Overview

• **Introduction: Molecular Transporters**

• Intracellular Cargo Delivery by Cell Penetrating Peptides Adapted to Target Cancer Cells through Cell Surface Protease Activation

• **Releasable Luciferin-Transporter Conjugates: Real-time Analysis of Uptake and Bioactivatable Release in Cells and Transgenic Reporter Mice**

• **Applications of this technology for delivery of real therapeutics**
Breaching Biological Barriers

Physical Property Control

Log P Box

POLAR DRUGS
siRNA

MOST DRUGS

NON-POLAR DRUGS
TAXOL

Log P
Breaching Biological Barriers

Facilitated Uptake

DRUG PROBE LEAD

Molecular Transporters

TRANSPORTER

CELL

Log P

POLAR DRUGS

NON-POLAR DRUGS

Log P Box

MOST DRUGS
Transporter Enabled Drug Uptake in Human Skin

UPTAKE OF BIOTINYLATED CYCLOSPORIN (A) AND BIOTINYLATED CYCLOSPORIN TRANSPORTER CONJUGATE (C) IN HUMAN SKIN GRAFTED ON IMMUNE-DEFICIENT MICE. THE SKIN WAS EXCISED, FROZEN, SECTIONED AND STAINED WITH FLUORESCEIN STREPTAVIDIN. PANELS B AND D ARE CONTROLS USING PROPIDIIUM IODIDE TO VISUALIZE ALL CELLS.

HUMAN TRIALS ESTABLISHED SAFETY AND THERAPEUTIC LEVELS OF CONJUGATE: T-L-CsA -> T-L + CsA
Real Time Quantification of Tissue Penetration and Release in Transgenic Reporter Mice

**Luc-RL-Trans** (Luciferin-Releasable Linker-Transporter)

Animals can be re-used!
Design Strategy for Real Time Dermal Uptake

GSH (mM)

GSH (mM)

GSH (mM)

GSH (mM)

GSH (mM)

GSH (mM)

GSH (mM)

GSH (mM)

GSH (μM)

GSH 10³ > in cell

Conjugate

hv

LUCIFERIN-RELEASABLE LINKER-TRANSPORTER

area of application

STRATUM CORNEUM

EPIDERMIS

DERMIS

t₁/₂ (37 °C, pH=7.4) = 1-38 hr

t₁/₂ (reduct.) = 0.5 - 3.5 min
Overcoming multidrug resistance of small-molecule therapeutics through conjugation with releasable octaarginine transporters
What is chemotherapy?

http://www.youtube.com/watch?v=vKIRWY-LMYc&feature=player_detailpage
Taxol is the best anticancer agent ever been isolated from plants. To date, Taxol is the best-selling cancer drug ever manufactured.

It is used for the treatment of ovarian, breast cancers, non-small-cell lung cancer, small-cell lung cancer, squamous cancers of the head and neck, and various other cancers.

However, to dissolve Taxol the drug formulation contains Cremophor (polyoxyethylate castor oil). Chremophor@ causes severe hypersensitivity reactions, such as an extreme allergic reaction called anaphylaxis. Therefore, Taxol has to be administered using pre-medications and by long infusions to patients with cancer.
Cell Cycle

M phase - mitosis, chromosomes drawn apart by molecular motors, cell divides. Many cancer drugs like taxol act here freezing the process and causing apoptosis.

G1 is entered when the cell senses growth signals or mitogens. These start the process of cell division which is linked to cell size. Proteins and mRNAs are synthesised in G1.

G2/M - cell arranges and checks chromosomes. There is a major checkpoint here to ascertain that DNA replication and chromosome segregation has successfully occurred. If not, a normal cell enters apoptosis.

Cell crosses restriction point c 8-10 hours into G1 - point of no return. Cell is committed to divide or die.

S phase - DNA is synthesised. Many cytotoxic drugs in cancer act here to destroy DNA.

G1/S checkpoint - cell arrest for cancer cells here leads to apoptosis.

http://www.youtube.com/watch?v=cvlpmmvB_m4&feature=player_detailpage
Ovarian cancer is a serious and under-recognized threat to women's health

• Ovarian cancer kills more women than all the Gynecologic Oncology combined.

• Ovarian cancer is the fourth leading cause of cancer death among women in Europe and USA.

• Ovarian cancer occurs in 1 in 57 women, up from 1 in 70 several years ago.

• More than 16,000 women will die this year alone and more than 25,500 will be diagnosed.
Treatment of Ovarian Cancer

- The initial treatment of ovarian cancer: drugs that contain platinum and taxane compounds (e.g., cisplatin, carboplatin, taxol)

- There are four ways to administer chemotherapy. The most common method is intravenous (through a vein) injection.

- Feb 17, 2006, FDA has approved treatment of ovarian cancer with the chemotherapy drugs given by intraperitoneal injection.

- The intraperitoneal method for delivery has been shown to increase survival.

  In this treatment, high doses of chemotherapy drugs are infused directly into the abdominal cavity to destroy cancer cells. These drugs eventually enter the bloodstream and may destroy any cancer cells that have spread.
Taxol: ideal drug?

**Problem:** Taxol is not soluble in water

Current solutions:
- Has to be given in Cremophor® (toxic, severe allergic and hypersensitivity reaction)
- Taxol has to be administered using pre-medications and by long infusions to patients with cancer.
Taxol SAR Summary

- Acetyl or acetoxy group may be removed without significant loss of activity. Some acyl analogs have MDR-reversing activity.
- Reduction improves activity slightly.
- May be esterified, epimerized or removed without significant loss of activity.
- Oxetane ring or close analog required for activity.
- Removal of acetate reduces activity; some acyl analogs have improved activity.
- Acyloxy group essential; certain substituted benzoyl groups and other acyl groups have improved activity.
- N-Acyl group required.
- Phenyl group or a close analog required.
- Free 2'-hydroxyl group, or a hydrolysable ester thereof required.
- Removal of 1-OH group reduces activity slightly.
Water Soluble Prodrugs of Taxol: an overview of *in vivo* activity
Prodrug is a compound that the body converts into active drug

Water Soluble Taxol Derivatives*

Prodrugs

Tested in vitro (cells)?

- yes
- no

- yes, Tested in vivo (animals)?
  - yes
  - no

- no, today
Why Water Soluble Taxol?

Taxol is the best anticancer agent ever isolated from plants. To date, Taxol is the best-selling cancer drug ever manufactured.

It is used for the treatment of ovarian, breast cancers, non-small-cell lung cancer, small-cell lung cancer, squamous cancers of the head and neck, and various other cancers.

However, to dissolve Taxol the drug formulation contains Cremophor (polyoxyethyleate castor oil). Chremophor™ causes severe hypersensitivity reactions, such as an extreme allergic reaction called anaphylaxis. Therefore, Taxol has to be administered using pre-medications and by long infusions to patients with cancer.

The Goal is to come up with a water soluble formulation of Taxol which would be therapeutically as efficient as Taxol itself given with Cremophor™.
Taxol SAR Summary

- Acetyl or acetoxy group may be removed without significant loss of activity. Some acyl analogs have MDR-reversing activity.
- Reduction improves activity slightly.
- May be esterified, epimerized or removed without significant loss of activity.
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## Water soluble phosphate prodrugs of Taxol

![Chemical structures of prodrugs](image)

### Table: % T/C and (mg/kg/inj)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Vehicle</th>
<th>Compound</th>
<th>% T/C&lt;sup&gt;b&lt;/sup&gt;</th>
<th>(mg/kg/inj)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>water</td>
<td></td>
<td>140% (25)</td>
<td>240 (25)</td>
</tr>
<tr>
<td>4</td>
<td>water</td>
<td></td>
<td>123% (30)</td>
<td>240 (25)</td>
</tr>
<tr>
<td>9</td>
<td>water</td>
<td></td>
<td>103% (30)</td>
<td>240 (25)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Murine lung carcinoma, i.p. (intraperitoneal) implant model

<sup>b</sup> T/C is mean survival time of treated group/ mean survival time of the control

<sup>c</sup> Dose administered i.p. on days 1, 5, and 9

<sup>d</sup> Administered in 10% Tween 80 in saline

Water soluble phosphate prodrugs of Taxol

- No taxol observed (HPLC) after incubation in dog or rat plasma after 24 hr at 37 °C
- Converted to free taxol after incubation with 10% alkaline phosphatase at 37 °C for 30 min

### Water soluble phosphate prodrugs of Taxol

![Chemical structure of water soluble phosphate prodrugs of Taxol](#)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Vehicle</th>
<th>% T/C&lt;sup&gt;b&lt;/sup&gt;</th>
<th>(mg/kg/inj)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>water</td>
<td>144% (100)</td>
<td>275 (30)</td>
</tr>
<tr>
<td>3b</td>
<td>water</td>
<td>156% (140)</td>
<td>275 (30)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Murine lung carcinoma, i.p. (intraperitoneal) implant model
<sup>b</sup> T/C is mean survival time of treated group/ mean survival time of the control
<sup>c</sup> Dose administered i.p. on days 1, 5, and 9
<sup>d</sup> Administered in 10% Tween 80 in saline

### Water Soluble C2’ Malic Acid Ester Prodrug of Taxol

![Chemical structure of the prodrug](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Vehicle</th>
<th>% T/C</th>
<th>Taxol (mg/kg/inj)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>water</td>
<td>105% (12.5)</td>
<td>204 (12.5)</td>
</tr>
<tr>
<td>4</td>
<td>water</td>
<td>122% (50)</td>
<td>204 (12.5)</td>
</tr>
</tbody>
</table>

**a** Murine Leukemia P388, i.p. (intraperitoneal) implant model  
**b** T/C is mean survival time of treated group/mean survival time of the control  
**c** Dose administered i.p. on days 1-4  
**d** Administered in ethanol:cremophore EL:water

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### Water Soluble C7 Dihydroxypropyl Prodrug of Taxol

![Molecular structure of the prodrug](image)

<table>
<thead>
<tr>
<th>Tumor Models</th>
<th>% T/C&lt;sup&gt;b&lt;/sup&gt;</th>
<th>(mg/kg/inj)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human prostate carcinoma PC-3</td>
<td>164% (40)</td>
<td>122 (16)</td>
</tr>
<tr>
<td>Human ovarian carcinoma OVCAR-3</td>
<td>138% (40)</td>
<td>120 (16)</td>
</tr>
<tr>
<td>Human mammary carcinoma MDA MB 469</td>
<td>128% (40)</td>
<td>111 (16)</td>
</tr>
<tr>
<td>Human colon carcinoma MT-39</td>
<td>147% (40)</td>
<td>130 (16)</td>
</tr>
</tbody>
</table>

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<sup>a</sup> Prodrug was administered in PBS  
<sup>b</sup> T/C is mean survival time of treated group/ mean survival time of the control  
<sup>c</sup> Dose administered i.p. on days 1,3,5 and 7  
<sup>d</sup> Administered in DMSO/ethanol/cremophore EL/PBS (8:6:6:80)

---

### N,N-dimethylglycyl and (N,N-diethylamino)propionyl Prodrugs of Taxol

**Formula:***

\[
\text{R} = \begin{cases} 
\text{NH} & \text{(4a)} \\
\text{O} & \text{(5b)} \\
\text{H} & \text{(6a, 9a)} 
\end{cases}
\]

---

<table>
<thead>
<tr>
<th>Compound</th>
<th>Vehicle</th>
<th>% T/C</th>
<th>(mg/kg/inj)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>water</td>
<td>178% (5.9)</td>
<td>CR (4.45)</td>
</tr>
<tr>
<td>5b</td>
<td>water</td>
<td>CR (4.5)</td>
<td>CR (4.45)</td>
</tr>
<tr>
<td>6a</td>
<td>water</td>
<td>170% (5.25)</td>
<td>CR (4.45)</td>
</tr>
<tr>
<td>9a</td>
<td>water</td>
<td>123% (8.25)</td>
<td>CR (4.45)</td>
</tr>
</tbody>
</table>

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**Notes:**

- **a.** Human Subrenal Mammary Carcinoma sc. (subcutaneous) implant model
- **b.** T/C is mean survival time of treated group/mean survival time of the control
- **c.** Dose administered i.p. on days 1,3, and 5
- **d.** Administered in 10% Tween 80 in saline

Taxol-Folic Acid Conjugate as Targeted Antineoplastics

<table>
<thead>
<tr>
<th>Compound</th>
<th>Vehicle</th>
<th>% T/C&lt;sup&gt;b&lt;/sup&gt;</th>
<th>(mg/kg/inj)</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>water</td>
<td>126% (32)</td>
<td>307 (17.2)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Murine Madison lung carcinoma M109, i.p. (intraperitoneal) implant mc
<sup>b</sup> T/C is mean survival time of treated group/ mean survival time of the control
<sup>c</sup> Dose administered i.p. on days 4,6,8,10, and 12 post implantation
<sup>d</sup> Administered in ethanol:cremophore EL:PBS

How do polymeric prodrugs work?

- Unlike the blood vessels in healthy tissues, those in tumor tissue have openings that make them porous.

- Polymeric prodrugs can flow through healthy blood vessels, and because the chemotherapy is stable when bound to polymer, lower levels of the drug are seen in the bloodstream. In tumor blood vessels polymeric drug leaks through the pores and is trapped in tumor tissue.

- The polymer is made of bio-digestible material and is metabolized inside the tumor, releasing the drug.

- Polymeric drugs are taken up by cells via endocytosis, that allows them to bypass MDR pump

Nanoparticle cancer treatment: http://www.youtube.com/watch?v=RBjWwInq3cA&feature=player_detailpage
# Water Soluble 2’ Poly(ethylene glycol) Ester Prodrugs of Taxol

<table>
<thead>
<tr>
<th>Compound</th>
<th>Vehicle</th>
<th>% T/C&lt;sup&gt;b&lt;/sup&gt;</th>
<th>(µmol/inj)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>water</td>
<td>113% (0.35)</td>
<td>150 (0.35)</td>
</tr>
<tr>
<td>9</td>
<td>water</td>
<td>126% (0.35)</td>
<td>150 (0.35)</td>
</tr>
</tbody>
</table>

*Murine Leukemia P388, i.p. (intraperitoneal) implant model

*T/C is mean survival time of treated group/mean survival time of the control

*Dose administered i.p. on days 1-5

*Administered in ethanol:cremophore EL:water

## Water Soluble Polyglutamatic Prodrug of Taxol

### Chemical Structure

![Chemical Structure of PG-TAX](image)

MW (Poly-L-Glutamic Acid) = 36,000

### Tumor Models

<table>
<thead>
<tr>
<th>Tumor Models</th>
<th>% T/Cb</th>
<th>(mg/kg/inj)</th>
<th>PG-TAXa</th>
<th>Taxold</th>
<th>PGa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murine hepatocarcinoma HCa-1</td>
<td>125%</td>
<td>113 (80)</td>
<td>125% (800)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Murine mammary tumor MCa-4</td>
<td>200%</td>
<td>183 (60)</td>
<td>142% (600)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Murine mammary tumor MCa-35</td>
<td>127%</td>
<td>180 (80)</td>
<td>145% (800)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Murine fibrosarcoma tumor FSa-II</td>
<td>183%</td>
<td>150 (80)</td>
<td>150% (800)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Notes

- **a** Prodrug was administered in saline
- **b** T/C is mean survival time of treated group/ mean survival time of the control
- **c** Dose administered i.v. (intravenous) on day when tumor reached 500 mm³
- **d** Administered in DMSO/ethanol/cremophore EL/PBS (8:6:6:80)

All implant models were generated intramuscular in the tights of legs

Clinical Trials

XYOTAX™ is in clinical trials that are open and enrolling patients.

- Colorectal cancer
- Esophageal and gastric cancer
- Lung cancer
- Non-Hodgkin's Lymphoma
- Ovarian cancer

Xyotax:
http://www.youtube.com/watch?v=X4YxFR4XUtw&feature=player_detailpage
Phase 2 clinical trials: TOCOSOL Paclitaxel has shown promising anti-tumor activity in breast, non-small cell lung, bladder and ovarian cancers.

Tocosol® Paclitaxel is currently being evaluated in phase III clinical trials for the treatment of women with metastatic breast cancer.
Conclusions

- A large variety of water soluble prodrugs of Taxol have been synthesized.
- Only a few of them have a therapeutic index comparable to Taxol itself.
- No “small” water soluble prodrug molecule is currently on the market.