Folate Receptor as a Target for the Management of Human Cancers

Christopher P. Leamon, Ph.D.
VP Research
Folates can principally be taken up by 2 distinct mechanisms: FR and RFC

1. Folic acid binds to the FR with high affinity
2. Upon binding to the FR (Kd=10^{-10} M), folic acid is internalized via endocytosis
3. FR releases folic acid inside the acidic endosome
4. Folic acid escapes endosome and enters cytosol
5. FR recycles back to cell surface

The reduced folate carrier binds folates with a low affinity (Kd=10^{-5} M). Folic acid is a very poor substrate.

Most antifolates enter cells this way
The Folate Receptor (FR)

- 38 kDa GPI-linked membrane protein (α and β isoforms)
- Enters cells via Endocytosis
  - Non-destructive pathway
- The FR is recycled, not destroyed
- Normal tissue expression
  - Kidney (PT), placenta, choroid plexus, lung, thymus
- Recognized tumor-associated protein
  - Predominant on epithelial cancers
  - Also found on CML, AML and lymphomas
- Binds folic acid, some antifolates, and folate-drug conjugates with high affinity ($K_d$ in low nM range)

*Can be exploited for cancer therapy*
Exploiting the Folate Receptor

*Proc. Natl. Acad. Sci. USA*
Cell Biology

**Delivery of macromolecules into living cells: A method that exploits folate receptor endocytosis**

*(drug delivery/folate uptake/vitamin endocytosis)*

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[CANCER RESEARCH 51, 5329–5338, October 1, 1991]

**Folate-binding Protein Is a Marker for Ovarian Cancer**

*Ian G. Campbell, Tania A. Jones, William D. Foulkes, and John Trowsdale*

*Imperial Cancer Research Fund, P.O. Box 123, Lincoln’s Inn Fields, London WC2A 3PX, England*
Macromolecules attached to folate are delivered into cancer cells via the folate receptor.

Endocytosis of folate-gold conjugates (analyzed by electron microscopy)

Gold particles linked to folate bind to surface of the cell and are internalized via endocytosis.

Within a few minutes, the folate-gold conjugates are released into the cytoplasm of the cell.
>1 million newly diagnosed cancer patients over-express the folate receptor (United States, Europe, and Japan)

Source: American Cancer Society and Endocyte estimates based upon imaging studies and tissue biopsies.

(1) Represents patients with at least one folate receptor positive lesion.
(2) Data for some indications based on IHC of alpha form of folate receptor (e.g., testicular, bladder). New data suggest some cancers express the beta form which is also targeted by Endocyte’s SMDCs and companion diagnostic imaging agent etarfolatide.

![Graph showing folate receptor positive cancers by cancer type](https://via.placeholder.com/150)

Vintafolide and etarfolatide target both FRα and FRβ
Prognostic impact of FR expression on human cancer


Disease-free survival by intensity of FR staining

Hartmann et al. (2007). Int. J. Cancer 121, 938-942

Overall survival by intensity of FR staining

Disease-free survival by intensity of FR staining
Specificity of folate targeting
Fluorescent imaging of metastatic NSCLC in the thoracic cavity of a mouse
Folate conjugates are highly specific for malignant FR-positive tissue in humans.

View of localized region in peritoneal cavity of an ovarian cancer patient as seen with the naked eye (left) or with the aid of a tumor-targeted fluorescence dye (right).
Real-time, Non-Invasive Assessment of FR Expression Using Companion Imaging Agents

$^{99m}$Tc-etarfolatide
($^{99m}$Tc-EC20)
Folate-targeted imaging agents specifically bind to FR-positive tissue

Folate-targeted agents are partially re-absorbed by FR-positive kidneys and transferred back into circulation.
Folate Receptors: healthy vs. cancer

Cancer-free Patient

Advanced Ovarian Cancer

Mets

Primary mass
Imaging brain lesions

Brain mets

Pituitary adenoma

Courtesy:
Drs. Nelson Oyesiku and James Galt
Emory University
Targeted SMDC Therapy

Small Molecule Drug Conjugates
Current therapeutic approaches in oncology

SMDC
- Cell specific
- Receptor-mediated
- Penetrates solid tumors
  Ex: vintafolide

ADC
- Cell specific
- Receptor mediated
  Ex: ado-trastuzumab emtansine, brentuximab

Farletuzumab
- Cell specific
- May block ligand binding
- May block signal transduction
- May trigger ADCC
  Ex: trastuzumab, cetuximab

Traditional Chemotherapy
- Not targeted
- Enters cancer and normal cells
- Narrow therapeutic window
  Ex: cisplatin, paclitaxel, vinblastine

Pathway Inhibitors
- Not cell specific
- Passive uptake
- Intracellular targets (mTOR, TKIs, ALK, BRAF)
  Ex: imatinib, sunitinib, everolimus

Leamon et al. (2013) Pharmacogenomics and Personalized Medicine 6, 113-125.
Our SMDC approach is modular in design.

**Ligand (Module 1)**
- High affinity
- Small size

**Hydrophilic Spacer (Module 2)**
- Separates the ligand from the drug payload
- Provides water solubility

**Drug (Module 4)**
- Highly potent
- Critical mechanism
- Derivatizable

**Releasable Linker (Module 3)**
- Stable in blood
- Cleaves in the endosome
- Self-immolative

**Ligand (Module 1)**
- High affinity
- Small size
Folate-SMDCs enter cells via the FR

1. Folate-conjugate binds the folate receptor

2. Upon binding to the folate receptor (Kd=10^{-10} M), the conjugate is internalized via endocytosis

3. The drug is cleaved inside endosome

4. Drug escapes endosome and exerts activity on cell

5. Folate receptor recycles back to cell surface

The reduced folate carrier binds folates with a low affinity (Kd=10^{-5} M). Folate conjugates will not enter cell through the reduced folate carrier. Most antifolates enter cells this way.
Vintafolide (EC145)
A folate-vinca alkaloid conjugate

Folic acid
(tumor-targeting ligand)

Peptide spacer
(hydrophilicity)

Bio-releasable
linker

DAVLBH
(active warhead)
Vintafolide receptor specific activity

**Activity of Vintafolide: 2 h/72 h Assay**

- **IC**$_{50}$ ~ 9 nM

- **% Viability vs. Concentration (Log M)**

<table>
<thead>
<tr>
<th>FR-Negative Cell Line</th>
<th>Vintafolide Conc. (nM)$^a$</th>
<th>Cell Kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td>100 nM</td>
<td>0%</td>
</tr>
<tr>
<td>4T1</td>
<td>100 nM</td>
<td>0%</td>
</tr>
<tr>
<td>HUVEC</td>
<td>100 nM</td>
<td>0%</td>
</tr>
<tr>
<td>LNCaP</td>
<td>100 nM</td>
<td>0%</td>
</tr>
</tbody>
</table>

$^a$2h pulse, 72 h total incubation

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Receptors are blocked with folate; Folate-drug cannot bind

Leamon et al. (2007) Int. J. Cancer 121:1585
Vintafolide clears quickly from circulation

Vintafolide PK in M109 Tumor-Bearing Balb/c Mice: 9.6 mg/kg i.v. bolus dose

*Concentration (ng/mL); Time (h); n=3

$t_{1/2} \sim 20 \text{ min}$

* No released DAVLBH identified in sera
Vintafolide is curative, specific and well-tolerated *in vivo*; the untargeted DAVLBH drug is not.

Re:etarfolatide

100-fold excess

*Vintafolide and DAVLBH tested at 1 µmol/kg, TIW 2 wk -regimen is MTD for DAVLBH and 1/5th MTD for Vintafolide*
Co-injected vintafolide blocks $^{99m}$Tc-etarfolatide uptake in FR-positive tissues

$^{99m}$Tc-etarfolatide

$^{99m}$Tc-etarfolatide + xs vintafolide

Balb/c-derived nu/nu mice bearing subcutaneous KB tumor xenografts. 500 nmol/kg of $^{99m}$Tc-etarfolatide i.v. without (panel A) or with (panel B) 50 µmol/kg of vintafolide. SPECT/CT performed on euthanized mice approximately 18 h post injection to identify tissue uptake. Green arrows, tumor; white arrows, kidneys. Residual radioactivity in the lower abdomen represents bowel clearance.
$^{99m}$Tc-etarfolatide predicts what tissues (lesions) will accumulate vintafolide
**FR expression, DAVLBH sensitivity, and vintafolide response in tumor models**

<table>
<thead>
<tr>
<th>Species</th>
<th>Tumor Line</th>
<th>Tissue origin</th>
<th>FR Quantity (pmol/mg protein)(^1)</th>
<th>Host</th>
<th>Sensitive to DAVLBH</th>
<th>Typical In Vivo response to Vintafolide</th>
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<tbody>
<tr>
<td>Human</td>
<td>A549</td>
<td>Lung</td>
<td>1.1</td>
<td>nu/nu</td>
<td>Yes</td>
<td>Unresponsive</td>
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<tr>
<td></td>
<td>Ov90</td>
<td>Ovarian</td>
<td>4.1</td>
<td>nu/nu</td>
<td>Yes</td>
<td>Durable PR; CR; cures</td>
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<tr>
<td></td>
<td>IGR-OV1</td>
<td>Ovarian</td>
<td>8.7</td>
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<td></td>
<td>KB</td>
<td>Nasopharyngeal</td>
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<td>nu/nu</td>
<td>Yes</td>
<td>Curative</td>
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<td>Mouse</td>
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<td>Breast</td>
<td>0.3</td>
<td>Balb/c</td>
<td>Yes</td>
<td>Unresponsive</td>
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<tr>
<td></td>
<td>J6456</td>
<td>Lymphoma</td>
<td>Not determined(^2)</td>
<td>DBA</td>
<td>Yes</td>
<td>Curative</td>
</tr>
<tr>
<td></td>
<td>M109</td>
<td>Lung</td>
<td>20</td>
<td>Balb/c</td>
<td>Yes</td>
<td>Curative</td>
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<tr>
<td></td>
<td>L1210A</td>
<td>Lymphocytic leukemia</td>
<td>95</td>
<td>DBA</td>
<td>No</td>
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</tbody>
</table>

\(^1\)Range for procured human OvCa specimens: 10-50 pmol/mg [Parker et al. (2005) Anal Biochem 338(2): 284-293.]

\(^2\)J6456 cells are FR-positive; we have not yet measured FR levels in xenograft tissue

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**Two requirements for an vintafolide response**

1) The tumor must be FR-positive \((^{99}\text{mTc-eta}rfo\text{lat}i\text{de})\)
2) The tumor must be sensitive to *vinca* alkaloids (DAVLBH)
Vintafolide is effective against triple negative MDA-MB-231 tumors.
Drug Combination Studies

Vintafolide can safely be combined with a wide variety of standard chemotherapeutic agents
Vintafolide + Doxil® combination is more active against M109 tumors than the individual drugs alone.

**M109 Tumor Growth**

- Control
- Vintafolide (3/5 cures)
- Doxil® (3/5 cures)
- Vintafolide + Doxil® (5/5 cures)

Vintafolide: 2 μmol/kg TIW, 2 wk
Doxil®: 4 mg/kg, weekly x 2

**Gross Toxicity**

%Weight Loss

*Supporting data for Endocyte’s PRECEDENT trial
Vintafolide + Doxil combination is effective against KB tumors
(human nasopharyngeal carcinoma)
Vintafolide + Doxil combination is effective against IGROV1 tumors (human OVCA)
Vintafolide combinations studies
Additive activity with Avastin® and topotecan; synergistic activity with docetaxel and cisplatin

Combination cohorts in blue

KB tumor model, nu/nu mice
EC145 synergizes with Taxotere® (docetaxel) against FR-positive KB tumors in nu/nu mice

*Similar results with paclitaxel (weekly or biweekly) and abraxane*
$^{99m}$Tc-Etarfolatide and Vintafolide

Clinical Utility
Selecting the right patient, for the right therapy, at the right time

$^{99m}$Tc-Etarfolatide: Companion Imaging

Vintafolide: Therapy
$^{99m}$Tc-Etarfolatide accumulates in FR-positive, not FR-negative lesions
Folate Receptor-Positive Lesions

Whole Body Scan

- **FR(100%)**
  - All target lesions positive
  - ~40% of pts are FR(100%)

- **FR(10-90%)**
  - Some target lesions positive
  - ~40% of pts are FR(10-90%)

- **FR(0%)**
  - No positive target lesions
  - ~20% of pts are FR(0%)
Reasons \(^{99m}\text{Tc}\)-etarfolatide (EC20) is better than IHC

1. Assessment of FR status
   a. EC20: Real-time analysis
   b. IHC: Archived tissue

2. Specimen analyzed
   a. EC20: Whole-body imaging
   b. IHC: Selected tissue specimen

3. Accessibility
   a. EC20: Reveals accessible tissue sites
   b. IHC: Indiscriminant (e.g. normal lung)

4. Functionality
   a. EC20: Binds only to functional FR
   b. IHC: Detects functional and non-functional FR
Vintafolide clinical trials

- **Single agent phase 2 studies**
  - FV-02: Recurrent ovarian cancer
  - FV-03: Recurrent NSCLC

- **Combination phase 2 studies**
  - PRECEDENT: Platinum-resistant ovarian cancer (Doxil/Caelyx)
  - TARGET: NSCLC

- **Combination phase 3 studies**
  - PROCEED: Platinum-resistant ovarian cancer

*Dr. Josep M. del Campo will discuss these studies following this presentation*
SMDC vs. ADC
Current therapeutic approaches in oncology

**ADC**
- Cell specific
- Receptor mediated
Ex: ado-trastuzumab emtansine, bretuximab

**SMDC**
- Cell specific
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- Penetrates solid tumors
Ex: vintafolide

**Farletuzumab**
- Cell specific
- May block ligand binding
- May block signal transduction
- May trigger ADCC
Ex: trastuzumab, cetuximab

**Traditional Chemotherapy**
- Not targeted
- Enters cancer and normal cells
- Narrow therapeutic window
Ex: cisplatin, paclitaxel, vinblastine

**Pathway Inhibitors**
- Not cell specific
- Passive uptake
- Intracellular targets (mTOR, TKIs, ALK, BRAF)
Ex: imatinib, sunitinib, everolimus

Many advantages for using small ligands versus receptor targeted antibodies

- SMDCs are rapidly cleared from body, reducing toxicity (e.g. non-specific uptake)
- Advanced linkers allows attachment of wide variety of molecules
- Receptor docking efficiency
- Superior manufacturing:
  - Synthetic ease
  - Homogeneity
  - Stability in storage and in vivo
  - Lower manufacturing cost
- Smaller size allows for better penetration of solid tumors
Better solid tumor penetration with smaller molecules

![Graph showing Rhodamine fluorescence (a.u.) over time (minutes) for Large MW payload and Small Molecule (1 min p.i.).]

- **Large Molecule**: Tumor Uptake 1st 65 min (red)
- **Small Molecule (1 min p.i.)**: Blood vessel

Endocyte

Tumor Uptake 1st 65 min (red)
Summary

- The folate receptor (FR)
  - Tumor biomarker
  - Expression is associated with aggressive disease

- $^{99m}$Tc-etagfolatide and vintafolide are highly specific for FR-expressing tissues

- Unlike ADCs, SMDCs (e.g. like etarfolatide and vintafolide) penetrate deeply into solid tumor tissue

- Vintafolide is very well tolerated
  - Can be combined with many standard anti-cancer agents due to its non-overlapping toxicity profile

- $^{99m}$Tc-etagfolatide is the best method for detecting active FR
  - Anatomically predicts where vintafolide will accumulate
  - Superior to alternative methodology (like IHC)
  - Real time detection
  - The only method for predicting possible response to vintafolide and other folate-based SMDCs
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Special thanks to our patients
Backup
The Importance of Folate

1. Role in human health
2. Role in cancer
3. Why dietary folate doesn’t interfere with vintafolide
Folate metabolism

DNA & RNA Synthesis

Methylation
- DNA
- RNA
- Protein
- Lipids

Folic Acid

DIETARY FOLATE

Supplements Fortified foods

DHFR Slow

DNA

R-methyl

R

THF

10-formyl-THF

Purine synthesis

10-formyl-THF

5,10-methenyl-THF

SHMT B6

5,10-methylene-THF

serine

glycine

B12

B6

B2

MTHFR

5-MTHF

TSH

dTMP

dUMP

5-formyl-THF

5,10-methenyl-THF

Betaine

Choline

Homocysteine

Cystathionine

Cysteine

SAM

SAH

Methionine

Enzymes:

DHFR Fast

5-MTHF
Oral folic acid is reduced and methylated to low affinity forms prior to entering the blood.
Bolus-administered SMDCs “displace” endogenous folates from the tumor surface

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Class</th>
<th>Relative Binding Affinity</th>
<th>Fold Weaker Affinity</th>
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<tbody>
<tr>
<td>Folic Acid (Pte-Glu)</td>
<td>Vitamer</td>
<td>1.00</td>
<td>1.0</td>
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<tr>
<td>5-formyl-tetrahydrofolate (Leucovorin)</td>
<td>Vitamer</td>
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<td>EC20 (etarfolatide)</td>
<td>Folate-based Imaging Agent</td>
<td>0.92</td>
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<td>EC145 (vintafolide)</td>
<td>Folate-SMDC</td>
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