TARGETING CANCER STEM CELLS WITH SELECTIVE INHIBITORS OF PI3K/MTOR & FAK

KEYSTONE SYMPOSIUM – STEM CELLS AND CANCER

Jonathan Pachter, VP/Head of Research, Verastem
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Disclosures

• I am an employee and shareholder of Verastem Inc.

• I will be discussing investigational drugs
Focus on Durable Response

- Objectives for a durable clinical response
  - Effectively target the bulk tumor
  - Overcome immune evasion
  - Remove the Cancer Stem Cells

1. Chemotherapy, radiation
2. Targeted therapy
3. Immunotherapy
4. Combinations
Reducing Cancer Stem Cells & Bulk Tumor for Durable Response

Current cancer treatments

Tumor reduction, but CSCs survive

Addition of CSC-targeting agent

Recurring tumor

Durable response
Absence of Cancer Stem Cells Correlates with Better Survival

- N = 115 breast cancer patients treated with AC → paclitaxel prior to primary surgery
- OS stratified by presence of CSCs [ALDH+ IHC] in residual disease

Sakakibara et al., Cancer 2012

Overall Survival in Breast Cancer Patients

No residual disease in lymph nodes
No cancer stem cells in residual disease in lymph nodes
Cancer stem cells in residual disease in lymph nodes

p < 0.001
VS-5584: Dual PI3K/mTOR Kinase Inhibitor

VS-5584

- PI3K/mTOR kinase inhibitor
  - Equipotent against mTORC1, mTORC2 and all four PI3K class I isoforms
  - Selective vs. other protein & lipid kinases
- Broad anti-tumor activity in xenograft models
- Phase I dose escalation in progress in patients with solid tumors & lymphomas
  - 3x/week intermittent dosing schedule
  - Finalizing Recommended Phase 2 Dose (RP2D)

<table>
<thead>
<tr>
<th>mTOR IC₅₀ (nM)</th>
<th>PI3K isoform IC₅₀ (nM)</th>
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<tr>
<td></td>
<td>Alpha</td>
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<tr>
<td>3.4</td>
<td>2.6</td>
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RTKs

PI3K

AKT

mTORC1

mTORC2

Elimination of CSCs
VS-5584 Preferentially Targets CSCs in Orthogonal CSC Assays

VS-5584 treatment, breast cancer cell lines:

**Weinberg HMLE assay**

**Aldefluor assay**

**Hoechst Dye Exclusion**
Dual PI3K/mTOR Inhibitor VS-5584 Preferentially Targets CSCs in vivo, in Contrast to mTORC1 Inhibitor Everolimus

VS-5584 treatment, MCF7 breast cancer xenograft model:

Treatment with VS-5584 reduces the population of CSCs and tumor initiating cells, and reduces the ability of remaining cells to generate new tumors

Source: Kolev et al. Cancer Res. 2015
Combined Inhibition of PI3K and mTOR is Critical for CSC Targeting

siRNA transfection of SUM159 cells: Phenocopies VS-5584

Source: Kolev et al. Cancer Res. 2015
VS-5584 Extends Efficacy After Discontinuation of Chemotherapy in SCLC Xenograft Models

**Front line + maintenance model**
VS-5584 in NCI-H841 SCLC xenograft model:

- Vehicle
- Cisplatin
- Cisplatin + VS-5584

**Maintenance only model**
VS-5584 in SCLC PDX model #051

- Control
- Cisplatin
- Cis/Etoposide
- Debulking
- VS-5584
- Cis/Eto → VS-5584
FAK is Critical for Multiple Aspects of Tumor Progression

• Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase that mediates signaling downstream of integrins & growth factor receptors

• Cancer Stem Cell Function
  – FAK is essential for both the survival & tumor-initiating capability of CSCs
  – Metastasis: FAK plays important roles in tumor cell migration, invasion & EMT which are all critical for the metastatic process

• Immuno-Oncology/Tumor Microenvironment
  – FAK inhibition reduces immune suppressive cell populations in tumor microenvironment: Tregs, M2 tumor-associated macrophages, MDSCs
  – FAK inhibition reduces stromal density: Facilitates entry of cytotoxic T cells into tumor

Novel Drugs Targeting Cancer Stem Cells
FAK is Critical for CSC Tumor-Initiating Capability

**Luo et al., Cancer Res 2009**
Targeted deletion of FAK eliminates tumor initiating capability

**Shibue et al. Cancer Discovery 2012**
FAK is critical for cancer cells undergoing EMT to become CSCs capable of generating macrometastases
### Verastem FAK/PYK2 Inhibitor Program: VS-6063 & VS-4718

#### VS-6063 (defactinib)

**Dosage in humans:** Oral, 400 mg BID
- Lead compound, studied in 300+ patients to date with good safety profile
- Several Ph 1/2 studies ongoing

![Graph showing FAK EC50 = 15 nM and PYK2 EC50 = 95 nM for VS-6063](image)

#### VS-4718

**Dosage:** Oral BID
- Structurally distinct from VS-6063
- Currently completing Ph 1 dose escalation

![Graph showing FAK EC50 = 6 nM and PYK2 EC50 = 20 nM for VS-4718](image)
FAK Inhibition Preferentially Reduces CSCs in Multiple Assays

### Aldefluor Assay

![Graph showing Aldefluor-Positive CSCs (% of Control) vs. VS-4718, nM](image)

### Tumorsphere Formation

![Bar chart showing Secondary Spheres (% of Control) for control and VS-4718 concentrations](image)

### Hoechst Dye Exclusion (SP)

![Flow cytometry plots for control and 1 μM VS-4718](image)

- **Control**: 14% CSCs
- **1 μM VS-4718**: 0.03% CSCs

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**Novel Drugs Targeting Cancer Stem Cells**
FAK Inhibitors Reduce CSCs in Tumor-Bearing Animals

**In vivo Treatment** → **Harvest Tumors** → **Dissociation** → **Viable Cells**

**VS-6063**  
*MDA-MB231 TNBC xenograft model*  

downarrow Tumorsphere growth

<table>
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<tr>
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<th>Control</th>
<th>VS-6063</th>
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<tr>
<td>Spheres per 2000 cells</td>
<td>150</td>
<td>50</td>
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<tr>
<td>TIC per 10^6 cells</td>
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**VS-4718**  
*MDA-MB231 TNBC xenograft model*  

downarrow CSCs (Aldefluor+)

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<thead>
<tr>
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<th>Control</th>
<th>VS-4718</th>
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<tbody>
<tr>
<td>Aldefluor+ Cells (% of Control)</td>
<td>150</td>
<td>50</td>
</tr>
<tr>
<td>TIC per 10^6 cells</td>
<td>40</td>
<td>10</td>
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**6-fold decrease in TIF**

**8-fold decrease in TIF**

EORTC, 2014
FAK Inhibitors Reduce CSCs Through a Wnt/β-Catenin-Dependent Mechanism

FAK inhibitor selectively inhibits TCF/LEF Wnt pathway reporter

Wnt target gene expression is decreased by FAK inhibitor

Expression of constitutively active β-Catenin attenuates the effect of FAK inhibitor on CSCs

AACR, 2015
**FAK Inhibitors Extend Efficacy After Discontinuation of Chemotherapy**

**Front line + maintenance model**
FAK inhibitor (VS-6063) in TNBC PDX model

**Maintenance only model**
FAK inhibitor (VS-6063) in Call51 TNBC xenograft model

AACR, 2015

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**Graphs:**
- **Front line + maintenance model:**
  - Tumor Volume vs. Days.
  - Paclitaxel vs. Vehicle vs. Paclitaxel + VS-6063.
  - P = 0.001

- **Maintenance only model:**
  - Relative Tumor Growth vs. Days.
  - Control vs. Cisplatin vs. Cisplatin + VS-6063.
  - p = 0.03

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**Notes:**
- Paclitaxel and VS-6063 are represented in the graphs.
- The effects of FAK inhibitors on tumor growth are statistically significant.
Adjuvant Treatment with FAK Inhibitors Blocks Metastatic Outgrowth

VS-6063 treatment, 4T1 adjuvant metastatic breast cancer mouse model:

Treatment after surgical removal of primary tumor

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<th>VS-6063 (FAKi)</th>
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<tr>
<td>Day</td>
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<td>3</td>
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<tr>
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Primary tumor is surgically removed

Treatment with FAK inhibitor

Total tumor burden

![Graph showing total tumor burden over days with control and VS-6063 treatments.](chart.png)

* P<0.05

AACR, 2015
VS-6063 Phase 1 Combo with Paclitaxel in Ovarian Patients

Why ovarian cancer?
- Role of cancer stem cells in disease progression
- Activity signal with VS-6063 in ovarian cancer in Phase 1
- VS-6063 re-sensitizes paclitaxel-resistant ovarian cancer tumor xenografts to paclitaxel
- High levels of tumor FAK (pFAK) expression correlate with poor survival

### Phase 1

*Completed: 200mg, 400mg BID*

- **VS-6063 (dose escalation BID) + paclitaxel (80mg/m²/week)**
- Patients dosed until disease progression or adverse event (continuation of VS-6063 permitted if AE related to paclitaxel)

*Manish Patel, ASCO 2014*

### Phase 1b

*Completed Recruitment*

- **VS-6063 run-in for 10 days (n=5)**
- **VS-6063 (400mg BID) + paclitaxel (80mg/m²/week)**

Sood et al., J Clin Invest 2010

![Graph showing survival comparison between low and high pFAK expression](image)
VS-6063 Inhibits FAK and Reduces CSCs in Tumors of Patients with Ovarian Cancer

Phase 1b Ovarian Cancer:

VS-6063 run-in for 10 days (n=5)

VS-6063 (400mg BID) + paclitaxel (80mg/m2/week)

Biopsy

Biopsy

Tumor pFAK

Cancer Stem Cells (SOX2 RNA)

Manish Patel, ASCO 2014
Durable Complete Response Shows Benefit of FAK Inhibitor Treatment

✓ 41% (9/22) disease control (objective response or SD ≥ 6 months; 2 CR; 3 PR; 4 SD ≥ 6 months)
✓ Patient with Stage IV platinum-resistant serous ovarian cancer with 5 prior lines of therapy

Stable disease (SD) with combination treatment

1. VS-6063 monotherapy at 4.5 months
2. Complete response (CR) at 11.8 months
3. Continues to tolerate VS-6063 well

MONTHS ON THERAPY

- carbo/taxol (adjuv)
- carbo/cytoxan/taxol
- cisplatin/gem
- measles vaccine
- alimta
- VS-6063 + Paclitaxel

CA-125 (U/mL)

Unlocked, in progress data as of 12/21/2015
VS-6063 Inhibits FAK and CSCs in Patients with Mesothelioma

Phase 2:

-CXCR2+/CD133+ mesothelioma cells have previously been shown to have 7-fold increase in tumor initiating capability over CXCR2-/CD133- cells
-Both CXCR2 and CD133 were increased in tumor samples from patients treated with Pem/Cisplatin
-Treatment with VS-6063 reduces CXCR2 and CD133 expression in patient tumors

Raphael Bueno, IMIG 2014
Cancer Stem Cells are Especially Resistant to T cell-Mediated Killing

*In vivo* breast tumor model

FACS analysis of tumor cells

*In vitro* T cell killing of tumor cells

David DeNardo, Wash U
FAK Inhibitors Increase Effectiveness of Adoptive T cell Transfer in Tumor Models *in vivo*

KP/Ova Pancreatic Cancer Xenograft

**Increased Anti-Tumor Efficacy**

**Increased Tumor Infiltration of Cytotoxic T Cells**

**Graphs and Bars**

- **Tumor Volume** mm$^3$
  - Vehicle
  - ACT
  - FAKi
  - FAKi+ACT

- **Normalized Flux** (photons sec/mm$^3$)
  - Veh+ACT
  - FAKi+ACT

**ACT = Adoptive T Cell Transfer**

*David DeNardo, Wash U*
FAK Knockout or FAK Inhibitor VS-4718 Induce Tumor Regression Through T Cell-Dependent Mechanism

SCC 7.1 chemical carcinogen-induced skin cancer model:

FAK Inhibitor Creates a More Favorable Tumor Microenvironment for Checkpoint Inhibitor Efficacy

Treatment with Verastem FAK inhibitor

Increases

Cytotoxic (CD8+) T cells
Tumor infiltration & tumor cell killing

Checkpoint inhibitor target presentation
Tumor PD-L1

Decreases

Immuno-Suppressive Cells
MDSCs, T-reg, M2 tumor-associated macrophages

More favorable tumor microenvironment for enhanced efficacy of Immuno-Oncology therapeutics

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Novel Drugs Targeting Cancer Stem Cells
Increased Efficacy of FAK inhibitor + anti-PD-1 in MC38 model Correlates with Decreased T-reg & Increased CD8+ T cells

MC38 colorectal cancer mouse model:

VS-4718 + Anti-PD-1 (n = 19; p < 0.0001)
VS-4718 (n = 10; p < 0.025)
Anti-PD-1 (n = 20; p < 0.025)
Vehicle (n = 10)

↓ T-reg

↑ Tumor CD8+ T cells

Mean fold change of vehicle:

- VS-4718: P < 0.03
- Anti-PD-1: P < 0.03
- VS-4718 + Anti-PD-1: P < 0.04
FAK Inhibitor Enables Efficacy of Checkpoint Inhibitors in Aggressive Pancreatic Cancer Transgenic Model

FAK inhibitor treatment, KPPC transgenic pancreatic cancer model:

Extended Survival

Entry of CD8+ Cytotoxic T cells into Tumor

“Immuno” = anti-PD-1 + anti-CTLA-4 + GEM (25 mg/kg)
Understanding the Cancer Stem Cell-Immunotherapy Interface

- CSCs appear to be especially resistant to T-cell mediated killing
  - Enriched in tumors growing out following T cell-mediated therapies

- Do CSCs actively mediate resistance of the tumor microenvironment to immune checkpoint antibodies?
  - Can we elaborate potential mechanisms?
    - e.g. CSCs $\rightarrow$ $\uparrow$TGFβ $\rightarrow$ $\uparrow$Tregs $\rightarrow$ blocks immunotherapy

- Only a subset of patients respond to checkpoint inhibitors
  - Might presence of CSCs drive resistance in some patients?
Clinical Testing of FAK Inhibitors in Combination with Checkpoint Inhibitors

- Clinical study initiated to test the combination of FAK inhibitor (VS-6063) with anti-PD-1 (pembrolizumab, Merck) + gemcitabine in patients with pancreatic cancer
  - Washington University, St. Louis

- New collaboration with Pfizer/Merck-KGaA to test combination of FAK inhibitor (VS-6063) with anti-PD-L1 (avelumab) in patients with ovarian cancer
Conclusions

• Combined inhibition of mTOR, PI3Kα and PI3Kβ suppresses CSCs
  —Consistent with the preferential effects of VS-5584 on tumor-initiating cells

• FAK inhibitors reduce CSCs both preclinically and in tumors from treated ovarian and mesothelioma patients
  —Potential role of downstream Wnt/β-catenin signaling
  —Durable responses in ovarian cancer patients treated with VS-6063 and paclitaxel

• Cancer stem cells are especially resistant to immunotherapies

• FAK inhibitors potentiate efficacy of checkpoint inhibitors for a more durable response
  —Now being tested clinically
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