“Destroy the seed of evil or it will grow up to your ruin.”
Aesop 6th Century BC

- “A revolution in Cancer Biology”
- “A disruptive idea overturning conventional diagnostic and therapeutic assumptions.”
- “Finally, the key to durable therapies!”
- “An artifact of the experimental system”
- “An old idea resurrected”
**Breakthrough**

**Stem Cell Treatment**

- Over 2000 patients treated
- No known negative side effects
- No age requirements
- Accepting patients from all over the world

**Contact US** To Get More Information

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**Actual Patient**

**Ricci - Spinal Cord Injury**

I was told by all my doctors that I would never be able to walk again. Three months after my Stem Cell Treatment, I was able for the first time after the accident feel my legs. Today I am walking.

*Ricci*
Cancer Stem Cells

- What are cancer stem cells?
- How do they relate to normal stem cells?
- Do they explain cancer treatment failure?
- When do cancer stem cells first emerge?
- How can cancer stem cells revolutionize breast cancer treatment?
- Why are cancer stem cells so controversial?
This behavior is identical to cancer metastasis!!!
CANCER STEM CELLS
Therapeutic implications of cancer stem cells.

Cancer stem cell

Tumorigenesis

Malignant tumour

Therapies that target cancer stem cells

Tumour growth

Therapies that kill non-tumorigenic cancer cells

Metastasis

Disseminated malignancies

Limited benign growth
How to make cancer stem cells

Differentiated cells

Differentiation

Growth

Xenograft generation and analysis

Spheroid cells produce tumors in mice
Spheres are tumorigenic
Prospective identification of tumorigenic breast cancer stem cells
• Mammary stem cells generate all the breast cell types
• Mammary stem cells are ER Negative and PR Negative
• They STILL require ER+PR hormones for proliferation and outgrowth

Feedback support of mammary stem cells by RANKL produced by PR POS Cells

Control of mammary stem cell function by steroid hormone signalling

Nature 2010
Cancer Stem Cells are sorted out using characteristic markers

ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome

Christophe Ginestier¹, Min Hee Hur², Emmanuelle Charafe-Jauffret³, Florence Monville³, Julie Dutcher¹, Marty Brown¹, Jocelyne Jacquemier³, Patrice Viens³, Celina Kleer¹, Suling Liu¹, Anne Schott¹, Dan Hayes¹, Daniel Birnbaum³, Max S. Wicha¹, and Gabriela Dontu¹.
The breast cancer stem cell marker ALDH functionally correlates with tumorigenesis and prognosis

ALDH1 Breast CSC marker elevation correlates with poor survival

Ginestier et al 2007

ALDEFLOUR POS / CD24 NEG/CD44 POS: only 20 breast cancer cells are needed to form xenograft tumors
Cancer Stem Cells are resistant to chemotherapy

- Erythroid cells
- Jurkat cells
- Glioma stem cells

Etoposide

Camptothecin

Cisplatin

Temozolomide

Parental

Spheroids

Untreated

Daunorubicin

Methotrexate

Cell death (%) vs. Hours
Why are cancer stem cells resistant to chemotherapy?

ABC transporters expel drugs

ALDH Aldehyde Dehydrogenase is known to confer resistance to chemotherapy by oxidizing aldehydes in drugs

BCL-2 /BAX/BAK prevents death

Altered cell cycle check points and DNA repair

Abdullah and Chow 2013
Cancer Stem Cell Controversies

• All previous drugs may have been designed to treat the wrong cells

• Diagnostic markers measured in the bulk tumor tissue may have no predictive value for the presumed tiny percentage of cancer stem cells

• Cancer stem cells are defined only by their ability to grow in animal models

• No one has cured a tumor in a patient by specifically treating the cancer stem cells

• Cancer stem cells are not true stem cells in the sense that they cannot differentiate into different tissue subtypes
BMI-1 inhibitors kill colon cancer stem cells

Self-renewal as a therapeutic target in human colorectal cancer

Antonija Kreso, Peter van Galen, Nicholas M Pedley, Evelyne Lima-Fernandes, Catherine Frelin, Thomas Davis, Liangxian Cao, Ramil Baiatzitov, Wu Du, Nadiya Sydorenko, Young-Choon Moon, Lianne Gibson, Yadong Wang, Cherry Leung, Norman N Iscove, Cheryl H Arrowsmith, Eva Szentgyorgyi, Steven Gallinger, John E Dick & Catherine A O’Brien

Affiliations  |  Contributions  |  Corresponding author

Targeting self-renewal, an Achilles' heel of cancer stem cells

Max S Wicha

When and where do Cancer Stem Cells originate?

Do Cancer Stem Cells arise in pre invasive lesions?
The genomic hallmarks of aggressiveness and invasiveness are selected out early and pre-exist within Ductal Carcinoma In Situ

Extensive similarities exist in the gene and protein expression level among the distinct stages of microdissected human breast cancer: At the gene expression level ADH is very similar to DCIS and IDC in the same patient.

Do Cancer Stem Cells arise in the hypoxic nutrient deprived intraductal niche?

Espina & Liotta, Nature Rev. Cancer 2010

Gatttenby 2004
Can we grow DCIS cells without viral immortalization?

Ginny Espina
Human DCIS generated ex vivo spheroids and 3D duct like structures
Human Ductal Carcinoma Xenografts in NOD SCID

- Xenograft generation and analysis
- Growth
- In vitro

33 patients

Vascularized tumors appear after 3 months

21/27 tumorigenic spheroid cultures from different patients

EpCAM-FITC (pseudo-colored green, 488 nm) and DAPI (pseudo-colored blue, 408 nm).
Differences in cell signaling proteins were a stable characteristic of the observed phenotype, and were maintained in an independent verification analysis.
Autophagy (self-eating) promotes survival of DCIS cells within the hypoxic nutrient deprived intraductal environment.

In order to survive, the cell digests its own contents thereby generating energy in the face of hypoxia and absence of nutrients.

Autophagy upregulated *in vivo* in DCIS.

**Images:**
- **DCIS:** Staining showing Atg5 positive cells within DCIS.
- **Lc3b:** Staining showing DCIS autophagosomes.
- **Atg2B:** Staining showing Atg5 positive cells within DCIS.
Autophagy is a complex catabolic program for lysosomal degradation of proteins and other subcellular constituents.
Chloroquine

• Rapidly accumulates in lysosomes to interfere with autophagy

• Radiotherapy sensitizing through lysosome permeabilization

• Combination therapy for glioblastoma

• Chemotherapy sensitizing by blocking autophagy mediated cell survival following DNA damage in myeloma

• Promotes therapy induced apoptosis in pancreatic carcinoma

• Suppresses autophagic mediated survival in melanoma

• Amplifies therapy induced apoptosis in myc induced lymphoma
Chloroquine (CQ) disrupts autophagy, induces apoptosis, kills the DCIS spheroid forming invasive cells, and blocks tumor xenograft growth.

Espina V and Liotta LA  Nature Rev Cancer 2010
PINC Trial: Preventing Invasive Breast Neoplasia With Chloroquine (oral) 30 days
PINC Trial: Preventing Invasive breast Neoplasia with Chloroquine

Trial endpoints
1. Shrinkage by MRI
2. Pathologic regression
3. Change in Proliferative Index
4. Elimination of genetically abnormal cells

Clinical PI: Kirsten Edmiston
### PINC Trial: Progress to date

<table>
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<tr>
<th>Patient ID</th>
<th>ER</th>
<th>PR</th>
<th>Nuclear Grade</th>
<th>Necrosis</th>
<th>Pathologic Area Reduction</th>
<th>Proliferation Index</th>
<th>Fold Change</th>
<th>MRI pre-treatment (LD in cm)</th>
<th>MRI post-treatment (LD in cm)</th>
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<td>Pos</td>
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<td>n/a</td>
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<td>41.4 (Ki-67)</td>
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<td>2</td>
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</tbody>
</table>

n/a: post-treatment residual DCIS was absent or insufficient for staining

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**IHC evidence of suppression of proliferation and autophagy post treatment**

**Before**

![Before Proliferating Cell Nuclear Antigen (PCNA)](image1)

**After**

![After Proliferating Cell Nuclear Antigen (PCNA)](image2)

**Before**

![Before LC3B Punctate Autophagosome Staining](image3)

**After**

![After LC3B Punctate Autophagosome Staining](image4)
Reduced DCIS intraductal cell Proliferation Index Pre/Post Chloroquine Treatment
Conclusions

• Cytogenetically abnormal spheroid forming stem like cells with invasive and tumorigenic potential exist within fresh human ADH and DCIS lesions and utilize autophagy for survival.

• Anti-autophagy Chloroquine treatment reduces the survival of cytogenetically abnormal invasive human breast neoplastic cells ex vivo and in animal xenografts.

• Open PINC trial neoadjuvant therapy trial for DCIS using Chloroquine to kill pre invasive cells by disrupting autophagy.

• Ten patients enrolled and treated to date. All patients with post TX residual lesion show reduction of proliferative index by greater than 75%. 2 patient had no residual disease for index assessment post treatment.
Targeting Cancer Stem-Like Cells for the Treatment of Preinvasive Breast Lesions

Kill the intraductal neoplastic cells before they can invade.