Reviewing the Mechanism of Action for Cancer Therapeutics (FDG, FLT, Other Tracers)

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Wayne State University
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Disclosures: Anthony Shields

Radiology Corporation of America advisor and stock owner

Siemens, GE, and Bristol-Myers Squibb consultant
Guiding Cancer Therapy: Clinical Needs

Pre-Rx

Therapy

Post-Rx

Early

Mid-Rx

Aggressive Dz? Rx Targets

Response? yes/no How much?

Residual Disease?

Relapse Survival
Role of Imaging
Prognostic and Predictive Markers

• Biopsies can be used to measure thousands of genes and proteins in a specimen, BUT

• Gene or protein levels may not always measure the activity of a pathway

• Biopsies represent only a small area of a tumor and may overlook tumor heterogeneity

• Repeated biopsies are difficult, so following changes with time may not be possible
Timing of Imaging for Treatment Response

TIMING

Imaging RATIONALE

Pre-Treatment
aggressiveness, metabolism, pathways, receptors

Immediate Post-Rx (hours-days)
effects on metabolism, blood flow, receptors

Early (1 month or cycle)
metabolic response or proliferation changes

End (2-6 months)
re-stage and assess residual lesions
### Functional Imaging in Therapeutic Trials

Functional imaging is now regularly utilized in phase I and II trials.

<table>
<thead>
<tr>
<th>Imaging Modality</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDG PET*</td>
<td>Metabolism</td>
</tr>
<tr>
<td>FLT PET*</td>
<td>Proliferation</td>
</tr>
<tr>
<td>Annexin</td>
<td>Apoptosis, stress</td>
</tr>
<tr>
<td>$^{64}$Cu-ATSM, $^{18}$FMISO*</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>Labeled Drugs</td>
<td>Pharmacokinetics, pharmacodynamics</td>
</tr>
<tr>
<td>DCE MRI</td>
<td>Perfusion</td>
</tr>
<tr>
<td>Vascularity</td>
<td>Integrin agents</td>
</tr>
</tbody>
</table>

* Commercially distributed
FDG PET to Monitor Breast Cancer Response to Therapy


![Graph showing Pre-Rx, Chemotherapy, Surgery (Path Response), Baseline, and Mid-Rx phases.](image)

- **Baseline**
- **Mid-Rx**

![Bar chart showing FDG SUL values for Responders and Non-Responders at Day 0 and Day 63.](chart)

- **Responders**: Day 0 (P = NS), Day 63 (P < .001)
- **Non-Responders**: Day 0, Day 63 (N=11)
Neo-Adjuvant Therapy of Pancreatic Cancer

Pre-Tx | Post Cycle 1 | Post XRT

MRI

PET

Hodgkin Lymphoma: Early FDG-PET and Prognosis

M Hutchings, Blood 2006;107:52-9
Ovarian Cancer Imaging FDG PET after 1 Cycle of Chemotherapy

N = 33 treated with carboplatin based therapy.
Treatment of GIST with Imatinib

Baseline

24 Hours

Rapid declines in FDG uptake demonstrate direct affects on metabolism

Courtesy A. Van Den Abbeele

FDG Avid Tumors in Liver
Imatinib Affects Glucose Uptake

- GIST tumors associated with KIT gain of function mutations.

- High glucose uptake regulated by PI3K/AKT pathway.

- Imatinib interferes with AKT and hence glucose transport (Glut 4).

- Cell growth is AKT independent and may not always be linked to glucose use.

C Tarn, Cancer Res. 66:5477-5486, 2006
“I don’t reimburse. I validate. I listen and acknowledge how difficult it was for you to find a place to park.”
# ACRIN 6678: FDG-PET/CT as a Predictive Marker of Tumor Response and Patient Outcome: Prospective Validation in Non-small Cell Lung Cancer

## Study Design

### Eligibility

Patients with advanced NSCLC scheduled to undergo palliative chemotherapy with a two drug chemotherapy regimen (bevacizumab or cetuximab allowed)

### Registration and Choice of Arms

<table>
<thead>
<tr>
<th>GROUP A:</th>
<th>GROUP B:</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 PET/CT scans prior to chemotherapy – at least 24 hours between scans</td>
<td>1 PET/CT at baseline--pre-cycle 1 of chemotherapy</td>
</tr>
<tr>
<td>1 PET/CT scan after the 1&lt;sup&gt;st&lt;/sup&gt; cycle</td>
<td>1 PET/CT scan after the 1&lt;sup&gt;st&lt;/sup&gt; cycle</td>
</tr>
<tr>
<td>Follow-up CT scans per care standard</td>
<td>1 PET/CT scan after the 2&lt;sup&gt;nd&lt;/sup&gt; cycle</td>
</tr>
<tr>
<td>Observational follow-up for 1 year.</td>
<td>Follow-up CT scans per care standard</td>
</tr>
<tr>
<td></td>
<td>Observational follow-up for 1 year.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GROUP C:</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 PET/CT scans prior to chemotherapy – at least 24 hours between scans</td>
</tr>
</tbody>
</table>
Hodgkin’s lymphoma
CS I/II untreated
15-70 yrs
no LP nodular

Favorable

UnFavorable

H10-trial Stages I/II (#20051)

ABVD x 2
FDG-PET
ABVD x 2
FDG-PET
any outcome of FDG-PET
negative
positive
any outcome of FDG-PET
negative
positive

ABVD x 1 IN-RT 30 Gy*
ABVD x 2
BEACOPPesc x 2 IN-RT 30 Gy*
ABVD x 2 IN-RT 30 Gy*
ABVD x 4
BEACOPPesc x 2 IN-RT 30 Gy*

* + boost 6 Gy to residual
Thymidine Utilization

UdR → dUMP

Thymidylate Synthase

dTMP → DNA

Thymidine Kinase

DNA Polymerase
Thymidine Kinase Activity During Cell Cycle Progression

Small Cell Lung Cancer

**BEFORE**

- CT

**AFTER THERAPY**

- CT

**THYMIDINE**
- 20-60 minutes
- $K_{Tdr}$ ml/min/g
- 0.12
- 0

**FDG**
- 30-60 minutes
- $MR_{FDG}$ μmoll/100g/min
- 20.7
- 13.9

Limitations of $^{11}$C-Thymidine: Rapid Catabolism

Thymidine

\[ \text{Thymidine} \rightarrow \text{Thymine} \rightarrow \text{Dihydrothymine} \]

\[ \text{NH}_3 + \text{CO}_2 \rightarrow \text{BAIB} \rightarrow \beta\text{-Ureidoisobutyric Acid} \]
Imaging of Non-small Cell Lung Cancer

mTOR Kinase Inhibition: S6 Phosphorylation vs. FLT PET

- Mice implanted with U251 & A431 cell lines
- Treat x 5 days with CCI-779
- Image with FLT PET & measure S6 phosphorylation day 5.

<table>
<thead>
<tr>
<th></th>
<th>Size at 14 days</th>
<th>S6 Phosphorylation</th>
<th>FLT PET</th>
</tr>
</thead>
<tbody>
<tr>
<td>U251</td>
<td>200 mm³</td>
<td>↓↓</td>
<td>↓53%</td>
</tr>
<tr>
<td>A431</td>
<td>600 mm³</td>
<td>↓↓</td>
<td>↓4%</td>
</tr>
</tbody>
</table>

Effect of MEK Inhibition on Implanted Melanoma Cells in Mice

FLT Images

A

SKMEL-28

Control

Pretreatment

Day 5

Day 12

PD0325901

25 mg/kg

B

%Change SUV

SKMEL-28

Control

PD0325901

FLT

FDG

DB Solit, Cancer Res, 2007
FLT uptake is high in the tumor and declines with treatment.
hENT1 Transport Increases in Proliferating Cells

- Tracer uptake in cycling and non-cycling A549 adenocarcinoma cells at 60 seconds

Human equilibrative nucleoside transporter 1 (hENT1) uptake predominates with Tdr and FLT, but not FMAU

**Imaging Proliferation**

Nucleoside degradation

( irreversibly in vivo)

Thymidine → DNA-pathway

Thymine → TdRPase → Thymidine → TK1 → Thymidine-5'-MP → DNA

CO₂ → TdRPase → X → FLT → TK1 → FLT-5'-MP

**Images:**

PRE-TX

22 days

113 days

**FLT**
FLT Model in Breast Cancer: Full Compartmental vs Simplified Patlak

Blood FLT \xrightarrow{K_1} \text{Tissue} \xrightarrow{TK} \text{FLT-MP} \xleftarrow{k_2} \xleftarrow{K_1} \text{Tissue} \xrightarrow{TK} \text{FLT-MP} \xleftarrow{k_4} \text{Tissue}


Compartmental: 18 blood samples, 4 w/ metabolites measured
Patlak: 8 blood samples, 1 metabolite measured
FLT PET Imaging of Neoadjuvant Treatment of Breast Cancer: After One Cycle

<table>
<thead>
<tr>
<th>Decrease SUVmean</th>
<th>CR</th>
<th>Pathologic Response</th>
<th>Pathologic Response</th>
<th>Pathologic Response</th>
<th>Pathologic Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;15%</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>≥15%</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
FLT PET Breast Imaging After One Cycle

CT at a mean of 3.3 months

FLT PET Breast Imaging After One Week

<table>
<thead>
<tr>
<th>Pre Therapy</th>
<th>Post Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>tumour</strong></td>
<td><strong>tumour</strong></td>
</tr>
<tr>
<td></td>
<td><strong>vertebra</strong></td>
</tr>
</tbody>
</table>

7 days post-therapy

**RESPONSE** in a patient with grade II lobular ca

7 days post-therapy

**NO RESPONSE** in a patient with grade II IDC

Aim: determine FLT-PET response at 1 wk in pts treated with chemo; determine the reproducibility of serial scans
- 17 discrete lesions in 13 stage II–IV breast ca pts
- Imaging prior to and at 1 wk after treatment with chemo
- Clinical response assessed 60 days after commencing chemo
- 6 pts significant clinical response at day 60;
- These pts also had a significant reduction in FLT uptake at 1 wk
- Decrease in SUV at 1 wk discriminated btw clinical response and SD
- FLT response preceded tm size changes

FLT PET Imaging of Response to Gefitinib in Lung Cancer

H-J Sohn, Clin Cancer Res 2008
FLT PET Imaging of Response to Gefitinib in Lung Cancer

Percent decrease of SUV\textsubscript{max}:

- Nonresponder
- Responder

>10.9
Sens: 92.9
Spec: 92.9

H-J Sohn, *Clin Cancer Res* 2008
Lung Cancer Imaged with FLT: Response to ChemoRadiation in Stage III

Pre-Treatment

SUV Mean 3.6

Last Day of Radiation

SUV Mean 1.2
FLT PET In Non-Hodgkin’s Lymphoma

6 Patients
Treated: CHOP or R-CHOP

Imaged at:
Baseline
Day 7
Day 40

K Herrmann, Clin Cancer Res, 2007
ACRIN6688 Breast FLT Study Objectives

Primary: To correlate the percentage change in SUVs between baseline (FLT1) and early-therapy (FLT2) with pCR (as a dichotomous variable) to neoadjuvant chemotherapy of the primary tumor in locally advanced breast cancer (LABC)

• FLT-1 (baseline PET) must be completed within 4 wks prior to chemo initiation
• FLT-2 (early PET) must be performed 5-10 days after initiation of the first chemo cycle
• FLT-3 (post therapy PET) will be performed after the completion of chemo and within 3 wks prior to surgery
Obtain pre-treatment proliferative Indices

Establish Eligibility

• Baseline organ function
• Pathologically confirmed disease
• Determine primary systemic Rx

Obtain post-treatment proliferative Indices

Ki-67, mitotic index on bx sample or re-biopsy (if available)

Baseline Imaging

18FLT PET/CT (FLT-1)

Chemotherapy cycle 1

Early therapy Imaging

18FLT PET/CT (FLT-2)

Chemotherapy last cycle

Post-therapy Imaging

18FLT PET/CT (FLT-3)

Surgical Resection

• Pathologic response,
• Ki-67, mitotic index,
• Surgical specimens

ACRIN6688 Breast FLT Study Outline
Biologic Consequences of Tumor Hypoxia

• Mediated through HIF-1 and other factors
• Established resistance factor for XRT
• Implicated in resistance to systemic therapy
  – Alters cell cycle kinetics
  – May select cells resistant to apoptosis
• Hypoxia may be imaged with $^{18}$FMISO, $^{60}$Cu-ATSM, $^{18}$FIAZA, $^{18}$F-EF5
Imaging Hypoxia as the Accumulation of a Radiopharmaceutical

\[
\begin{align*}
\text{H}_2\text{O}_2 & \rightarrow \cdot\text{OH} & \cdot\text{O}_2^- \\
\cdot\text{O}_2^- & \rightarrow \text{R-NO}_2 \\
\text{R-NO}_2 & \rightarrow \text{R-N=O} & \rightarrow \text{R-NH}_2
\end{align*}
\]

\text{Nitroreductase enzymes} + e^- \rightarrow \text{R-N=O} + 4e^- \rightarrow \text{R-NH}_2

\text{Radical Anion}

\text{covalent bonding to macromolecules}

[F-18]-fluoromisonidazole

University of Washington

KA Krohn
Tumor Hypoxia Quantified by PET Predicts Survival

**FMISO PET**

- brain tumor
- H & N cancer
- cervical cancer

Spence
Clin Can Res, 2008

Rajendran
Clin Can Res, 2007

Dehdashti
Int J Radiat Oncol Biol Phys, 2003
[\textsuperscript{18}F]FMISO Sequential PET Imaging Studies

Tissue:Blood Ratio

Pre-treatment CT

Pre-treatment

After 1 fraction

Mid-radiation

End of radiation

D. Mankoff et al.
Tissue Hypoxia in Advanced Axillary Breast Cancer

[F-18]-FDG
Glucose Metabolism
SUV max = 10.2

[F-18]-Fluoromisonidazole (FMISO)
Hypoxia
Tumor/Blood max = 1.8

Significant FMISO uptake seen in ~ 30% of large breast cancers

JG Rajendran, Clin Cancer Res, 2004
Head and Neck Cancer: PET Imaging with FDG and FMISO

FDG Pre-TX
Primary and node visible

FMISO Pre-TX
Hypoxia in node

FDG 12 weeks
Post-radiation
Node active, primary not visible

Head and Neck Cancer: Time to Local Failure

Hypoxia vs Normoxia
Treated with XRT+ Cisplatin/5FU or Cisplatin-Tirapazamine
Tirapazamine is active in hypoxic tumors

Pharmacodynamic Imaging with Thymidine PET

- 5FU and other agents such as nolatrexed (AG337, Thymitaq) inhibit thymidylate synthase and thymidine synthesis.

- This results in a decline in thymidine in the cell, but an increase in deoxyuridineline.

- Tumor cells may compensate by a rapid increase in the activity of thymidine kinase (TK1), to use the salvage pathway.
Chemotherapy Effect on Thymidine Kinase Activity in Glioma Cells

Chemotherapy Effect on FLT Uptake in Glioma Cells

• Treated patients with GI cancers using nolatrexed
• Imaged with $^{11}$C-thymidine before and 1 hour after treatment
• Fractional retention of thymidine (FRT) increased 38% and SUV 43%

FLT Pharmacodynamics: One day of Capecitabine Treatment

Baseline

Day 1

SUV

4.6

12.8
Study Design for: Predictive Assays

Assay

+  -

Response Rate  Response Rate

• Examples of in vitro assay
  ER - Endocrine therapy for breast cancer
  TS - 5-FU for colon cancer
  HER2 - Trastuzumab for breast cancer
Breast Cancer: Imaging ER Receptors

Assessment of ER status is routinely done on breast cancer biopsies:

- ER staining with FISH or immunochemistry is not perfect
- ER status may change with time or metastases
\(^{18}\text{F-Fluoroestradiol (FES)}\): PET Estrogen Receptor (ER) Imaging

Provides a Quantitative Estimate of ER Expression

\[ \text{ER Concentration (fmoles/mg protein)} \times 10^{-4} \]

vs Radioligand Binding

vs IHC

F - Fluoroestradiol

Kieswetter


Mintun

Radiology 169:45, 1988

Peterson

FES Uptake Predicts Breast Cancer Response to Hormonal Therapy

**Example 1**
- Recurrent sternal lesion
- ER+ primary
- Recurrent Dz strongly FES+

**Example 2**
- Newly Dx’d met breast CA
- ER+ primary
- FES-negative bone mets

University of Washington

FES Uptake Predicts Response of Advanced Breast Cancer to Hormonal Therapy

Mortimer, J Clin Oncol, 19: 2797 2001

Linden, J Clin Oncol, 24: 2793, 2006

(P < .01 for both)
Breast Cancer: Imaging ER Receptors

PET Imaging of Fluoroestradiol (FES) can assess tumor levels:

H Linden, *J Clin Oncol*, 2006

47 patients ER+ treated with aromatase inhibitors

| Response Rate | Overall 23% 11/47 | PET ER negative 0% 0/15 | PET ER+ 34% 11/32 | PET ER+/Her2- 46% 11/24 |

ER present and active still <50% respond.

How do you show tumors in which ER drives growth?
Predicting Response to Hormonal Therapy: Metabolic Flare

FDG-PET before and after 7-10 days tamoxifen in 40 patients with advanced ER+ cancers

With change >10%:
PPV 91%
NPV 94%
for predicting response

Breast Carcinoma: FDG-PET

Prediction of Response to Hormonal Therapy

Before Hormonal Therapy

Responder

SUV=4.7

Non-responder

SUV=5.7

After Hormonal Therapy

SUV=7.5

SUV=5.5

Courtesy of Dehdashti & Siegel
Predicting Response to Hormonal Therapy: Estradiol Challenge → “Metabolic Flare”

- 51 women with advanced ER+ breast cancer
- FDG-PET before and after 30 mg estradiol × 1d
- With change ≥ 12%:
  - PPV 100%
  - NPV 94%
- for predicting response

Breast Cancer: Imaging ER Receptors

ER/PR negative tumors have a very poor response to anti-estrogen treatment.

PET imaging of ER improves assessment, but still only about $\frac{1}{2}$ respond.

Metabolic flare response with FDG further improves the assessment.

Late response assessment can also be done with FDG.

How do we best use these approaches in clinical and research settings?
Labeled drugs can be used to help monitor pharmacokinetics and pharmacodynamics.

Many investigators and companies are working to develop new drug delivery systems (such as nanoparticles).

PET can be used to monitor the in vivo biodistribution of such agents.
Labeled FAU for Phase 0 and Phase I Studies

The Model of FAU Retention Pathway

FAU $\xrightarrow{TK}$ FAU-P $\xrightarrow{TS}$ FMAU-P

DNA
Phase 0 Study of $^{18}$F-FAU with PET

Marrow

Tumor

FAU Blood Level

Time (min)

H Sun, *Cancer Chemother Pharmacol*, 2006
Increased FAU Retention Shown in Primary Breast Cancer (n=3)

Transverse

FAU Retention Curve
(Tumor, BKGD & Marrow)
Phase I Trial of FAU Treatment and Imaging $^{18}$F-FAU Pharmacodynamics

- Phase I dose-escalation protocol of FAU treatment and imaging now open
- FAU imaging done prior to therapy
- FAU imaging repeated on the first day of treatment at the complete of a 60 minutes infusion of therapeutic FAU
  - Comparing tumor FAU uptake and blood clearance at tracer and therapeutic doses
  - Comparing tumor FAU uptake and response to enzymes in pyrimidine metabolism
- FMAU imaging to assess clearance of the major metabolite of FAU
# FAU Therapy and Imaging Study Schema

<table>
<thead>
<tr>
<th>Screening</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAU Treatment</td>
<td>X X X X X</td>
<td>X X X X X</td>
</tr>
<tr>
<td>Biopsy</td>
<td>🔴</td>
<td>🔴</td>
</tr>
<tr>
<td>$^{18}$F-FAU PET</td>
<td>🔵</td>
<td>🔵</td>
</tr>
<tr>
<td>$^{18}$F-FMAU PET</td>
<td>🔵</td>
<td></td>
</tr>
</tbody>
</table>

**Enrollment**

- 1 2 5 29 56

**Toxicity Assessments**

- A. Shields, P. LoRusso
Imaging $^{18}$F-FAU Pharmacodynamics

A. Shields, P. LoRusso
FLT and FDG Can Be Used to Assess Treatment Response

- Studies have shown the FDG can be used to measure response in a number of tumors

- The use of FDG and FLT may depend in the tumor and treatment being used

- Further multi-center trials using FDG to assess treatment are being done and more are needed
The Usual Drug Development Pathway

Multiply patients needed for Phase I - III

X

Number of Tumor Types

X

Number of Different Schedules

> $1,000,000,000

to bring a drug to market

X about 500 new drugs
Development of New Therapies Is Very Expensive

- To develop CI-1040 (ONYX-015) Adenovirus would require a new plant.
- A phase II trial of 18 patients showed no response by CT or FDG PET.
- Further development was stopped by Pfizer.
- The cost of additional imaging can more than be made up by decreases in development costs.
- About 300 - 500 new drugs are in the development pipeline.

Phase II Trial of Intravenous CI-1042 in Patients With Metastatic Colorectal Cancer

By Oday Hamid, Mary L. Varteresian, Scott Wadler, J. Randolph Hecht, Al Benson III, Evanthia Galanis, Margaret Uprichard, Charles Omer, Paul Bycott, Robert C. Hackman, and Anthony F. Shields
Conclusions: Metabolic Imaging of Cancer Treatment

• A number of tracers and approaches are available and for metabolic imaging.

• Some approaches will provide predictive information prior to therapy.

• Imaging of pharmacodynamics can be done with labeled drugs and assessing flare responses.

• Imaging can complement measurements of gene, mRNA and protein measurements.

• Integrating new imaging approaches into routine treatment and research remains a challenge.
PET Requires a Group Effort

KCI:
T. Mangner
O. Muzik
S. Nimmagadda
K. Douglas
J. Lawhorn-Crews
U. Vaishampayan
P. Lorusso

E. Heath
S. Gadgeel
A. Duric
M.A. Reinoehl
H. Sun
O. Tehrani

NCI:
J. Collins

Support from NCI, DOD, DOE.
Incorporation of FLT PET into Early Clinical Trials

• A number of tracers and approaches are available and for metabolic imaging.

• One can image energetics, blood flow, proliferation, cell stress, and specific pathways.

• FLT imaging complements imaging with FDG and other tracers.

• Early changes in FLT, increased and decreased retention, can reflect PD changes.

• The best timing of imaging after therapy needs to be determined for each tracer and disease.
Lung Cancer: Pemetrexed + Cediranib Study

18F-FLT PET

cediranib-daily

-7 1 22 43

Pemetrexed

X X X X X
### Lung Cancer: Pemetrexed + Cediranib Study

Pemetrexed

<table>
<thead>
<tr>
<th>Scan</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>%Diff S1 vs S2</th>
<th>%Diff S2 vs S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUV Mean</td>
<td>6.78</td>
<td>3.37</td>
<td>5.33</td>
<td>-50.3%</td>
<td>58.2%</td>
</tr>
<tr>
<td>SUV Max</td>
<td>10.8</td>
<td>5.64</td>
<td>10.5</td>
<td>-47.8%</td>
<td>86.2%</td>
</tr>
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</table>
Lung Cancer: Pemetrexed + Cediranib Study

SUV mean

Baseline 1-week 4-weeks

AZD 217

AZD & Pem