CCKK Drug Delivery Technologies
CCKK Drug Delivery Technologies:
Monoclonal Antibodies for Delivery of Chemotherapy Drugs for Cancer Therapy

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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

http://www.clevelandclinicmeded.com/diseasemanagement/neurology/braintumor/braintumors.htm

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

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

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

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

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)


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

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.

Vector aids in transport across the BBB by transcytosis.

Transcytosis – transport of substance across epithelium by uptake into and release from coated vesicles.

OX26 is a MAb

OX26 undergoes receptor-mediated transcytosis

- Targets transferrin
- Transferrin receptor is highly expressed on brain capillary endothelial cells

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

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

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

Investigation of Immunomicelles

- Torchilin et al. used immunomicelles loaded with Taxol® to treat Lewis lung carcinoma

- Drug delivery by cell engulfing the micelle

- Micelle made of polyethylene glycol–phosphatidylethanolamine conjugates

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

Micelle-Antibody Attachment

$\text{Micelle} - \text{CH}_2\text{CH}_2 - \text{O} - \text{CO} - \text{O} - \text{NH}_2 - \text{Ligand}$

aqueous buffer, pH 8-9.5

$\text{Micelle} - \text{CH}_2\text{CH}_2 - \text{O} - \text{NH} - \text{Ligand}$

Micelle-Antibody attachment reaction
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.

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

\[
\frac{dC_{\text{blood}}}{dt} = -kC_{\text{blood}}
\]

- Separating variables and integrating yields

\[
\ln\left(\frac{C_{\text{blood}}}{C_0}\right) = -kt
\]
The half life of Temodar® is 1.8 hours

Half life can be used to find a k value of 0.38hr\(^{-1}\)

The final model is described by the equation

\[
\frac{C_{\text{blood}}}{C_0} = e^{-0.38 \cdot t}
\]

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?

![Graph showing comparison between oral dosage and Micelle half-life models.](image-url)
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

\[
\text{Drug concentration} = \frac{\text{dosage}}{(\text{flowrate} \times \text{time})}
\]

*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 microvessel.
- Amount of blood in capillaries can be used to determine amount of drug per capillary.

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

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/mm³ brain/tumor tissue

\[
\frac{\text{capillaries}}{\text{volume tumor tissue}} \times \frac{\text{drug delivered}}{\text{capillary}} = \frac{\text{drug delivered}}{\text{volume tumor tissue}}
\]

Injection Delivery Standard Curve

Drug Delivered upon Initial Injection
(350 mg dosage)
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

- Compartment 1: Brain
- Compartment 2: Rest of Body
Brain Concentration Model

www.4um.com/tutorial/science/pharmak.html
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
- \( \alpha \) and \( \beta \) are the hybrid rate constants (units T\(^{-1}\))

Brain Concentration Model Derivation

- $\alpha$ and $\beta$ 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

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

Alpha Equilibration Model

Graph showing concentration over time (min) for the Alpha Phase in the brain and body.
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.
From model parameters, k-values can be determined.
Brain Concentration Model

The graph illustrates the concentration of a substance in the brain over time. It shows two distinct phases:

1. **Redistribution Phase**: This phase occurs immediately after exposure, where the concentration drops sharply.
2. **Elimination Phase**: This phase follows the redistribution phase and shows a gradual decline in concentration over time.

The x-axis represents time in hours (hr), while the y-axis represents brain concentration in mg/ml.
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
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

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<tr>
<th>Grant</th>
<th>Average Amount per fiscal year</th>
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<tr>
<td>Small Business Technology Transfer (Phase I)</td>
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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

Micelle Begins Approval Process

Phase I Trials (1½ Years)

Safe

Unsafe

Phase II Trials (3 Years)

More effective with lower side effects than current treatments.

More effective with lower side effects than current treatments.

Same effectiveness with lower side effects than current treatments.

Less effective with more side effects than current treatments.

Project Fails

Phase III Trials (4 Years)

Long term severe side effects, with less effectiveness.

Long term mild side effects, with same effectiveness.

Long term mild side effects, with more effectiveness.

Project Fails

Side Effects Unacceptable

Side Effects Acceptable

Project Fails

Approved
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.
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.

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

http://www.usnews.com/usnews/health/hosptl/rankings/specihqcanc.htm
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

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

\[ p_1d_1 = p_2d_2 \]

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

\[ p_1d_1 = \alpha p_2d_2 \]

Knowledge multiplier \( \alpha \) 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 expenditures 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

- Minimum Marketing: $500,000
- $500,000: Red
- $1,000,000: Green
- $1,825,000: Blue
- $2,250,000: Purple
- Maximum Demand: Dotted Line
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 \( \beta \)
  - Decreased treatment side effects decreases \( \beta \)
- 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

- Minimal R&D Investment
- Increased Pre-FDA Spending
- Increased FDA Spending
- Increased Pre-FDA & FDA Spending
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 -80°C)

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 50°C in a 5mM sodium citrate buffered saline at pH 5.0 and vortex for 5 minutes to reform micelles.

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%