Targeting Angiogenesis in Ovarian Cancer: Overcoming Adaptive Responses

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THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Making Cancer History®
Disclosure

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- Ovarian and Uterine Cancer SPORE
- OCRF
- NCI (CA177909, CA109298)
- GSK
Overview

- Clinical observations from anti-angiogenesis trials
- Mechanisms of adaptation
  - Tumor rebound – role of platelet extravasation
  - Adaptive resistance
# Angiogenesis as a target: Ovarian cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Agent</th>
<th>Target</th>
<th>HR-PFS (95% CI)</th>
<th>HR-OS (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOG 218¹</td>
<td>Bevacizumab</td>
<td>VEGF Ligand</td>
<td>0.72 (0.63-0.82)</td>
<td>0.89 (0.75-1.04)</td>
</tr>
<tr>
<td>ICON7²</td>
<td>Bevacizumab</td>
<td></td>
<td>0.81 (0.70-0.94)</td>
<td>0.99 (0.85-1.14)</td>
</tr>
<tr>
<td>AURELIA⁵</td>
<td>Bevacizumab</td>
<td></td>
<td>0.48 (0.38-0.60)</td>
<td>0.85 (0.66-1.08)</td>
</tr>
<tr>
<td>OCEANS⁷</td>
<td>Bevacizumab</td>
<td></td>
<td>0.53 (0.41-0.70)</td>
<td>0.96 (0.76-1.21)</td>
</tr>
<tr>
<td>GOG-213⁹</td>
<td>Bevacizumab</td>
<td></td>
<td>0.61 (0.52-0.72)</td>
<td>0.83 (0.68-1.005)</td>
</tr>
<tr>
<td>AGO-OVAR12³</td>
<td>Nintedanib</td>
<td>VEGFR, FGFR, PDGFR</td>
<td>0.84 (0.72-0.98)</td>
<td>NR</td>
</tr>
<tr>
<td>AGO-OVAR16⁴</td>
<td>Pazopanib</td>
<td></td>
<td>0.77 (0.64-0.91)</td>
<td>0.99 (0.75-1.32)</td>
</tr>
<tr>
<td>ICON6⁸</td>
<td>Cediranib</td>
<td>VEGFR</td>
<td>0.57 (0.44-0.74)</td>
<td>0.70 (0.51-0.99)</td>
</tr>
<tr>
<td>TRINOVA-1⁶</td>
<td>Trebananib</td>
<td>Ang ligand</td>
<td>0.66 (0.57-0.77)</td>
<td>0.86 (0.69-1.08)</td>
</tr>
</tbody>
</table>

4. du Bois A et al. LBA ESGO 2013 Liverpool, UK
6. Monk BJ, et all., LBA ESGO, Liverpool, UK
9. Coleman, RL, SGO 2015 LBA3
Frontline Anti-VEGF Therapy: GOG-0218

HR: 0.73
10.4 vs. 13.9 mos
Median Δ: 3.5 mos

Burger et al., NEJM 2011
Frontline ICON7: Similar findings for PFS

HR = 0.81
(95% CI 0.70–0.94)
p=0.0041
GOG-111: PFS curves on chemotherapy trials

RR: 0.7; 95% CI: 0.5 – 0.8

McGuire et al., NEJM 1996
PFS curves on chemotherapy trials

**GOG-111**

*Proportion Progression-Free*

- **Cisplatin + cyclophosphamide**
  - No. Progression-free: 28
  - No. with Treatment Failure: 174
  - Total: 202
  - Median Progression-free Survival (mo): 13

- **Cisplatin + paclitaxel**
  - No. Progression-free: 45
  - No. with Treatment Failure: 139
  - Total: 184
  - Median Progression-free Survival (mo): 18

*RR: 0.7; 95% CI: 0.5 – 0.8*

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**JGOG**

*HR 0.71 (95% CI: 0.58 – 0.88); p < 0.015*

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McGuire et al., NEJM 1996

Katsumata et al., Lancet 2009
Questions to consider

• Combination vs. sequential therapy
• Therapy for life?
  ➢ Angiogenesis escape vs. continued benefit
  ➢ Impact of prior target class exposure
• Mechanisms of adaptive changes
• Is there accelerated tumor growth following cessation of anti-angiogenic therapy?
Questions to consider

• Combination vs. sequential therapy
• Therapy for life?
  ➢ Angiogenesis escape vs. continued benefit
  ➢ Impact of prior target class exposure
• Mechanisms of adaptive changes

• Is there accelerated tumor growth following cessation of anti-angiogenic therapy?
Effect of anti-angiogenic therapy withdrawal on tumor outgrowth

A

withdrawal

necropsy

days

0 7 14 28 35

withdrawal

long (continuous)
treatment: twice weekly, i.p. (Bevacizumab, B20);
daily, p.o. (Pazopanib)

i.p. injection of cells

B

C

D

Tumor weight (g)

No. of Nodules

Tumor weight (g)

Control
Paz withdrawal
long Paz

Control withdrawal long Bev

* ***
Adaptive-evasive responses: Future targets

Platelets
- PDGF↑
- HGF↑
- Plasminogen↑
- Fibrinogen↑
- MIP-1α↑
- PAI-1↑
- Vimentin↑
- RANTES↑
- U-PA↑
- Factor V↑
- IL-8↑
- Osteonectin↑
- Laminin-8↑
- GRO-α↑
- HRG↑
- Factor IX↑
- ENA 78↑
- MMP-4↑
- Multimerin↑
- MCP3↑
- Thrombospondin-1↑
- Gas6↑
- APO-1↑
- EGF↑
- IGF-1↑
- IL-1β↑
- VEGF↑
- IGFBP3↑
- FGF↑
- CDYOL↑

Endothelial Cells
- PDGF↑
- VEGF↑
- Vascular Stability↑

Pericytes
- VEGF↑
- Vascular Stability↑

Tumor Cells
- p53↑
- PHD↑
- MET↑
- PIGF↑
- PDGF↑
- P13k/Akt/MTOR↑
- VEGF↑
- MCP-β↑
- EGF↑
- APO-1↑
- SDF-1↑
- IGFI-1↑
- APO-2↑
- Hifl-α↑
- IL8↑
- DLL4↑
- Glut-1↑
- GCSF↑
- FGF↑
- AldoA↑
- Osteopontin↑
- Ephrin↑
- AKT↑

White Blood Cells
- Tie-2↑
- Bv8↑
- VEGF↑
- CD11b↑

Fibroblasts
- Retraction of vessels toward tumor
- JAK/STAT↑
- HGF↑
- cMET↑

Bottsford-Miller et al., JCO, 2012; Stone et al., NEJM, 2012
Role of Platelets in the tumor microenvironment
Alpha Granule Contents

**Pro-angiogenic**
vascular endothelial growth factor
platelet-derived growth factor
basic fibroblast growth factor
epidermal growth factor
transforming growth factor
insulin-like growth factors
angiopoietin-1
sphingosine-1-phosphate
matrix metalloproteinases
epidermal growth factor

**Anti-angiogenic**
thrombospondin I
platelet factor 4
plasminogen activator inhibitor I
endostatin
angiostatin

Dense Granule Contents
serotonin
ATP/ADP
histamine
Ca++

Lysosomal Contents
proteinases
glycosidases
Thrombocytosis and patient survival

**Progression-Free Survival**
- Platelets WNL
- Thrombocytosis

**Overall Survival**
- Platelets WNL
- Thrombocytosis

Platelets in the tumor microenvironment

Tumor hypoxia and vascular leakage following withdrawal of anti-VEGF therapy (2774 model)

Haemmerle et al., JCI, In Press
Revascularization following withdrawal of anti-VEGF therapy

Haemmerle et al., JCI, In Press
ADP production after hypoxia

**In vitro**

**In vivo**

Haemmerle et al., JCI, In Press
Platelet extravasation into the tumor

A

Extravasated platelets (per HPF)

Control  Bev-treated  Paz-treated

***  *

B

GP1b-β  CD31  DNA

Control  Bevacizumab

RBC  RBC  PLT  EC  EC

Haemmerle et al., JCI, In Press
Effects of platelets on tumor growth

**Proliferation**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Increased proliferation (%, rel. to control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeyA8</td>
<td>***</td>
</tr>
<tr>
<td>SKOV3ip1</td>
<td>***</td>
</tr>
<tr>
<td>OVCAR5</td>
<td>**</td>
</tr>
<tr>
<td>ID8</td>
<td>*</td>
</tr>
</tbody>
</table>

**Apoptosis**

<table>
<thead>
<tr>
<th>Condition</th>
<th>% Apoptotic cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>normoxia</td>
<td></td>
</tr>
<tr>
<td>normoxia + pls</td>
<td></td>
</tr>
<tr>
<td>hypoxia</td>
<td>***</td>
</tr>
<tr>
<td>hypoxia + pls</td>
<td>***</td>
</tr>
</tbody>
</table>

**Invasion**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Invasion (no. of cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>normoxia</td>
<td></td>
</tr>
<tr>
<td>normoxia + pls</td>
<td>***</td>
</tr>
<tr>
<td>hypoxia</td>
<td>***</td>
</tr>
<tr>
<td>hypoxia + pls</td>
<td>***</td>
</tr>
</tbody>
</table>

Haemmerle et al., JCI, In Press
Role of platelets in tumor outgrowth after anti-angiogenic therapy withdrawal

APA = Anti-platelet antibody

Haemmerle et al., JCI, In Press
Roles of focal adhesion kinase (FAK) in megakaryopoiesis and platelet function: studies using a megakaryocyte lineage–specific FAK knockout

Ian S. Hitchcock, Norma E. Fox, Nicolas Prévost, Katherine Sear, Sanford J. Shattil, and Kenneth Kaushansky

Department of Medicine, University of California San Diego, La Jolla

Focal adhesion kinase (FAK) plays a key role in mediating signaling downstream of integrins and growth factor receptors. In this study, we determined the roles of FAK in vivo by generating a megakaryocyte lineage–specific FAK-null mouse (P14-Cre/FAK-floxed). Megakaryocyte and platelet FAK expression was ablated in P14-Cre/FAK-floxed mice without affecting expression of the FAK homologue PYK2, although PYK2 phosphorylation was increased in FAK−/− megakaryocytes in response to fibrinogen. Megakaryopoiesis is greatly enhanced in P14-Cre/FAK-floxed mice, with significant increases in megakaryocytic progenitors (CFU-MK), mature megakaryocytes, megakaryocyte ploidy, and moderate increases in resting platelet number and platelet recovery following a thrombocytopenic stress. Thrombopoietin (Tpo)–mediated activation of Lyn kinase, a negative regulator of megakaryopoiesis, is severely attenuated in FAK-null megakaryocytes compared with wild-type controls. In contrast, Tpo–mediated activation of positive megakaryopoiesis regulators such as ERK1/2 and AKT is increased in FAK-null megakaryocytes, providing a plausible explanation for the observed increases in megakaryopoiesis in these mice. In P14-Cre/FAK-floxed mice, rebleeding times are significantly increased, and FAK-null platelets exhibit diminished spreading on immobilized fibrinogen. These studies establish clear roles for FAK in megakaryocyte growth and platelet function, setting the stage for manipulation of this component of the Tpo signaling apparatus for therapeutic benefit. (Blood. 2008;111:596-604)

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Blood, 2008
Effect of FAK silencing in platelets (HeyA8)

Systemic siRNA delivery (chitosan NP)

Haemmerle et al., In Press; Krzeszinski et al., Nature, 2014
Role of FAK in platelets (ID8)

*FAK k.o. in platelets*

Haemmerle et al., JCI, In Press
Diminished platelet extravasation after FAK knock out

⇒ No effect on blood platelet counts
Dual anti-FAK and anti-VEGF therapy in tumor rebound model

OVCA-432 model

HeyA8 model

*p<0.01

Haemmerle et al., JCI, In Press
Adaptive response to anti-VEGF therapy

Hypoxia

ADP ↑

Withdrawal of anti-angiogenic therapy

Tumor angiogenesis ↑
Tumor growth ↑
Pericyte coverage ↓
Platelet infiltration ↑

Continuous anti-angiogenic therapy & FAK inhibition

Platelets
Pericytes
Tumor vessels
Leaky tumor vessels
Adaptive resistance to bevacizumab
Summary

• Multiple adaptive changes occur in the tumor microenvironment in response to anti-angiogenesis therapy

• Extravascular platelets play an important role in accelerated tumor regrowth following anti-VEGF therapy

• FAK inhibition may represent an important combination with anti-VEGF drugs

• Macrophages promote adaptive resistance to anti-VEGF drugs
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