imgres?imgurl=http://www.skcc.org/n_images/transcytosis.jpg&imgrefurl=http://www.skcc.org/schnitzer.html&h=253&w=500&sz=118&tbnid=7FtvVYAfLg0J:&tbnh=64&tbnw=126&start=1&prev=/images%3Fq%3Dtranscytosis%26hl%3Den%26lr%3D%26sa%3DG

Bickel, Ulrich, et al. *Pharmacologic effects in vivo in brain by vector-mediated peptide drug delivery.* Proceddings of the National Academy of Science. 1992.

Suggested Vector: OX26

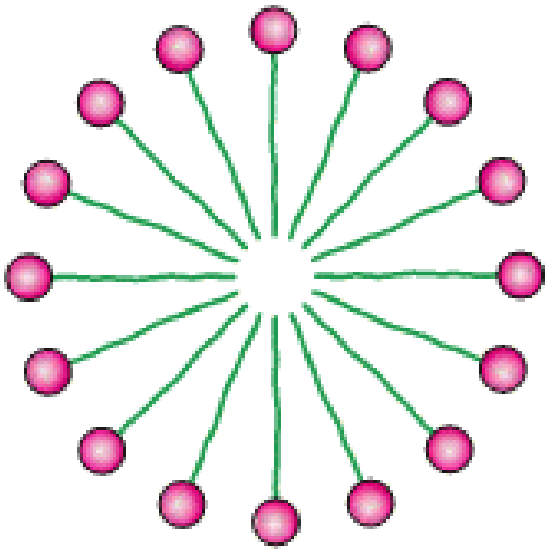
- OX26 is a MAb

- OX26 undergoes receptor-mediated transcytosis
  - Targets transferrin
  - Transferrin receptor is highly expressed on brain capillary endolthelial cells

Bickel, Ulrich, et al. *Pharmacologic effects in vivo in brain by vector-mediated peptide drug delivery.* Proceddings of the National Academy of Science. 1992.

What is a Micelle?

- Globular structure made of a charged head group with a lipid tail
- Hydrocarbon tails located on the inside of the micelle to reduce interactions with water



Cross-sectional View of a Micelle

Berg, Jeremy, et al. Biochemistry. W.H. Freeman and Co. 2002.

Micelle and MAbs For Drug Delivery: Immunomicelle

- Structure
  - MAb attached to the head group of micelle
  - Toxin will be stored inside the micelle

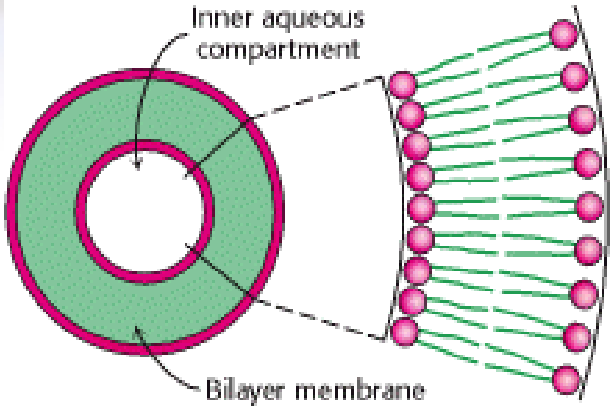
- Toxin Delivery
  - Immunomicelle will be transported across the BBB by vector mechanism
  - Toxin delivered to the tumor when immunomicelle is engulfed

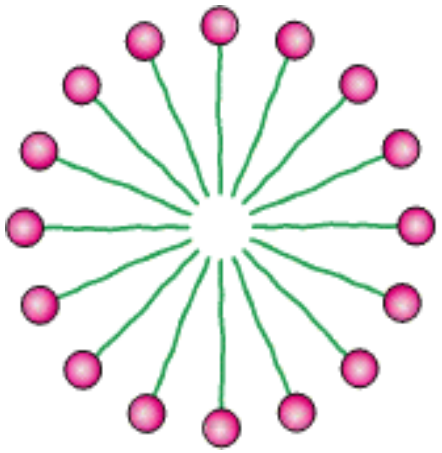
Previous Studies: Immunoliposomes

- Huwyler et al. utilized immunoliposomes
  - Deliver the daunomycin to the rat brain
  - Liposome did not cross the BBB without a vector
  - Drug delivery successful when the vector OX26 was used
  - Higher density of vector → more immunoliposomes cross BBB

Huwyler, Jorg, et al. *Brain Drug Delivery of Small Molecules Using Immunoliposomes.* Proceedings of the National Academy of Sciences. Neurobiology. 1996.

How does a Micelle Compare to a Liposome?





- Micelles and Liposomes can be made of the same material
- Hydrophilic environment inside the liposome and hydrophobic environment inside the micelle
- Superficially micelles and liposomes are indistinguishable
- More toxin can be inserted inside a micelle than a liposome of the same diameter

Pictures Courtesy of: Berg, Jeremy, et al. Biochemistry. New York. W.H. Freeman and Co.: 2002

Investigation of Immunomicelles

- Torchilin et al. used immunomicelles loaded with Taxol® to treat Lewis lung caracinoma
- Drug delivery by cell engulfing the micelle
- Micelle made of polyethylene glycol–phosphatidylethanolamine conjugates

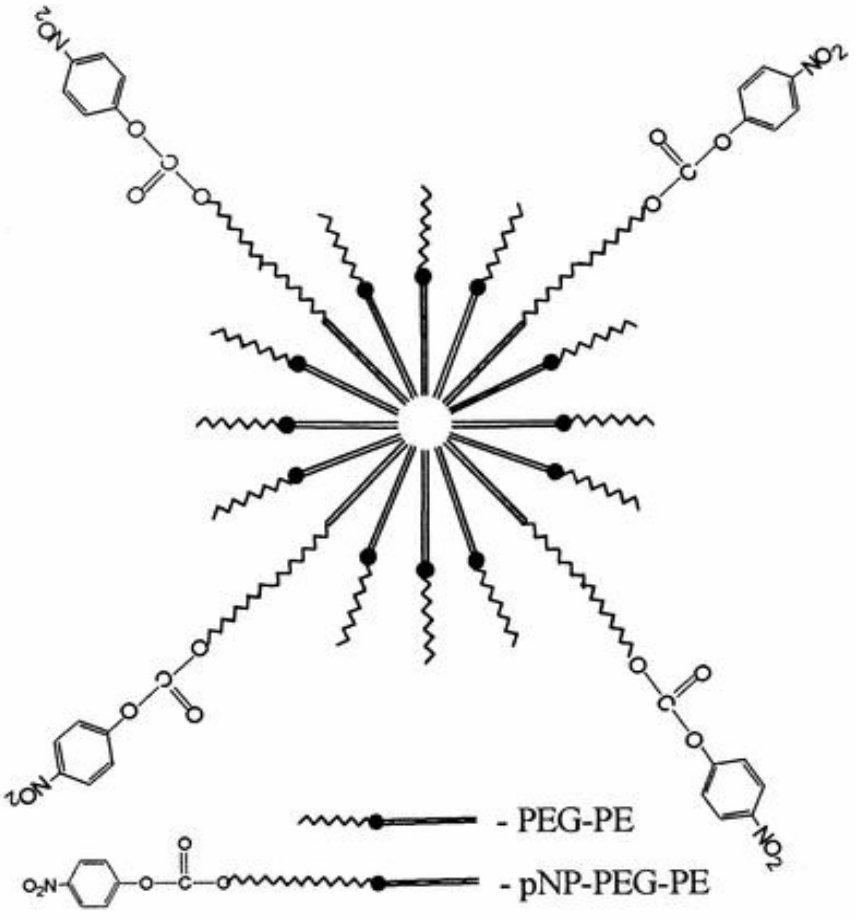
Torchilin, Vladimir P., et al. “Immunomicelles: Targeted pharmaceutical carriers for poorly soluble drugs.” Proceeding of the National Academy of Sciences. May 13, 2003.

Investigation of Immunomicelles

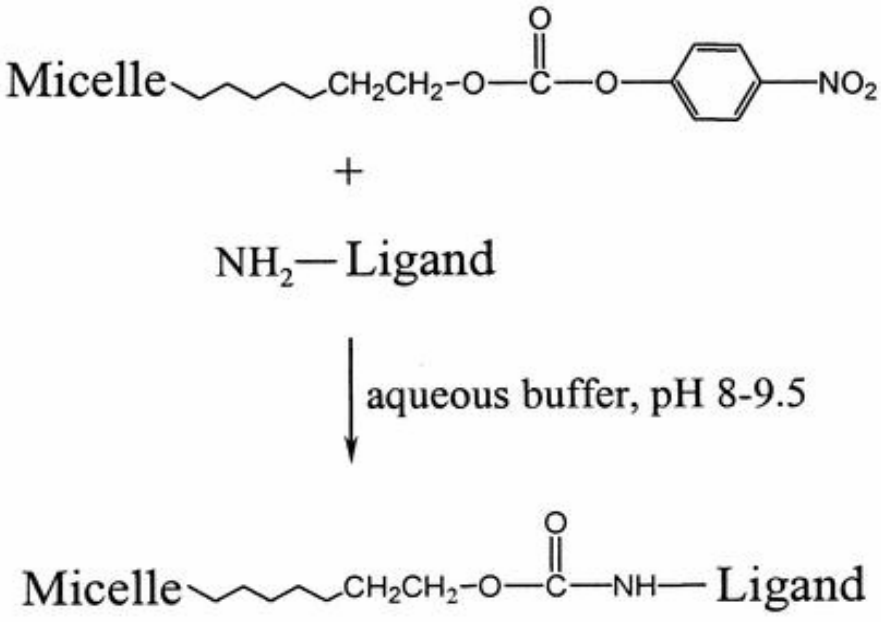
- Use amphiphilic derivative of PEG: pNP-PEG-DOPE
- pNP-PEG-DOPE readily incorporates into the micelle
- Binds primary amino group ligands via water-exposed pNP groups
  - Forms stable, nontoxic urethane bonds
- Several dozen MAbs can be attached to one micelle

Torchilin, Vladimir P., et al. “Immunomicelles: Targeted pharmaceutical carriers for poorly soluble drugs.” Proceeding of the National Academy of Sciences. May 13, 2003.

Micelle-Antibody Attachment



Micelle



Micelle-Antibody attachment reaction

Size Considerations with Drug Delivery to Tumors

- Diffusion and accumulation inside the tumor depend on the cutoff size of the tumor blood vessel arrangement
- This cutoff size varies for different tumors
- MAbs attached to micelles does not significantly affect the size of the micelle

Torchilin, Vladimir P., et al. “Immunomicelles: Targeted pharmaceutical carriers for poorly soluble drugs.” Proceeding of the National Academy of Sciences. May 13, 2003.

Selected Drug for Encapsulation

- Drug of choice for micelle delivery is Temodar®
  - Similar side effects as other chemotherapeutic agents
  - Smaller molecular diameter
  - Acts through alkylation of DNA of replicating cells
  - Standard dosages of 100 and 500 mg daily

Blood Concentration Model

- Generally, the concentration of a drug in the body is modeled by a half life function with the equation

$$\frac{dC_{blood}}{dt} = -kC_{blood}$$

- Separating variables and integrating yields

$$\ln\left(\frac{C_{blood}}{C_0}\right) = -kt$$

Temodar® Blood Concentration Model

- The half life of Temodar® is 1.8 hours
- Half life can be used to find a k value of $0.38 hr^{-1}$
- The final model is described by the equation

$$\frac{C_{blood}}{C_0} = e^{-0.38\,t}$$

Temodar Product Information, Schering Corporation, 2003

Micelle Blood Concentration Model

- Micelle Half Life Model Assumptions:
  - 20-25% of the blood in body is received by kidneys
  - 10-15% of blood received is cleaned
  - 50% micelles removed from the blood in each circulation
  - Blood is recirculated through the body every minute

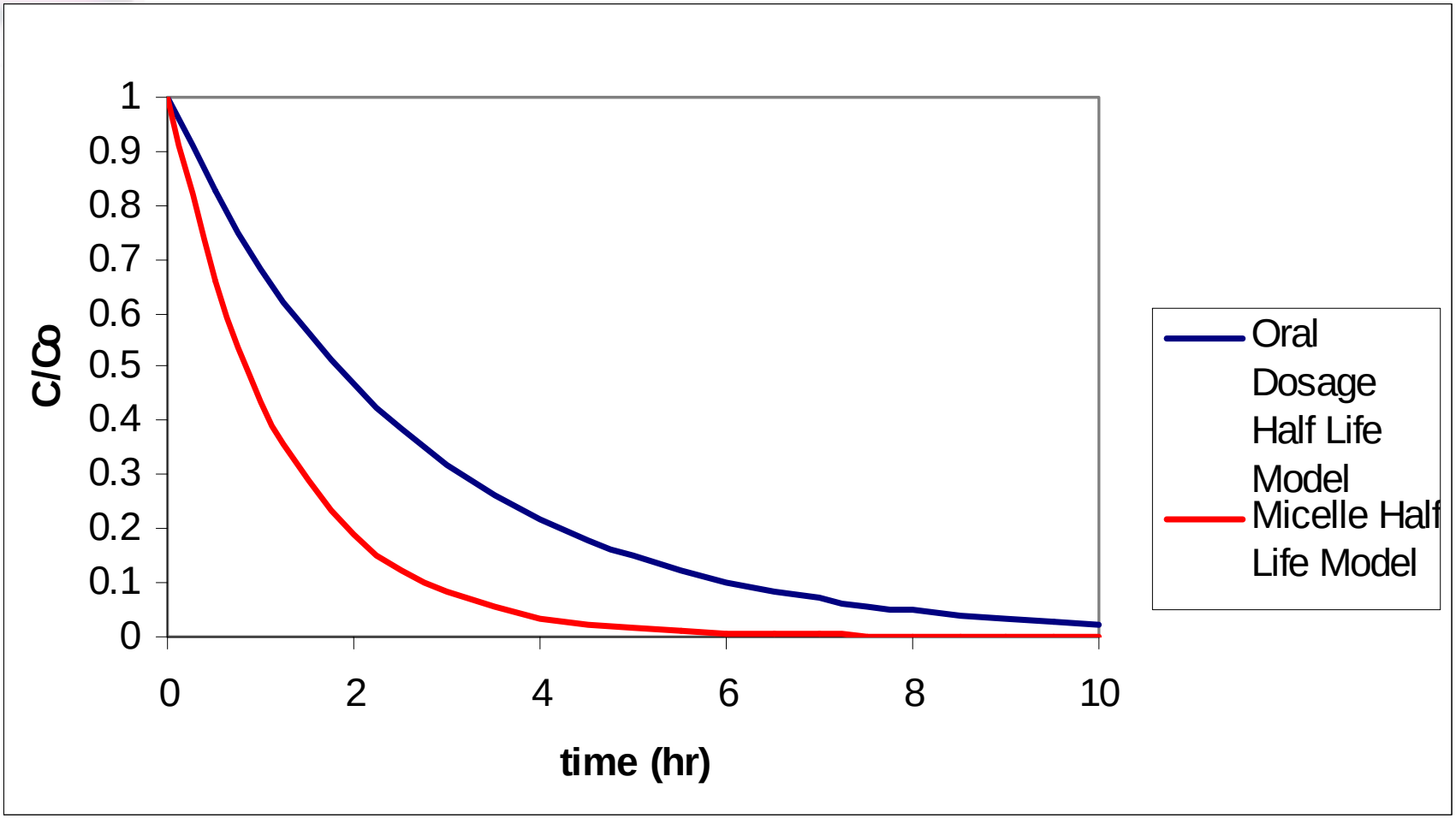
$$\%cleaned = \%received \times \%processed$$

- Micelle half life is 50 min.

- This is used to find a k of 0.83hr^{-1}

Koeppen, Bruce M. and Bruce A. Stanton, *Renal Physiology*, ed. 3, St. Louis, MO, 2001

Why is Micelle better than Temodar® alone?

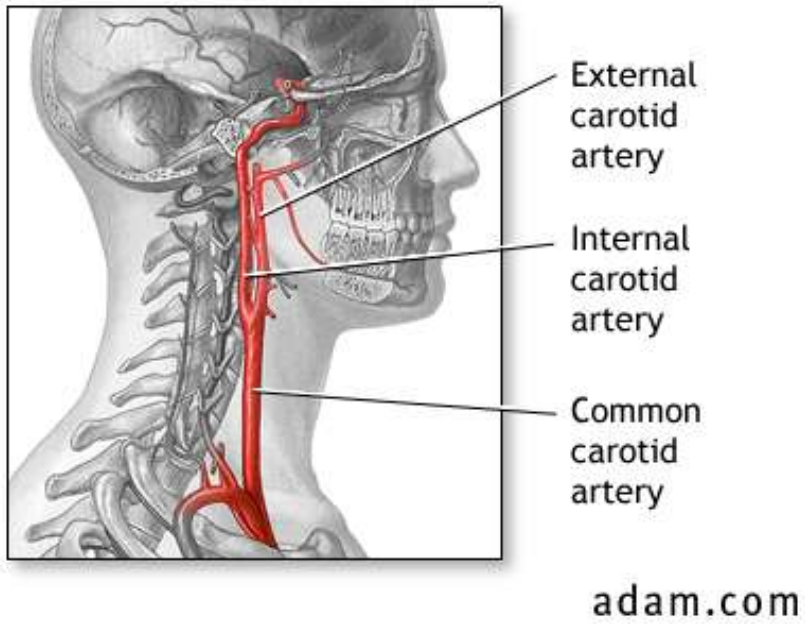


Micelle Concentration Model Considerations

- Model is accurate for determining the blood concentration of an oral dosage
- Micelles will be injected to deliver the micelles directly to the tumor in the brain
- Additional model needed to determine how the concentration of micelles in brain changes with time

Injection Delivery and Dosage Model

- Drug is administered by injection into the internal carotid artery (ICA)
- Average flow rate within carotid artery is 370 mL/min
- Assume all of drug enters artery in 5 seconds



*Drug concentration = dosage/(flowrate*time)*

Image courtesy of www.pennhealth.com/

Injection Delivery and Dosage Model

- Estimated number of capillaries in the portion of interest in the brain can be used to determine the amount of blood contained in each mircovessel
- Amount of blood in capillaries can be used to determine amount of drug per capillary

$$\frac{plug\ volume\ in\ ICA}{number\ of\ \ capillaries} \times plug\ concentration = \frac{drug\ \ delivered}{capillary}$$

Blinkov, Samuil M., and Il-ya I. Glezer, eds. The Human Brain in Figures and Tables: A Quantitative Handbook. Moscow: Basic Books, Inc, 1968

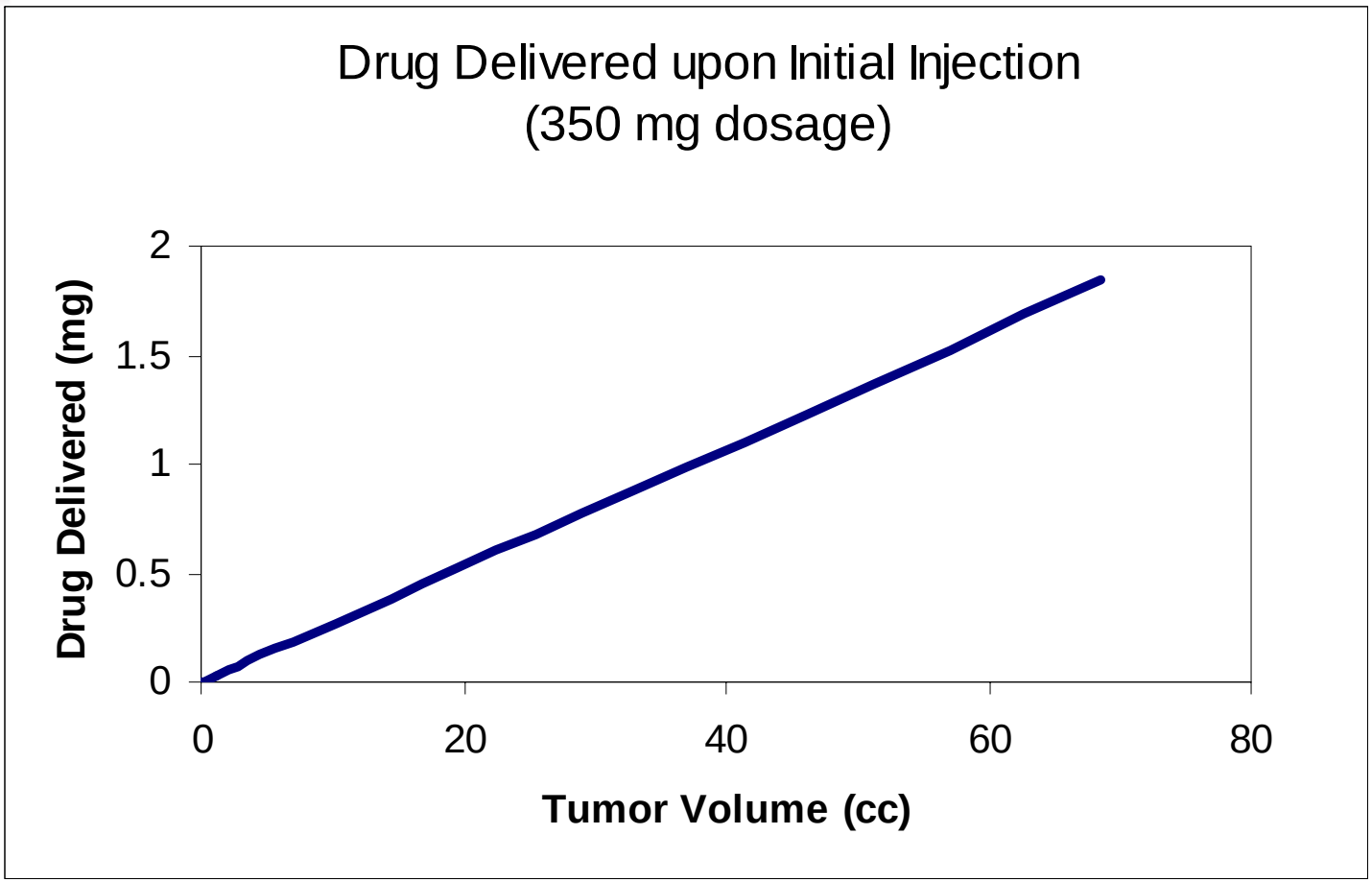
Injection Delivery and Dosage Model

- Assumptions
  - 50% of initially injected drug will penetrate BBB
  - 50% of drug that crosses will bind to tumor cells
  - 3000 capillaries/mm^3 brain/tumor tissue

$$\frac{capillaries}{volume\ tumor\ tissue} \times \frac{drug\ delivered}{capillary} = \frac{drug\ delivered}{volume\ tumor\ tissue}$$

Blinkov, Samuil M., and Il-ya I. Glezer, eds. The Human Brain in Figures and Tables: A Quantitative Handbook. Moscow: Basic Books, Inc, 1968

Injection Delivery Standard Curve



Why is Injection Superior to Oral Dosage?

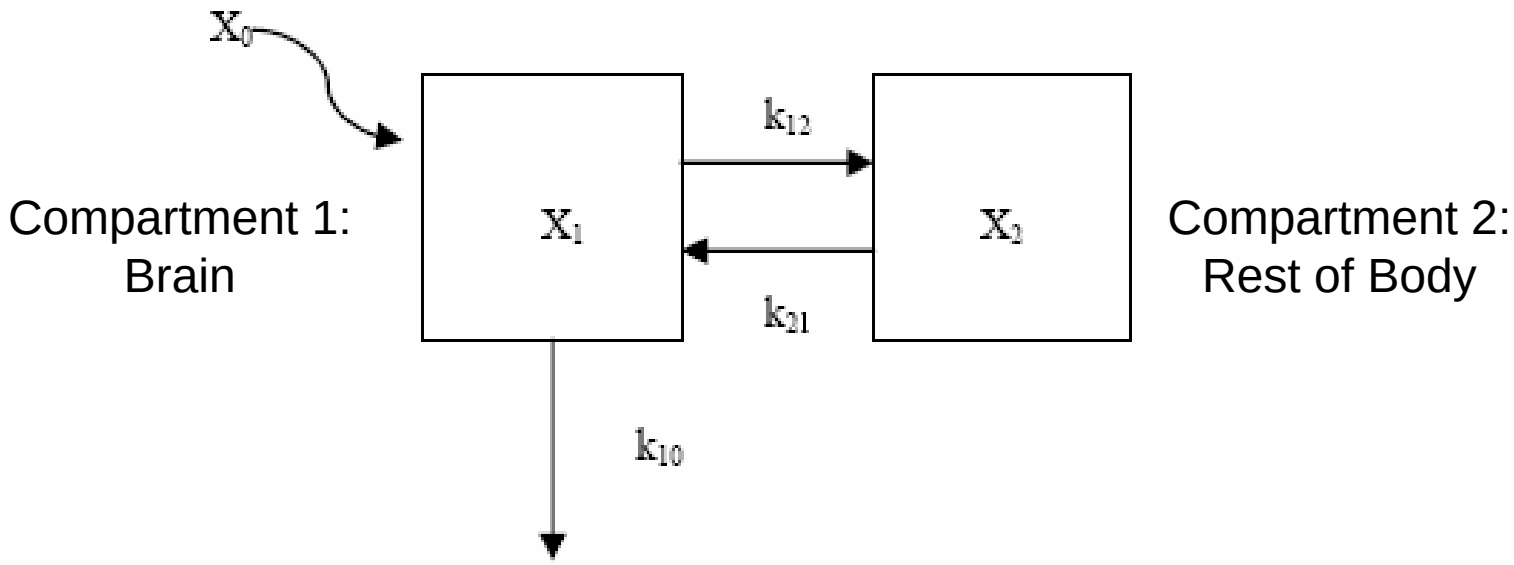
- Delivery model shows a 250 fold efficacy improvement from injection over oral delivery
- Dosage can be kept to a minimum with equal tumor reduction as current treatments, which will subsequently lower side effects

What are the Applications of the Dosage Model?

- Dosages can be determined for individual patients based on tumor size and location
- Necessary dosage for effective treatment can then be incorporated into the blood concentration model in order to determine dosage regimen
- Initial and final brain concentrations can be used to estimate parameters for brain elimination models

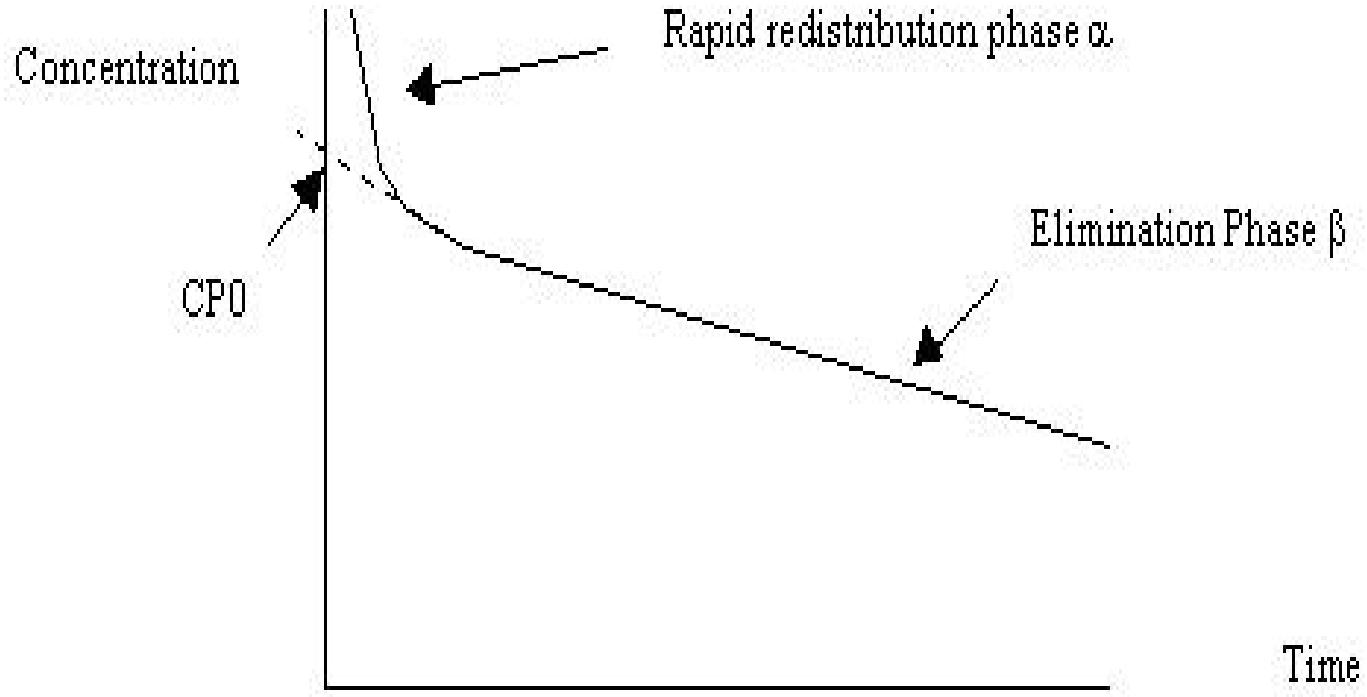
Brain Concentration Model

- Analyzed as a two compartment membrane model



- k values are rate constants, X values are concentrations

Brain Concentration Model



www.4um.com/tutorial/science/pharmak.html

Brain Concentration Model

- Plasma Concentration in brain decays bi-exponentially with time and will fit the following equation:

$$C_{brain} = A_1 e^{-\alpha T} + B_1 e^{-\beta T}$$

- T is time
- A_1 and B_1 are the intercept constants
- α and β are the hybrid rate constants (units T^{-1})

Welling, Peter G., *Pharmacokinetics: Processes and Mathematics*, American Chemical Society, Washington DC, 1986

Brain Concentration Model Derivation

- α and β are functions of phase half life

- The half life for the elimination phase was assumed to be the half life of the micelle in the body in the oral dosage model

$$\beta = \frac{\ln(2)}{(t_{1/2})_{\beta}}$$

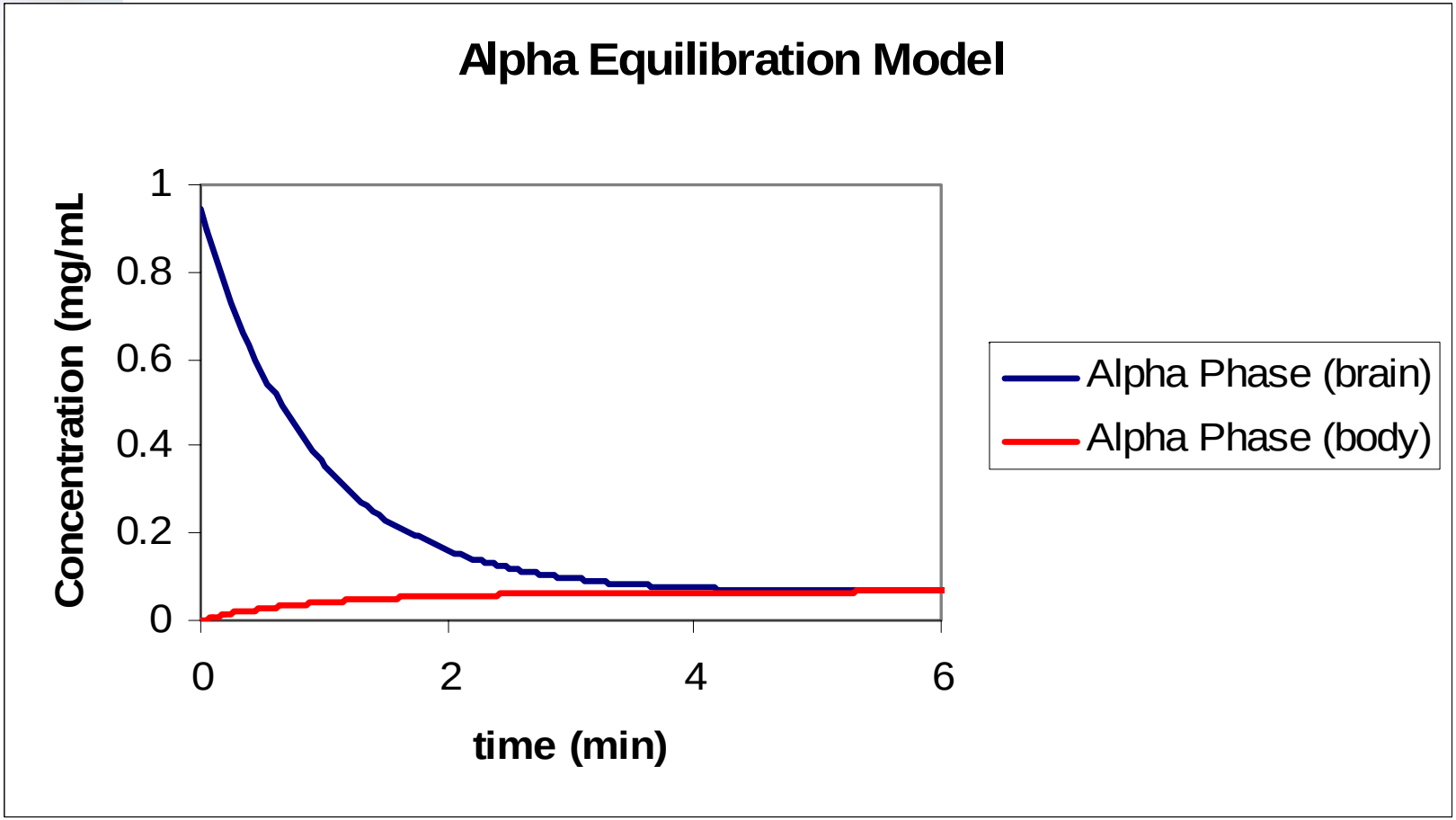
Brain Concentration Model Derivation

- Half life for redistribution phase is determined from the injection dosage model

$$\alpha = \frac{\ln(2)}{(t_{1/2})_{\alpha}}$$

- Mass balances on brain/body system were used to determine how the concentrations in the body and brain change during this phase to determine half life

Brain Concentration Model Derivation



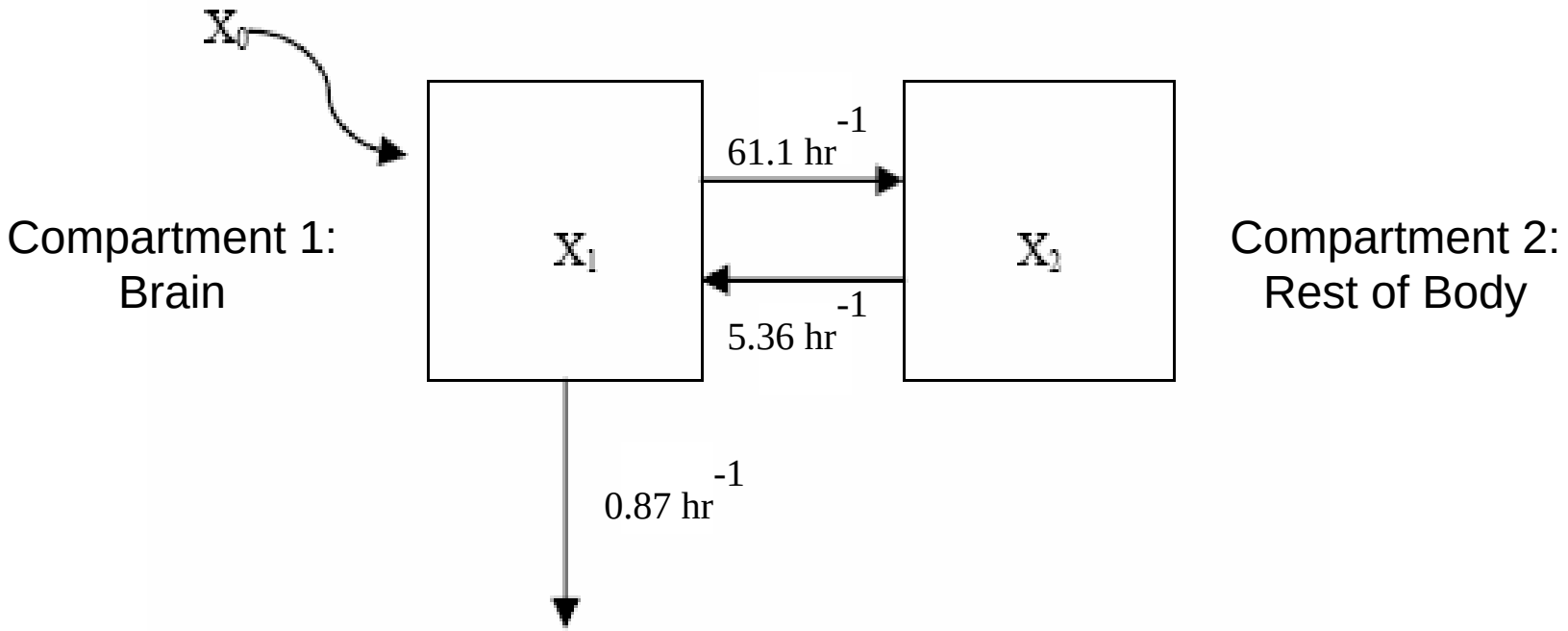
Brain Concentration Model Derivation

- A_1 was determined from concentration of drug leaving brain after tumor delivery

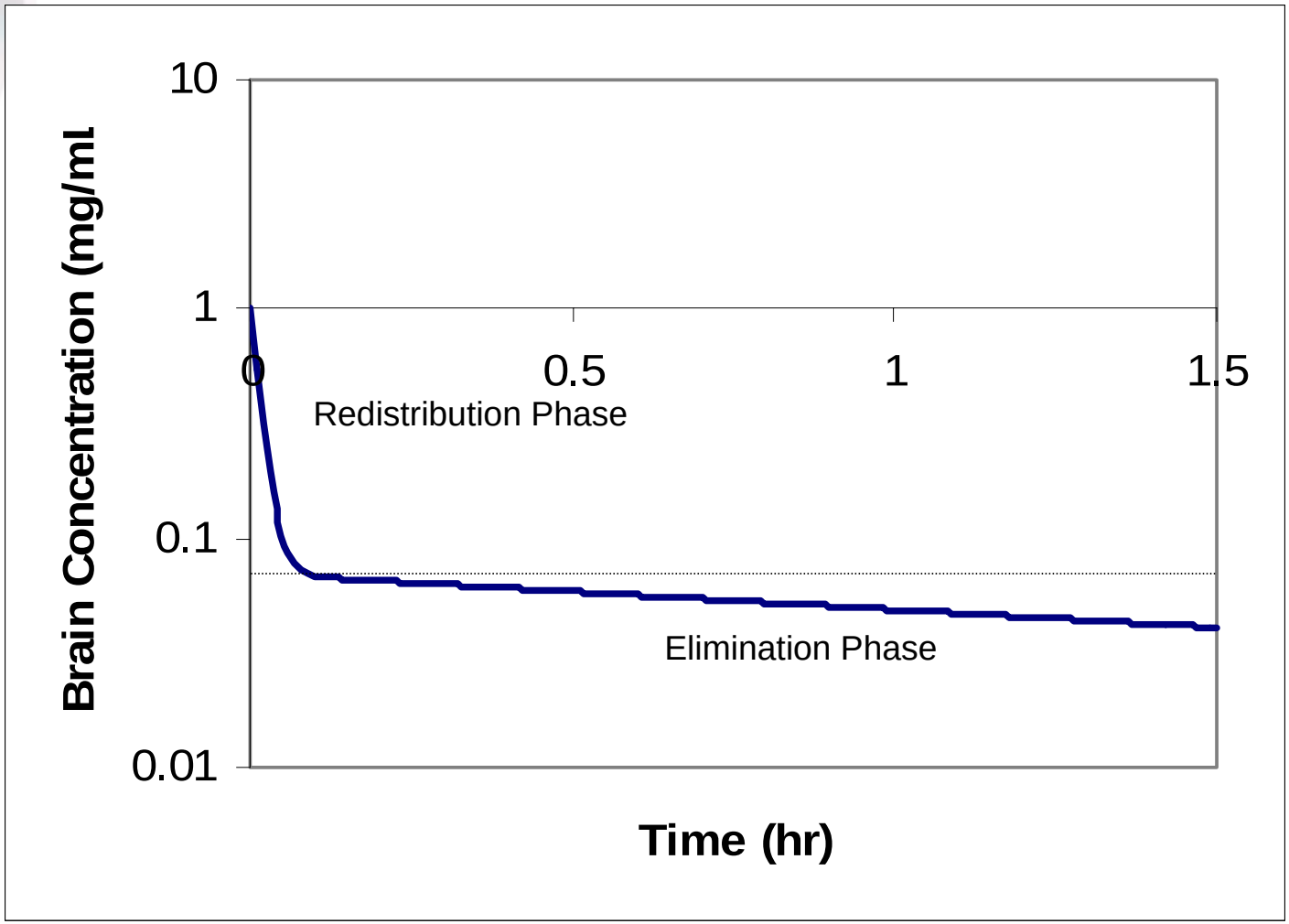
- B_1 was determined from estimate of theoretical body concentration as a function of mass of drug exiting the brain after initial injection

Elimination Model Rate Constants

- From model parameters, k-values can be determined



Brain Concentration Model



Pre-FDA Model Verification

- Before FDA testing begins, this model will be verified by animal testing
  - Determine % micelles eliminated from the blood by kidneys
  - Determine percent of micelle to cross BBB
  - Determine percent of micelle to bind to cancer cells
  - Determine rate constants for the redistribution phase and elimination phase from experimental half life results

FDA Approval

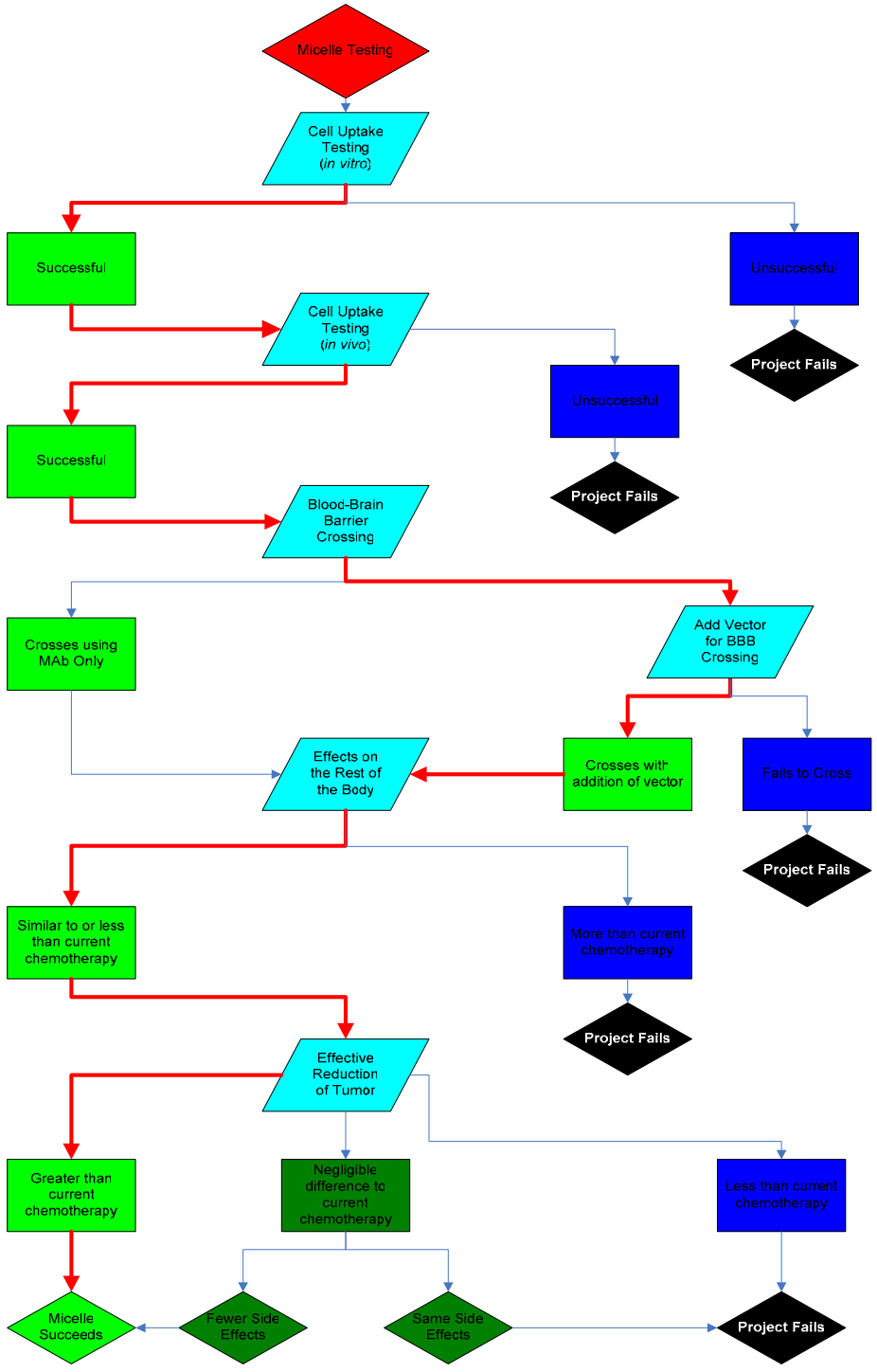
- There are 3 major phases of FDA approval for a new drug or therapeutic:
  - Pre Clinical Trials (~13 years)
  - Clinical Trials (~8.5 years)
  - Validation (~2 years)

- The FDA approval process will take around 23 years

What occurs during Pre-FDA Testing?

- Testing in mice to determine treatment safety and efficacy
- Testing to confirm drug delivery mechanism parameters

Pre-FDA Testing Flow Chart



Pre-FDA Funding

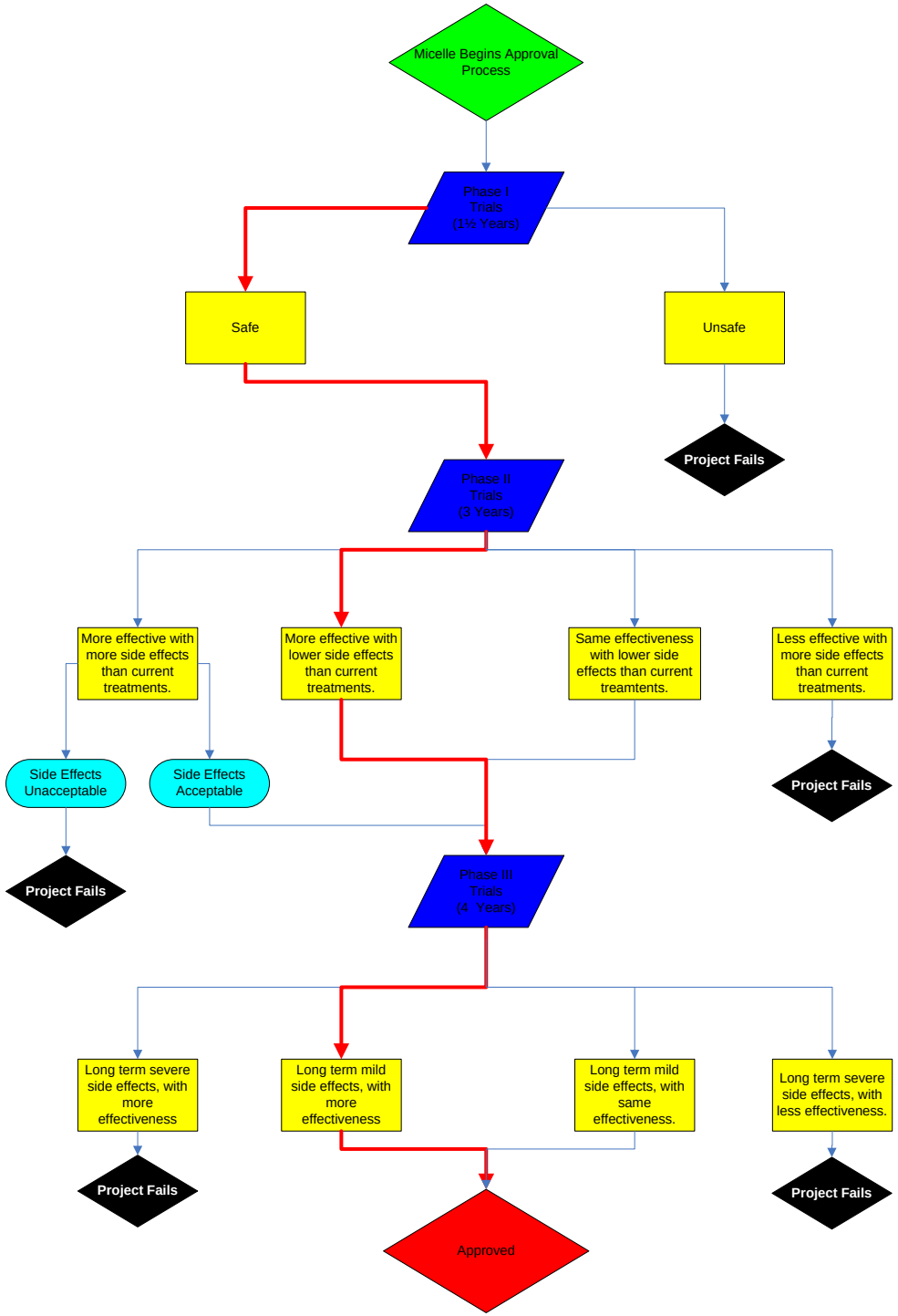
- Major source of funding for Pre-FDA testing is the National Institute of Health (NIH)
- These grants are renewable annually
- Pre-FDA testing will be performed in conjunction with universities

Grant	Average Amount per fiscal year
Small Business Technology Transfer (Phase I)	$140,700
Small Business Technology Transfer (Phase II)	$318,492
Small Business Innovation Research (Phase I)	$149,261
Small Business Innovation Research (Phase II)	$425,517
Animal (Mammalian and Non-mammalian) Model, and Animal and Biological Material Resource	$716,044
Biotechnology Resource Grant Program	$1,628,377
Exploratory Grants	$1,134,298

What occurs during FDA Clinical Trials?

- Phase I Trials
  - Determine immediate treatment safety and dosage
  - 1½ Years
- Phase II Trials
  - Determine potential short-term side effects
  - 3 Years
- Phase III Trials
  - Determine potential long-term side effects
  - 4 Years

FDA Clinical Trials Flow Chart



FDA Approval

- At the completion of clinical trials a New Drug Application (NDA) is filed with the FDA
- The final stage of FDA approval is the review and post-marketing analysis by the FDA
- At the end of this period, one drug is approved out of many that enter the FDA approval process

MAb Market in Cancer Therapy

- The market for cancer treatments is growing at a tremendous rate
- In 1997 the first MAb for cancer was approved by the FDA (Rituxan)
- Annual average growth over 2003 and 2004 for the MAb market in cancer therapy is 60%

MAb Market in Cancer Therapy

- Revenues for MAb products increased by $2 billion since 2001
- $3 billion in revenue in 2003 for MAb's for Cancer Therapy
- By 2008 Projected Sales are more than $12 billion.

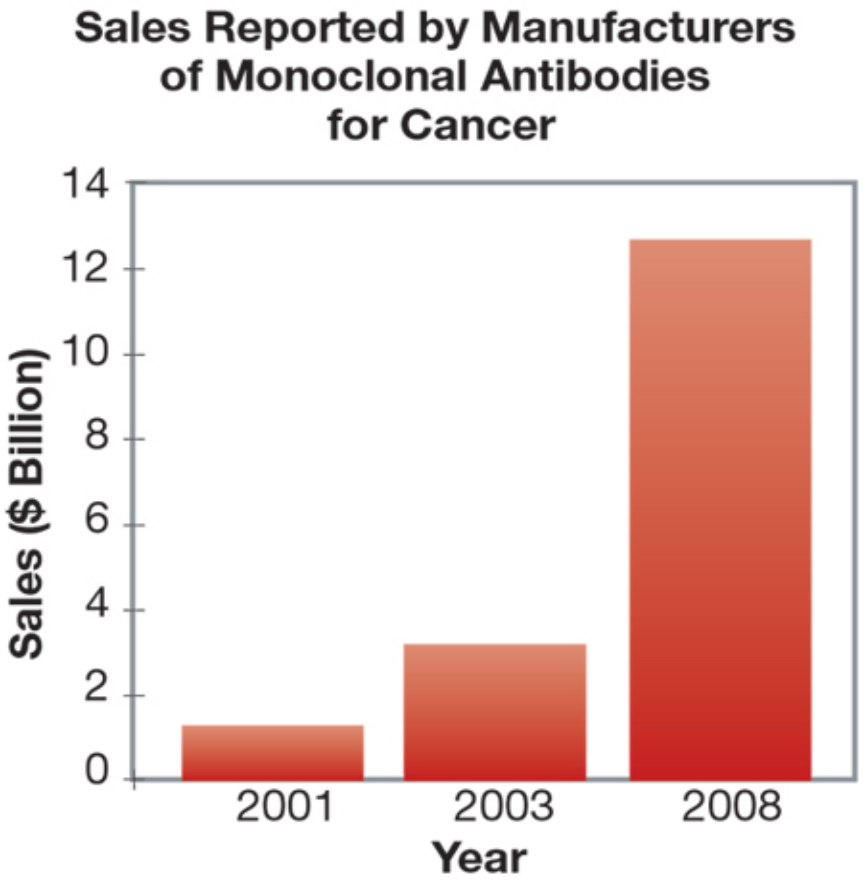


Image courtesy of: Elder, Melissa. *Monoclonal Antibodies for Cancer*. Biopharm International. Volume 17, Number 11. pp 66. Advanstar Communications Publication. November 2004.

Facilities

- The facilities needed are:
  - Laboratories (for Quality Control and research)
  - Warehouses
  - Manufacturing plants
    - MAb and Vector Production
    - Micelle Production

Cost Estimation

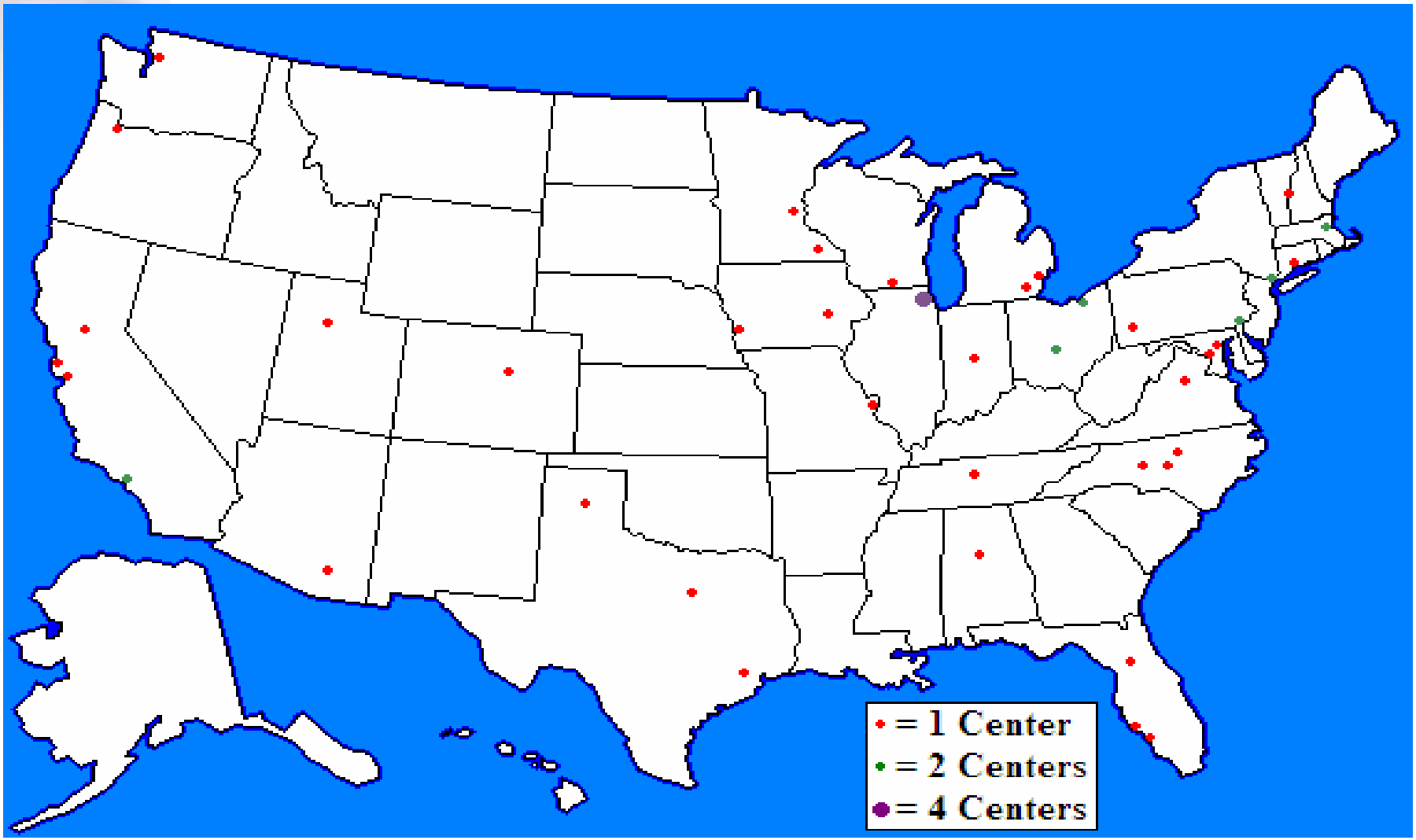
- FCI: $31.3 million
- TCI: $45 million
- Manufacturing are assumed as $1,000/g MAb produced per year
- Product will sell for around $15,000/per treatment

Plant Location

- Top 50 cancer research hospitals in the US plotted on map
- 33 of the top 50 hospitals are east of the Mississippi River
- 3 of top 5 hospitals are in New England from Washington D.C to Boston

Best Hospitals 2004: Cancer. USNews.com.
http://www.usnews.com/usnews/health/hosptl/rankings/specihqcanc.htm

Cancer Research Locations



Plant Location

- Best place to build project plant is outside New York City
- 9 top 50 hospitals are within 200 miles of New York City
- Market strategy will be to supply these 9 hospitals with treatment

Incidence of Gliomas in the US

- Population of the US determined by report issued by the Census Bureau
  - 295 million as of January 1, 2005
  - Population growth of 1%/year
- 2 to 3 cases per 100,000 people per year
- Cases in US per year: 5900-8850
  - Mean number of patients per year assumed to be 7350

Stevens, Glen. Brain Tumors: Meningiomas and Gliomas. April 22, 2003. http://www.clevelandclinicmeded.com/diseasemanagement/neurology/braintumor/braintumors.htm

Inferiority Function

- For a given market, two products of equal quality and prominence follow the equation:

$$p_1 d_1 = p_2 d_2$$

- Two products of equal quality and unequal prominence follow the equation:

$$p_1 d_1 = \alpha p_2 d_2$$

- Knowledge multiplier α is function of marketing expenditure

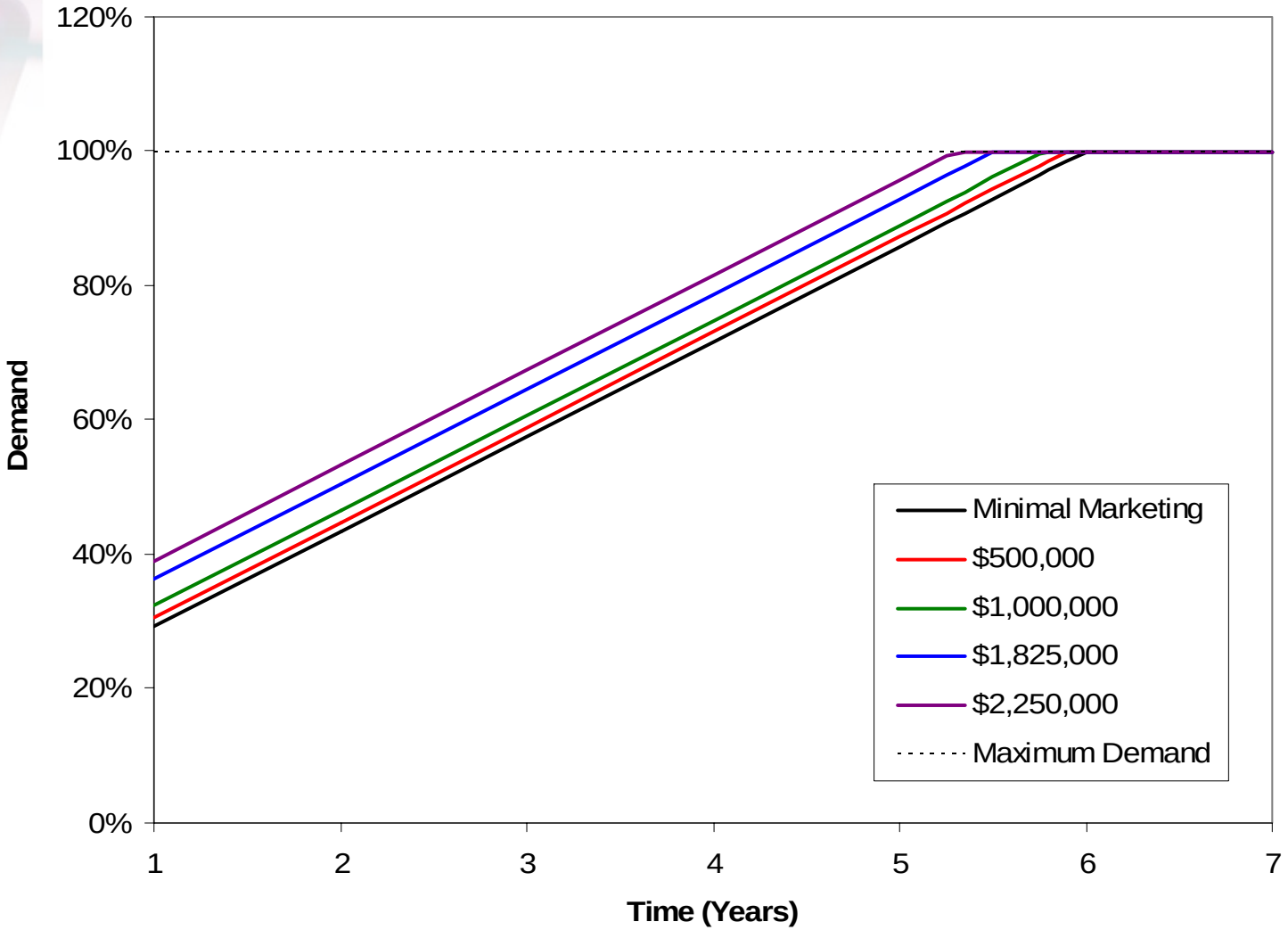
Minimum Proposed Marketing

- Marketing strategy is geared toward the 9 cancer research centers located near suggested plant location
- During Pre-FDA testing and Phase I and II Trials $150,000/year will be spent on marketing to oncologists at these hospitals
- Marketing will increase to $400,000/year when Phase III Clinical Trials begin

Potential Demand Model

- An increase in post-production marketing expeditures causes a negligible difference in rate of increase of demand
- Varied pre-production marketing spending changes the initial percent of potential demand
- Effects of increased marketing modeled with inferiority function $\alpha(t)$

Inferiority Function Plot



Superiority Function

- For a given market, two products of unequal quality and prominence will follow the equation:

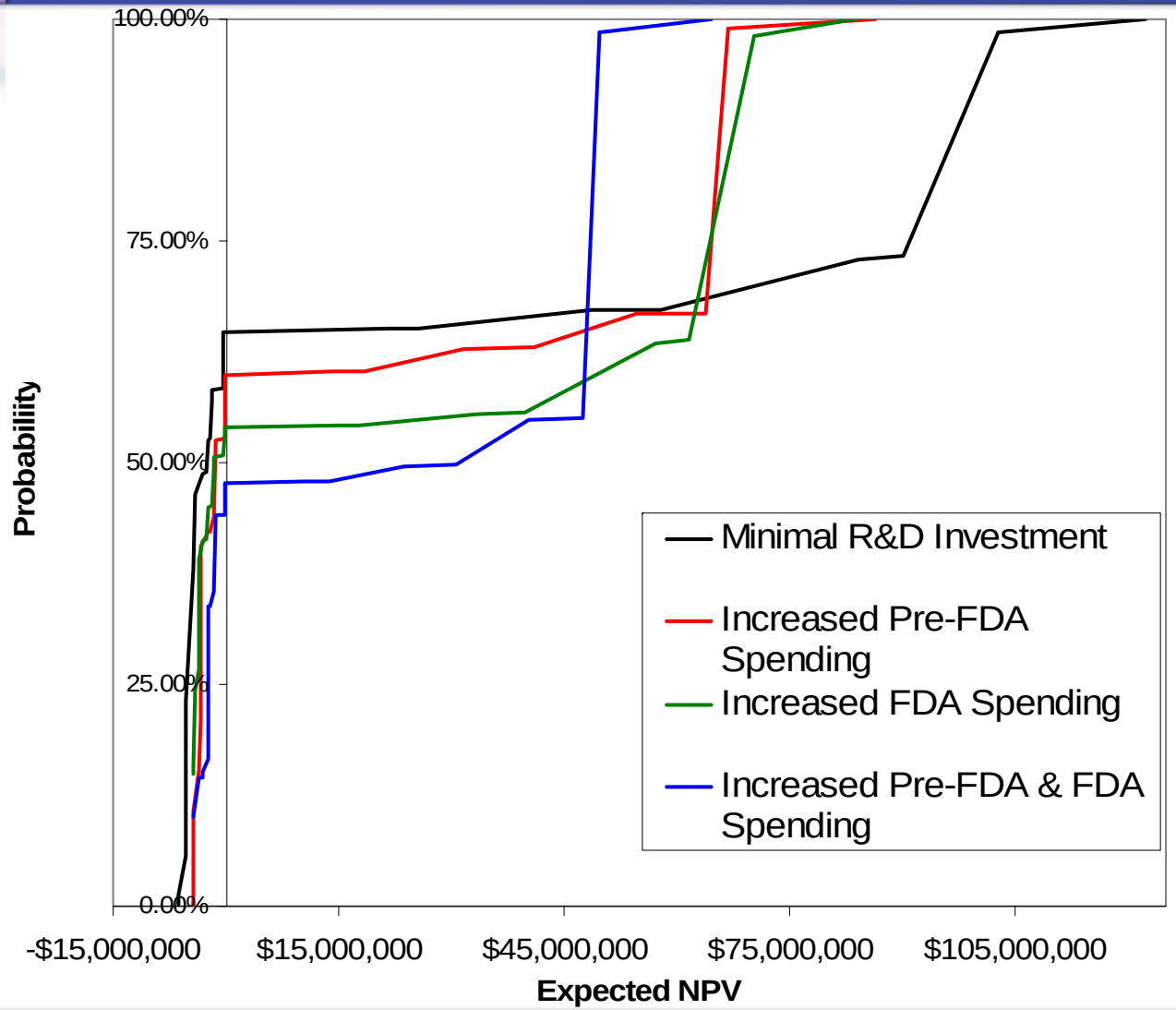
$$\beta p_1 d_1 = \alpha p_2 d_2$$

- Treatment demand is a function of treatment effectiveness and side effects
  - Increased treatment effectiveness decreases β
  - Decreased treatment side effects decreases β
- 25% demand increase assigned to each facet of product superiority in financial analysis

Financial Analysis

- Treatment cost to recover the investment in 3 years is based on the most likely successful pathway in FDA testing
- Treatment cost determined as $9,000 per patient based on assumed sales of 7350
- Treatment cost fixed at $15,000 to ensure an acceptable profit margin

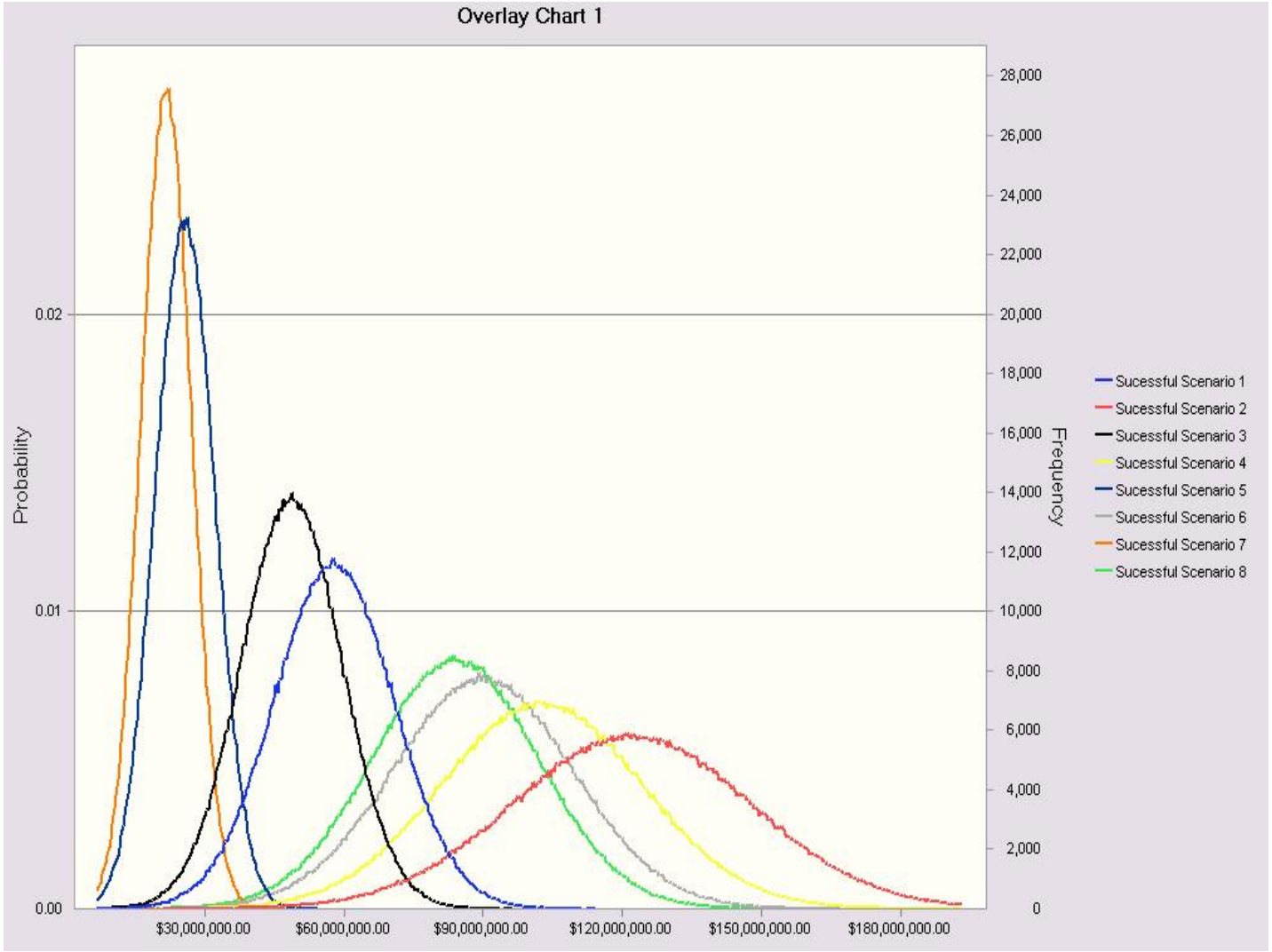
Expected NPV Risk Curves



Uncertainties in Financial Analysis

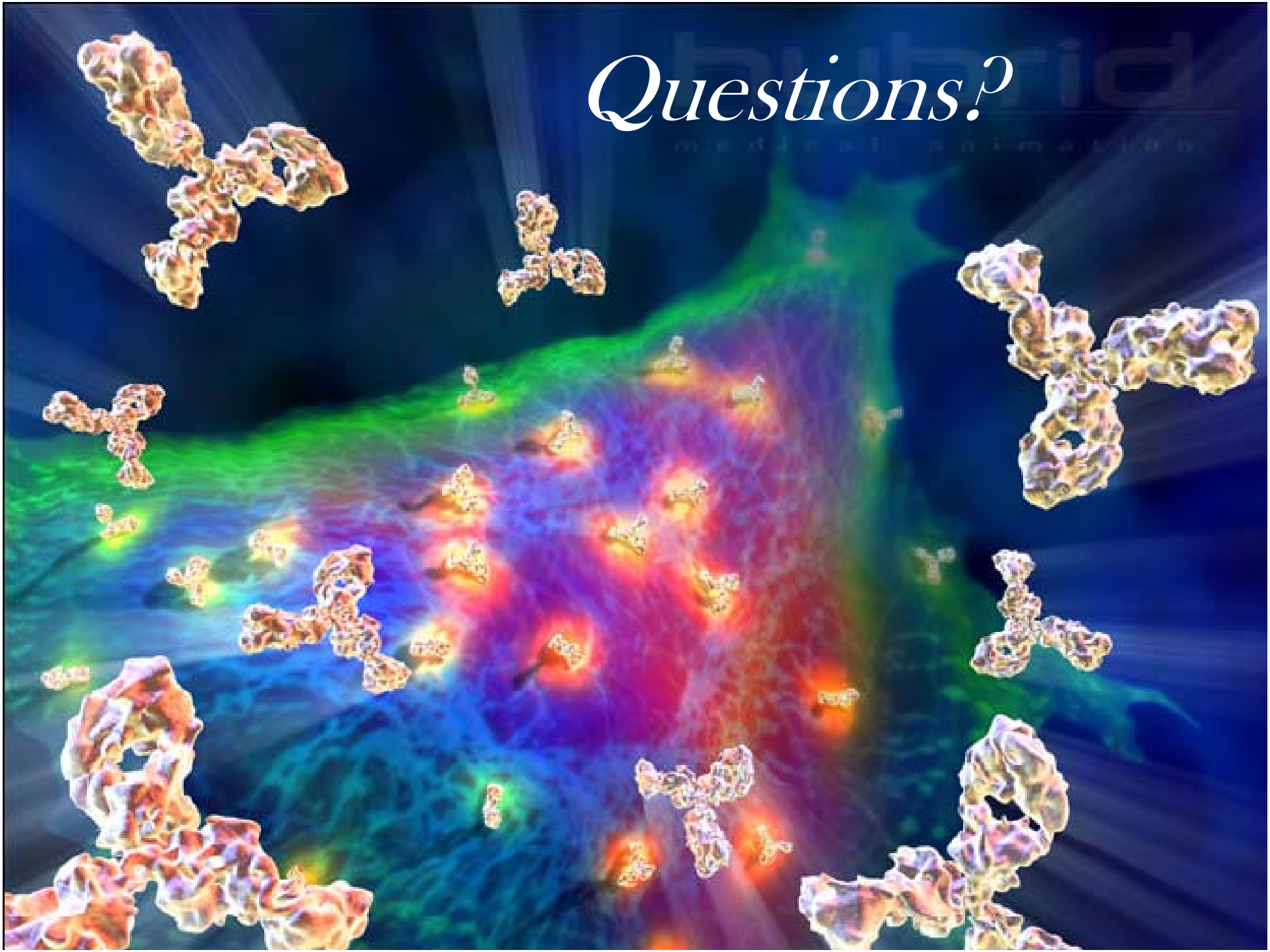
- Number of Sales per Year
- Facility Costs
- Pathway Probabilities

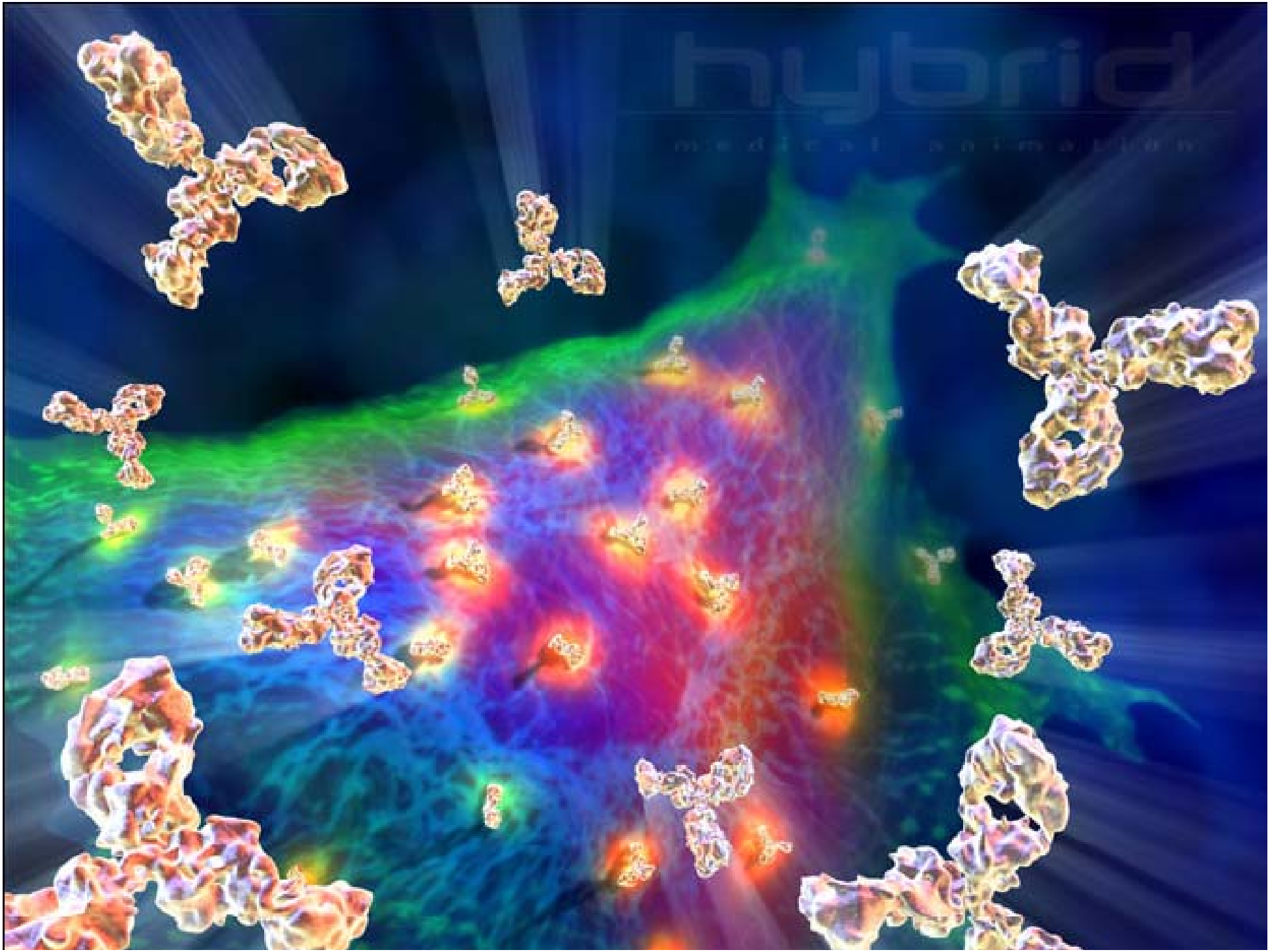
NPV Distributions of Successful Pathways



Conclusions

- Financial Analysis indicates that the profitability for this project is high
- Potential losses are minimal when compared to the potential gains
- CCKK recommends that this project be commercialized as soon as possible


Questions?



Synthesis of pNP-PEG-PE

- Add PE to a 10x excess of PEG-$(pNP)_2$ in chloroform in the presence of triethylamine
- Remove organic solvents
- Separation from free PEG and pNP on using a CL-4B column
- Freeze dry the pNP-PEG-PE
- Extract using chloroform (storage should be at -80ºC)

Torchilin, Vladimir, et al. *TAT peptide on the surface of liposomes affords their efficient intracellular delivery even at low temperature and in the presence of metabolic inhibitors.* National Academy of Sciences. 2001

Loading the Micelle with Toxin

- Prepare lipid film by putting the mixed solution of PEG-PE/pNP-PEG-PE under vacuum
- Add drug dissolved in methanol to chloroform solution of the pNP-PEG-PE/PEG-PE solution
- Rehydrate at 50ºC in a 5mM sodium citrate buffered saline at pH 5.0 and vortex for 5 minutes to reform micelles

Torchilin, Vladimir P., et al. “Immunomicelles: Targeted pharmaceutical carriers for poorly soluble drugs.” Proceeding of the National Academy of Sciences. May 13, 2003.

Attaching MAb to Micelle

- Procedure:
  - Add 1mg protein per 10mg of pNP-PEG-PE
  - Increase pH to 8.5
  - Incubate 2 hours to attach MAb and hydrolyze pNP
  - Purification - gel filtration chromatography
- Yield: 60%

Torchilin, Vladimir P., et al. “Immunomicelles: Targeted pharmaceutical carriers for poorly soluble drugs.” Proceeding of the National Academy of Sciences. May 13, 2003.