Suppression of Metastatic Disease with Integrin $\alpha\nu\beta3$
Targeted Nanoparticle Drug Delivery

David A. Cheresh
Normal vessels

Tumor vessels
\(\alpha\nu\beta_3\) is Preferentially Expressed on Tumor Neovasculature

---

Adjacent normal tissue

Breast cancer tissue

NP accumulation at tumor site

αvβ3-negative

αvβ3-positive

Tumor Mass
Liposome Formulation:
Cholesterol : DOPE : DSPC: mPEG2000-DSPE : **R-DSPCE** : BODIPY-DOPE
29.5 : 29.5 : 29.5 : 5 : 5 : 1.5 mol%

<table>
<thead>
<tr>
<th>Day</th>
<th>Hydrodynamic Diameter (nm)</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>104.8</td>
<td>-1.52</td>
</tr>
<tr>
<td>4</td>
<td>107.8</td>
<td>2.22</td>
</tr>
<tr>
<td>10</td>
<td>109.6</td>
<td>3.97</td>
</tr>
</tbody>
</table>
Competitive Binding to Endothelial Cells (HUVEC) with Nanoparticle

Targeted Liposome

\[ \text{20-fold molar excess of peptide} \]

\[ \text{Negative control} \]

**10 uM (cRGDfK)-DSPE in Liposome**
Dorsal Skin Fold Chamber for Studying Tumor Vascularization
Targeting the Tumor Neovascularature
(Intravital Imaging at 5h)

NP (Non-targeted)

NP (ανβ3-targeted)
What is effect of targeting Doxorubicin to the tumor vasculature?

- Doxorubicin is a genotoxic drug that inhibits tumor growth at 10-20 mg/kg when given systemically.
- At this dose Doxorubicin also produces severe cardiotoxicity.
- Can we deliver a metronomic dose of this drug to the tumor that spares normal tissue?
- To address this question we implanted pancreatic tumor cells into the mouse pancreas and monitored the growth of both the primary tumor and spontaneous metastases after systemic injection of $\alpha_\nu\beta_3$-NP-Dox.
cRGD-Dox blocks bFGF-induced angiogenesis by 58%.

Male C57BL6 mice
N=4 each group
8wks old, BW=19-26g
Treated every 2 days with particle
FITC-lectin content of plugs on Day 7
Raw OD +/- SEM
Respective PBS group subtracted

<table>
<thead>
<tr>
<th>Treatment</th>
<th>bFGF-induced Angiogenesis (Raw OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>1000 ± 200</td>
</tr>
<tr>
<td>cRAD-Dox</td>
<td>750 ± 100 (-24%)</td>
</tr>
<tr>
<td>cRGD-Dox</td>
<td>440 ± 50 (-58%)</td>
</tr>
</tbody>
</table>

p value
Vehicle vs. cRGD 0.004
cRAD vs cRGD 0.076
cRGD-targeted doxorubicin creates vessel fragments and reduces angiogenic response after 7 days

- Networks of vessels (through 50μm z-series)
- Significant branching

- Fragments of vessels (through 50μm z-series)
- Little branching
Empty particle and free peptide do not block bFGF-induced angiogenesis

P-values:

- cRGD: P=0.04, 69%
- Empty particle: 0.14, 33%
- Free peptide: 0.12, 30%

Male C57BL6 mice
10wks old
22.3g initial BW
7-day experiment
Mouse orthotopic pancreatic cancer and metastasis model

Tumor cells injected in the tail of the pancreas

Metastasis to the Hepatic Hilar Lymph Node

Image From: http://pathology2.jhu.edu/pancreas/mets.cfm
Nanoparticle Distribution in the Orthotopic Pancreatic Cancer Model:

- Blood vessels
- cRGD

Liver, Heart, Brain, Kidney, Lung, Pancreas (adjacent), Pancreas (tumor)
Orthotopic Pancreatic Carcinoma Model: Primary Tumor Growth and Metastasis

Day 0

Inject Pancreatic Carcinoma Cells

Day 5

iv injection of RGD-Dox NP at 1mg/kg

Day 7

Day 9

Day 11

Harvest Tumors

IHC
Targeted Nanoparticle Delivery of Doxorubicin (1 mg/kg) Prevents Metastasis

Primary Tumor (pancreas)

- 43%

P=0.03

Metastasis (hilar lymph node)

- 90%

P=0.001
Targeted Nanoparticle Delivery of Doxorubicin Prevents Metastasis

**Primary Tumor**  
(pancreas)  

- **23%**

**Metastasis**  
(hepatic hilar lymph node)  

- **p = 0.02**
- **p = 0.01**

*Treatment on Day 5, 7, 9  
Euthanized on Day 11  
n=6 for PBS, n=7 remaining groups*
Dose-Response Curve of Free Doxorubin in the Orthotopic Pancreatic Carcinoma Model

**Primary**

- PBS vs 1 mg: $P = 0.629$ vs $P = 0.227$
- PBS vs 7.5 mg: $P = 0.012$ vs $P = 0.127$
- PBS vs 15 mg: $P = 0.00043$ vs $P = 0.014$

**Metastasis**

- PBS vs 1 mg
- PBS vs 7.5 mg
- PBS vs 15 mg

Treatment on Day 5, 7, 9

$n = 7$
RGD-Dox-NP Treatment Reduces both the Incidence of Metastasis and the Total Metastatic Burden in the RCC model

**Primary**

- **RAD**: Primary tumor weight (mg)
- **RGD**: Primary tumor weight (mg)

**Metastatic**

- **RAD**: Metastatic burden
- **RGD**: Metastatic burden

<table>
<thead>
<tr>
<th>Tissue</th>
<th>RGD-DOX-NP</th>
<th>RAD-DOX-NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>1/8</td>
<td>4/8</td>
</tr>
<tr>
<td>Spleen</td>
<td>3/8</td>
<td>5/8</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>1/8</td>
<td>4/8</td>
</tr>
<tr>
<td>Pancreas</td>
<td>5/8</td>
<td>6/8</td>
</tr>
<tr>
<td>Lungs</td>
<td>1/8</td>
<td>2/8</td>
</tr>
<tr>
<td>Gut</td>
<td>4/8</td>
<td>6/8</td>
</tr>
</tbody>
</table>

**Note:** Dosed day 8 to 42, 2 mg/kg liposomal doxo
Orthotopic Renal Cell Carcinoma – Metastasis to Various Organs

RGD RAD

Pancreas

Spleen

Diaphragm

Liver

Dosed day 8 to 42, 2 mg/kg liposomal doxo
Apoptosis only near $\beta_3$-positive blood vessels on tumor margin with cRGD-Dox
Absence of Apoptosis (or $\beta_3$ Expression) in cRGD-Dox
Apoptosis: Only near β3-positive blood vessels on tumor margin with cRGD-Dox-NP

Endothelial cells
beta3 integrin
(overlap)

Viable cells
Apoptotic cells
Summary

- The cRGD nanoparticles targeted the tumor neovasculature in vivo in both the dorsal skin-fold chamber and orthotopic pancreatic carcinoma model, and disrupted angiogenic vasculature in the mouse matrigel model.

- Systemic administration of targeted nanoparticles containing 1 mg/kg of doxorubicin prevents metastasis in orthotopic models of renal cell and pancreatic cancer.

- Apoptosis is induced by the targeted nanoparticle containing doxorubicin only in areas of the tumor with β3 positive vessels.

- The 1 mg/kg dose is 15-fold below the dose of free doxorubicin required to suppress metastasis.
Acknowledgements

Eric Murphy
Bharat Majeti
Leo Barnes
Milan Makale
Kimberly Lutu-Fuga
Sara Weis
Wolfgang Wrasidlo