Opening the Black Box

Black Box

Black Box
Dissecting Tumors using Intravital Microscopy

- Molecular imaging
- Cellular imaging
- Anatomical imaging
- Functional imaging
- Therapeutic response

Molecular probe or engineered cells

Genetically engineered mice & Tumor models

Computer assisted image analysis

Deconstructing Solid Tumors

- Blood vessel
- Lymphatic vessel
- Matrix
- Cancer cells
- Host cells
3D imaging of normal vasculature

3D imaging of tumor vasculature

Tumor blood vessels: abnormal structure and function
Mean interstitial profile of \( pO_2 \) and \( pH \) in tumors

Interstitial Fluid Pressure in Human Tumors

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal cell carcinoma</td>
<td></td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td></td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td></td>
</tr>
<tr>
<td>Metastatic melanoma</td>
<td></td>
</tr>
<tr>
<td>Colorectal liver mets</td>
<td></td>
</tr>
<tr>
<td>Head/neck carcinoma</td>
<td></td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td></td>
</tr>
<tr>
<td>Metastatic melanoma</td>
<td></td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td></td>
</tr>
<tr>
<td>Brain tumor</td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td></td>
</tr>
<tr>
<td>Normal skin</td>
<td></td>
</tr>
<tr>
<td>Normal breast</td>
<td></td>
</tr>
</tbody>
</table>

N=1

N=13

N=26

N=14

N=8

N=27

N=8

N=12

N=26

N=17

N=7

N=5

N=8
Bench to Bedside and Back
Regression of LS174T by anti-VEGF antibody

Before treatment  3 days  7 days

Yuan et al. PNAS (1996)
Normalization of tumor vasculature by hormone withdrawal

Jain et al. PNAS (1998)

Vessel Diameter (µm)

Permeability

Sham

Castration
Can Herceptin normalize tumor vessels?

Control

Herceptin

Treatment

in vitro
C H

in vivo
C H

VEGF

TSP-1

VEGF Blockade Normalizes Tumor Vasculature

Normalization Hypothesis

Tong et al. (Cancer Research 2004)

Normal arteriole

control

DC101

Decrease in interstitial fluid pressure (IFP) by VEGF blockade

DC101

Bevacizumab


Fluorescent BSA penetration in tumor tissue after VEGFR2 blockade

<table>
<thead>
<tr>
<th></th>
<th>Perfused lectin</th>
<th>Extravasated BSA</th>
<th>Vessel</th>
<th>ImageJ macro</th>
<th>Quantification</th>
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</thead>
<tbody>
<tr>
<td><strong>IgG control</strong></td>
<td><img src="image1" alt="IgG control Perfused lectin" /></td>
<td><img src="image2" alt="IgG control Extravasated BSA" /></td>
<td><img src="image3" alt="IgG control Vessel" /></td>
<td><img src="image4" alt="IgG control ImageJ macro" /></td>
<td><img src="image5" alt="IgG control Quantification" /></td>
</tr>
<tr>
<td><strong>DC101</strong></td>
<td><img src="image6" alt="DC101 Perfused lectin" /></td>
<td><img src="image7" alt="DC101 Extravasated BSA" /></td>
<td><img src="image8" alt="DC101 Vessel" /></td>
<td><img src="image9" alt="DC101 ImageJ macro" /></td>
<td><img src="image10" alt="DC101 Quantification" /></td>
</tr>
</tbody>
</table>
Human colon cancer casts

Jain,
Sci Am 1994
Protocol of Clinical Trial

Avastin:
Day: -3 1 3 12 15 29 32 43 93 103

Blood/urine
Tumor Biopsy
IFP
Imaging

50.4Gy EBRT / CTX

Surgery

Effect of BV plus chemoradiation in rectal cancer patients (BV: 5mg/kg)

Willett et al., Nature Medicine 2004
Endoscopic IFP Measurements


low dose | high dose

Human IFP data
Sagittal PET scans: Patient #1

Pre-Tx  Day 12  Pre-Surgery

Tumor response after first BV infusion

Immunohistochemistry in biopsy tissues

- Microvascular density
- Tumor cell apoptosis
- Tumor cell proliferation

### Pre-clinical and clinical data

**Effects of anti-angiogenic therapy**

<table>
<thead>
<tr>
<th></th>
<th><strong>Pre-clinical data</strong></th>
<th><strong>Clinical data</strong></th>
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</thead>
<tbody>
<tr>
<td>Blood volume</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Vascular density</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Permeability (high MW)</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>PS product (low MW)</td>
<td></td>
<td>- (no changes)</td>
</tr>
<tr>
<td>Interstitial fluid pressure</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Perivascular cell coverage</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Apoptosis/ Proliferation</td>
<td>↑ /?</td>
<td>↑ ↑</td>
</tr>
<tr>
<td>Plasma VEGF /PIGF</td>
<td>↑ ↑</td>
<td>↑ ↑</td>
</tr>
<tr>
<td>EPC/CEC</td>
<td>↓ ↓</td>
<td>↓ ↓</td>
</tr>
</tbody>
</table>

Winkler, Kozin et al. (Cancer Cell 2004)
Willett et al. (J. Clin. Oncol. 2005)
Willett et al. (Nat. Med. 2004)
Duda et al. (J. Clin. Oncol. 2006)
Tong et al. (Cancer Res. 2004)
Cranial Model – Orthotopic Tumor Model
The Vascular Normalization Time Window

Normalization Time Window

- Vascular density
- Diameter

- Vascular Morphology
- Pericyte Coverage
- Basement Membrane Thickness
- Ang1 MMPs

Day 0

Day 8

Winkler, Kozin et al., Cancer Cell 2004
DC101 Decreases Tumor Hypoxia During the Vascular Normalization Time Window

Winkler, Kozin et al., Cancer Cell 2004
Radiation Therapy Acts Synergistically with VEGFR2 Blockade During the Normalization Time Window

Winkler, Kozin et al., Cancer Cell 2004
## Study Design

<table>
<thead>
<tr>
<th>Day</th>
<th>-5 to -3</th>
<th>-1</th>
<th>8 hours</th>
<th>1-2</th>
<th>9-10</th>
<th>28</th>
<th>56</th>
<th>84</th>
<th>112</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI</td>
<td>●</td>
<td>●</td>
<td></td>
<td>●</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>●</td>
<td>●</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Urine *</td>
<td>●</td>
<td>●</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

AZD treatment, 45 mg QD

* Batchelor et al, Cancer Cell, 2007
Radiographic responses in the first 16 patients
Post-contrast T1-weighted MRI

Relative Vessel Size

Permeability ($K_{\text{trans}}$)
Re-emergence of White Matter Tracts

Day -1  Day +27
Functional consequences of normalization

Value relative to pretreatment (%)

Study Days

-1 1 28 56 112

Ve

ADC

FLAIR
Normalization time window in patients

- Vessel size
- Vascular permeability
- Vasogenic edema

Day 1 to Day 28
Post-contrast T1-weighted MRI

Relative Vessel Size

During Drug Holiday

After Drug Holiday

-1  1  28  55  111
## Blood biomarkers

<table>
<thead>
<tr>
<th></th>
<th>Effect of tumor progression</th>
<th>Effect of drug holidays</th>
<th>Effect on vessel size</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>NS</td>
<td>-43%</td>
<td>NS</td>
</tr>
<tr>
<td>PIGF</td>
<td>-32% [-49% - -9%]</td>
<td>-58%</td>
<td>NS</td>
</tr>
<tr>
<td>sVEGFR2</td>
<td>[0% - +26%]</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>bFGF</td>
<td>[+2% - +148%]</td>
<td>NS</td>
<td>0.138</td>
</tr>
<tr>
<td>SDF1α</td>
<td>[+2% - +22%]</td>
<td>NS</td>
<td>[0.008 - 0.268]</td>
</tr>
<tr>
<td>Viable CECs</td>
<td>+53% [+3% - +128%]</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CPCs</td>
<td>NS</td>
<td>+75% [+15% - +167%]</td>
<td>NS</td>
</tr>
</tbody>
</table>

Batchelor et al, Cancer Cell 2007
Potential mechanisms of action of anti-VEGF agents on tumor vasculature

Jain RK et al. (2006) Lessons from phase III clinical trials on anti-VEGF therapy for cancer
Collaborators

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