Targeting microtentacles on circulating breast tumor cells to reduce metastasis.

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The limits of clinical imaging shape our understanding of cancer recurrence

Cigarette smoke exposure can increase metastatic risk for **ALL** cancers.

**Dynamic CTC responses**

**Primary Tumor**

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**Circulating Tumor Cells (CTCs)**

- PET/CT
- MRI

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**Seeding**

Below clinical detection threshold

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**Dormancy**

- Foci > 10 million tumor cells

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**Metastatic Recurrence**

- PET/CT
- MRI

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**Eradicate these cells**

**Delay or Eliminate Recurrence**
“Dormant” cells produce dynamic membrane protrusions when detached

MCF10A mammary epithelial cells

Frame / 1 sec.
Cytoskeletal support of membrane protrusions


Inhibit actin polymerization

Destroys Filopodia and invadopodia

Phase Contrast

α-tubulin

Actin
Microtentacles promote tumor cell aggregation (Live confocal imaging)

**Microtentacles increase in invasive/metastatic breast tumor cell lines**

Confocal imaging of live tumor cell attachment to endothelial cells
Death from fragmentation

Death from apoptosis

Fates of circulating cells?

Inhibit microtentacles

[1] ADHESION
Microtentacles

[2] EXTRAVASATION
Actin-dependent

Primary tumor

Metastatic tumor
Current working model of microtentacle (McTN) structure

- MTOC
- Vimentin
- KINESINS
- Glu-tubulin microtubules
- Unidentified Carboxypeptidase (TCP)
- Tyr-tubulin microtubules
- Actin cortex

Microtubule expansion is counteracted by actin cortical contraction

EB1-GFP imaging

Actin Tense

ECM-attached cell

Actin Loose

Detached cell
Kinesin inhibitors reduce microtentacles

Jennifer Yoon et al., *Breast Cancer Research and Treatment* (2011)

Tetracaine (125µM)
Drugs targeting cell division can enhance microtentacles


Tumor Growth

Vehicle

Jas + Paclitaxel

CTC Metastasis?
Chemotherapy before surgery and CTC levels

(from Hekimian et al., 2012)

Neoadjuvant therapy

- CTCs decrease
- CTCs increase

 Cumulative relapse-free survival vs Years

P = 0.028
HR = 0.036
Surgical samples from breast cancer patients (membrane dynamics)
Surgical samples from breast cancer patients (membrane dynamics – 600x)
Drug responses can be measured quickly in patient-derived tumor cells
(30 minute Colchicine response)
Automated measurement of McTN characteristics

Confocal imaging of patient tumorgraft cells (HCI-001)

Automated McTN Analysis (MATLAB)

<table>
<thead>
<tr>
<th>#McTNs/cell</th>
<th>Avg. McTN length (µm)</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>11</td>
<td>8.8</td>
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<tr>
<td>4</td>
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</tbody>
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HCl-001 cell population
McTN frequency = 34%
Avg.# McTNs/cell = 9.0
Avg. McTN length = 9.1 µm
Department of Physiology

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Eric Balzer
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Lindsay Hessler

Susette Mueller (Georgetown)
Jing Yang (UCSD)
Josef Kas (Leipzig)
John Olson (Surgery)

UM-College Park
Wolfgang Losert
Chris Jewell
Ben Shapiro

Recruited from Harvard with CRF startup funds
Yielded 16 new grants to date
DoD Era of Hope Scholar – only 3 awards nationwide

$601,019 CRF funds → $11,138,912 new grant funding

1,853% return on CRF investment

Many new research and administrative jobs supported
(now and for years to come – through 2020 at least)

New collaborations and grant applications with:
College Park Engineering
Johns Hopkins
Local Maryland companies (SBIR)