Immunoliposomes for Targeted Radionuclide Therapy

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Outline

• Liposomes
  - Overview of structure and function
  - Enhanced Permeability and Retention (EPR) effect
  - PEG, size, charge
  - Doxil, Myocet
• Immunoliposomes
  - Composition and structure – different types
  - Mode of targeting/contrast w/ untargeted liposomes
• Immunoliposomes in targeted alpha-emitter therapy

Liposome - structure

Liposome – tumor localization

Enhanced Permeability and Retention (EPR)
Liposomes – Composition

- **Phospholipids chosen**
  - Surface Charge - interaction with cells, cluster penetration
  - Membrane fluidity - rigidity
- **Size**
  - Diameter > 250 μm, localize to spleen
- **Long polymeric chains on surface (PEGylated)**
  - Evade RES system, increase circulation half-time
  - Typically 5% mole of 2000 MW PEG
  - Too much (>10%) – micelles, too little - phagocytosed

Biodistribution of Liposomes (PEG vs nonPEG) vs In-111

At 650 nm, a very rapid sequestration to spleen is shown resulting in spleen/liver & spleen/lung of ~30% as early as 1 hr PI and lasting to at least 6 hr PI.

Tumor penetration of small unilamellar liposomes – dependence on composition.

Confocal microscopy equatorial slice (~100 μm) images of tumor spheroids following 2-hr incubation with SUVs containing (a) DMPC:DC-chol, (b) DMPC:DOPE:DC-chol and (c) DMPC:DOPE:DOTAP.

Kostarelos, et al., Int J Cancer, 2004
**Doxorubicin**

Cardiac Toxicity 4 to 20 Years After Completing Anthracycline Therapy

Doxil®

- **Size = 100 nm**
- **Type = large unilamellar vesicles**
- Hydrogenated soy phosphatidyl choline (PC), cholesterol, PEG-2000, distearoyl phosphatidyl ethanolamine
- **Drug/lipid weight = 0.125**
- **Drug loading: ammonium ion gradient**
- Sulfate salt precipitate – “striated gel”

**Immunoliposome - types**

- **Type A**
- **Type B**
- **Type C**

- Liposome
- Antibody or antibody fragment
- PEG chain


**Immunoliposomal - targeting**

- **No difference in overall tumor concentration**
- **Gross localization to tumor based on EPR effect**
- **Significant difference in drug delivery**
  - Increased interaction with target cell population
  - Increased drug delivery to target cell population
Immunoliposome - targeting

Lipid (tritiated cholesterol)
- Lipid (tritiated cholesterol)
- fluorescence

A

B

C

D

In vivo uptake of liposomes and immunoliposomes in the U87/EGFRvIII tumor xenograft model

Ab targeting – Metastatic dissemination

Therapeutic efficacy of anti-EGFR immunoliposome-doxorubicin in EGFR-overexpressing tumor models

MDA-MB-468 (5 x 10^5 sites/cell)

U87 (1-2 x 10^5 sites/cell)
Alphas vs Betas

**alphas**
- He nucleus (4amu)
- 80 keV/µm
- 2 to 3 tracks kill cell
- Irreparable DNA damage
- Potent single cell, cluster kill

**betas (electrons)**
- 0.2 keV/µm
- $10^2$ to $10^4$ tracks to kill cell
- DNA damage is repaired
- Cross-fire required

Animal Model: LCV injection

**Left**: Image of excised liver, 4-wks after LCV injection. **Right**: Section of the liver containing a metastatic tumor mass. At the periphery of the mass is a thick rim of viable tumor cells that infiltrate and compress adjacent hepatic parenchyma. At the center, a large area of tumor necrosis with remnants of congested vascular and lesser amounts of hemorrhage and inflammatory cells.

**Note**: Survival for 3.5 to 4.0 weeks, 90% successful inoculation.
**Alpha-Immunoliposome construction**

- DMPE + Cholesterol + DSPE-PEG → Liposomes (~100nm)
- Liposomes + DTPA, pH 5.9 → Liposomes-DTPA
- DSPE-PEG-maleimide + thio-Ab → DSPE-PEG-Ab
- Liposomes-DTPA + DSPE-PEG-Ab → Immunoliposomes-DTPA
- Immunoliposomes-DTPA + Bi-213 → Immunoliposomes-Bi-213

**1¹¹In-Ab targeting of metastases**

- 1h PI
- 6h PI
- 24h PI
- 48h PI

**α-Radioimmunotherapy**

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<th>α particles</th>
<th>β particles</th>
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<td>Dimension / range (µm)</td>
<td>35-100</td>
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<td>LET in tissue (keV/µm)</td>
<td>80-150</td>
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<td>No of DNA hits required to inactivate a cell</td>
<td>1-5</td>
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Therapy
- Short penetration and short half-life
- Wide penetration and extended damage

**Specific activity of antibody**

1 Bi-213 / ~1900 antibody

**Liposome Immunoreactive fraction**

- 1/cell
- Total/specific activity

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<th>Liposome</th>
<th>Immunoliposome</th>
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| >90% stability after 4h in 10% serum | }
**In-vitro Cell Kill Assay**

![Graph showing cell kill assay results](image)

- D0 values:
  - Liposome, 14 µCi/ml
  - Immunoliposome, 8 µCi/ml
  - Antibody, 3.3 µCi/ml

**Metastatic BCa Targeted alpha-radiotherapy**

![Graph showing metabolic activity](image)

- Median Survival Time:
  - Control: 29.5 days (n=4)
  - Cold Liposome: 28 days (n=5)
  - Liposome-Bi-213: 39 days (P<0.002) (n=5)
  - Immunoliposome-Bi-213(7.16.4): 47.5 days (P<0.008) (n=4)
  - Antibody: 41 days

**Acknowledgments**

- Hong Song
- Andy Pribaux
- Yael Har-El
- Mohana Lingappa
- Rizik Hebbes
- Caroline Escasas
- Reihaneh Shafieverdi
- Kostas Kostarelos
- Stevenita Safou
- Todd Reilly
- Elizabeth Jaffe
- David Huso
- Kathy Gabrielson

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*NIH:*
- IRB Study
- Diane Milenic

*ITU:*
- Institute for Transuranium Elements (ITU)
- Karlsruhe, Germany
- Carlos Apostolides
- Alfred Morgenstern

*NH:
- Joel D. Hero
- Beth Allen

*DO:
- Erik Brady
- Mark Brechbiel
- Diane Milenic

*Institute for Transuranium Elements (ITU),* Karlsruhe, Germany.
- Carlos Apostolides
- Alfred Morgenstern

*Acknowledgments*