Cancer stem cells and therapeutic targeting via CCR5

11th Annual Conference on Stem Cell and Regenerative Medicine
October 15
Richard G Pestell

President Pennsylvania Cancer and Regenerative Medicine Center
DISCLOSURE

• The flying FINNS

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DISCLOSURE

• Supported from NIH (R01CA70896, R01CA75503, R01CA107382, R01CA8607 P30CA56036)
• Supported from Breast Cancer Research Foundation
• Supported from Marian Falk Foundation
• Supported from State of Pennsylvania Department of Health
• Founder and CSO of AAA Phoenix and ProstaGene
• CMO CytoDyn
• External Advisor to 7 NCI cancer centers
• President PCARM
  • Blumberg Institute,
  • European Cancer Stem Cell Institute,
  • Weizmann Institute
  • University of Stuttgart
  • Karolinska Institute
  • University of Helsinki
Precise Medicine

- 2015 Precision Medicine Initiative
- Public trust
- Accountability
- Data sharing, quality integrity
Precise Medicine
Targeting Cancer Stem Cells

A. Conventional therapy

1. Heterogeneous tumor tissue
2. Reduction of tumor bulk
3. Increase of the cancer stem cell fraction
4. Recurrence

B. Cancer stem cell-targeted therapy

1. Heterogeneous tumor tissue
2. Cancer stem cells are targeted
3. Cancer stem cell ablation: no source of differentiated cells
4. Regression

C. Perivascular niche-targeted therapy

1. Heterogeneous tumor tissue
2. Cancer stem cell niche is targeted
3. Cancer stem cell ablation: no source of differentiated cells
4. Regression

Legend:
- Transit Amplifying cells
- Different progeny of cells
- Niche
- Cancer stem cell
Genetic Determinants of Mammary Cancer Stem Cell

Year of Publication by Pestell Lab

p21<sup>CIP1</sup> Determines Mammary Cancer Stem Cell

Dach1 Governs Stem Cell

2. Jiao, .. Pestell R. Cell Stem Cell Reports, 2018
Therapies Targeting CSC

![Diagram of Therapies Targeting CSC]

Legend:
- Stromal cell
- Chemotherapy
- WNT ligands
- Hedgehog ligands

**SELF-RENEWAL PATHWAY ANTAGONISTS**

- WNT ligand inhibitors
- β-Catenin destruction complex stabilizing agents
- WNT transcription complex inhibitors
- Anti-DLL4 antibody
- γ-Secretase inhibitors
- SMO antagonists
- Hedgehog ligand inhibitors

**CHEMoresistance-Reverting AGENTS**

- MDR inhibitors
- DNA repair pathway inhibitors

**Key Pathways**

- Wnt/β-catenin pathway
- Notch pathway
- Hedgehog pathway

**Functions**

- Inhibitors target specific pathways to disrupt CSC function.
Cancer Stem Cell Niche

CCL3 MIP1α
CCL4 MIP1β
CCL5 RANTES
CCL8
CCL11 Eotaxin1
CCL13
CCL14 HCC-1
CCL16 LEC, MTN-1
The HIV CCR5 Receptor Signaling and Function
Breast oncogene induction of CCR5 Receptor Signaling and Invasion

MCF-10A: Vector  Neu-T  Ras  Src

Control  CCL5

Invasion

Distance invaded (µm)

P<0.05  P<0.05

P>0.05
CCR5 Receptor expression in Breast Cancer
Cancer Stem Cell Characteristics

- Minor population in tumor: 0.1 - a few percent
- Self-renewing: infinite proliferative potential.
- Enhanced resistance to drugs, radiation, cell stress.
- Tumorigenic; give rise to other cell types in tumor.
- Associated with metastasis and relapse.

Metastasis and relapse are involved in more than 90% of all cancer deaths

Strategies to eradicate CSCs are an urgent topic in cancer research.
CCR5 - Minor population in Breast Cancer - Associated with Relapse

Node-negative Breast Cancer
Logrank p=0.002

Overall Survival

Low CCR5 (N=226)  High CCR5 (N=323)
CCR5 activates calcium influx in Breast Cancer

CCL5

FBS

MDA-MB-231

RFI

Time (s)

FBS

CCL5
Single cell sequencing of CCR5$^+$ cells
Increased “volatility” of gene expression
Single cell sequencing of CCR5+ cells
Increased “volatility” of gene expression

A

B

CCR5+ cells

CCR5- cells

Fold Change (Log2)
P Value

0.05
0.003
0.01
0.001
0.0

EFNA5
FGF8
STFD2
KAT7
RAD54L1.2
TNC4R
CNNM2
ANK3
RP1-59D14.9
RP11-294N21.2
RP11-118A1.2
SNHG5
IGHV1-30-34

0 -1 0 1 2
and histonism

Value
CCR5+ cells give rise to mammospheres
CCR5+ cells give rise to tumors

CCR5+

CCR5−
Reintroduction of CCR5 into CCR5- BCa cells give rise to tumors in vivo

SUM159-Vector

SUM159-CCR5

Photon flux
\((x10^9 \text{p/sec/cm}^2/\text{sr})\)

\(P<0.05\)
CCR5 Receptor Expression Promotes Breast Cancer Cell invasion

P=0.004
CCR5 Receptor Inhibition blocks Breast Cancer cellular invasion
CCR5 antagonists block Breast Cancer metastasis
CCR5 restrains DNA damage response in breast tumors

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<tr>
<th>SUM-159</th>
<th>Vector</th>
<th>CCR5</th>
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<tr>
<td>0</td>
<td>100</td>
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Doxorubicin (nM)

p-γH2AX

Vinculin

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<tr>
<th>SUM-159</th>
<th>DMSO</th>
<th>Doxorubicin (100 nM)</th>
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<tbody>
<tr>
<td>Control</td>
<td>CCL5</td>
<td>Maraviroc</td>
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</table>

p-γH2AX

Vinculin
CCR5 Receptor induces DNA damage repair
CCR5 inhibitors increase breast cancer cell killing

**PARP inhibitors**

**Doxorubicin**

**Maraviroc**

Doxorubicin

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<th>0.039</th>
<th>0.078</th>
<th>0.156</th>
<th>0.312</th>
<th>0.625</th>
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</table>
Chemotherapy induces CCR5

CCR5 governs DNA damage repair and breast cancer stem cell expansion. Cancer Res. Pestell et al 2018 78:7, 1657
CCR5 function in Breast cancer Metastasis

- CCR5 signaling induced in human basal breast cancer
- Oncogenes induce CCR5 signaling
- CCR5 overexpressed in basal breast epithelial cells
- CCR5 induces invasion and metastasis
- CCR5 inhibition blocks basal breast cancer metastasis in pre-clinical models
- CCR5/CCL axis induced in >50% of human BCa
- **Cancer.**
  - Breast, Prostate, colorectal
  - esophageal, kidney,
  - Leukemia, liver, lymphoma
  - Myeloma brain, bladder.

<table>
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<th>Analysis Type by Cancer</th>
<th>Cancer vs. Normal</th>
<th>Outlier</th>
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<tr>
<td>Breast Cancer</td>
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<tr>
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<tr>
<td>Sarcoma</td>
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**CCR5 is expressed in many different cancer types**
Prostate Cancer

- Most common cancer in American men (ACS 2012) (1/6 men)
- 241,740 men will acquire this year
- 28,170 deaths/per year
- 2.5 M current survivors - no reliable predictors of survivors
- Increasing incidence globally
- Death from metastasis (bone, brain)
- No reliable pre-clinical testing models
Acute GVHD are 38.5% for grade II to IV disease and 21.9% for grade III or IV disease.

CCR5 inhibitors block PCa metastasis
CCR5 in Prostate Cancer

- CCR5 signaling activated in vivo in immune competent animals
- Reliable metastasis of isogenic prostate cancer lines
- CCR5 inhibitors reduce metastasis in immune-competent mice in vivo (total body, lung, bone and bone)

- BUT serious adverse events with Maraviroc in HIV patients…-
CCR5 specific monoclonal antibody therapy for cancer stem cells

- **Leronlimab**
- Humanized monoclonal antibody
- Used in HIV treatment (Mono and combination therapy)
- No serious adverse events (SAEs)
  - >600 patients in 8 clinical trials for HIV
- Once weekly, easy, subcutaneous
  - self administration
Leronlimab blocks Breast Cancer Ca^{2+} signaling
Leronlimab blocks Breast Cancer cellular invasion

A

Control  PRO140 (1/500)  Vicriviroc

B

Distance of Invasion (µm)

Control  PRO140  Vicriviroc

P<0.001

C

Control  1/500  1/1000

D

Distance of Invasion (µm)

Control  1/500  1/1000

P<0.001
Leronlimab blocks Colon Cancer tumor growth

SW480 Human Colon Carcinoma Xenografts in NCr Nude Mice
PRO 140, 2 mg i.p. twice/week, started day 1, n=16 tumors/group
advanced-stage metastatic colorectal cancer who are refractory to standard chemotherapy, including regorafenib.
1. April 1, 2018, Pfizer, Phase I, Pembrolizumab + Maraviroc + MSS CRC
2. September 2018, Merck, Phase 2, Pembrolizumab + Vicraviroc + MSS CRC

**CCR5 inhibitors objective response**

![Before and after CHT+CCR5 inh. images](image)

![Change in SLD vs baseline (%)](chart)
Leronlimab Breast Cancer study

November 2018-March 2019
Phase II

Pro-140 525 mg 1sc/week
Carboplatin AUC 2q week x3
28 days cycle

Endpoints
1. OS
2. PFS'
3. Decreased CTC

Breakthrough (unmet need)
April 2019-July 2021 (Phase III)
CCR5+ cells in Breast Cancer
Stem Cells

- Stem like cells – (mammospheres)
- Give rise to new tumors
- Contribute to therapy resistance
- Promote metastasis (necessary and sufficient)
- Single cell sequencing - volatility
- Enhanced DNA repair
- CCR5 inhibitors increase DDR cell death - >200%
Spend more time with your family and friends, eat your favorite foods, visit the places you love.