Imaging Cancer Cell Proliferation: A Matter of Life or Death in Treatment Selection

Hubert Vesselle, Ph.D., M.D.

Division of Nuclear Medicine
Department of Radiology
University of Washington

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Why Image Tumor Proliferation?

- Increased cell proliferation is specific to tumors
- Tumor proliferation is evaluated at pathology by: mitotic figure counting (mitotic index), Ki-67 or PCNA IHC, S-phase fraction at flow cytometry
- Pathology limitations: 1) Requires a tissue sample (invasive); 2) a biopsy sample cannot reflect the heterogeneity of cellular proliferation across a primary tumor or multiple tumor sites
Why Image Tumor Proliferation?

- Tumor growth rate can be assessed by serial anatomic imaging (CT) but the correlation with tumor proliferation is poor because overall tumor size depends on non-tumoral and tumoral components.
- Tumoral component depends on proliferation and cell death.
- Advantages of PET imaging:
  - Can demonstrate heterogeneity of proliferation within a primary tumor or across multiple tumor sites (metastases).
  - Non-invasive.
  - Can be performed prior to surgery to allow treatment planning (ex: selection of neo-adjuvant chemotherapy in NSCLC).
Why Image Tumor Proliferation?  
The Case of NSCLC

- Tumor proliferation differentiates benign non-growing lesions from malignant lung lesions
- Tumor proliferation rate (PCNA, Ki-67, S-phase fraction) is prognostic in resectable NSCLC
- Assessing tumor proliferation in-vivo permits the evaluation of tumor response to therapy
- The hallmark of successful tumor response to therapy is the tumor’s inability to sustain proliferation
Thymidine Analogs

2-[C-11]Thymidine or [C-11]methyl-Thymidine

BUdR, IUdR

FFUdR

2'-Deoxy-pseudo-thymidine

[C-11]methyl-FMAU or [F-18]FMAU

AZT

[F-18]FLT
Enzymes of Thymidine Biochemistry

- **Uridine-PO₄ (dUMP)**
  - thymidylate synthase

**De novo pathway**:

- Thymidine
  - thymidine kinase
  - Thymidine-PO₄ → dTDP → dTT

**Salvage pathway**:

- Thymidine
  - thymidine phosphorylase
  - Thymine + ribose 1-phosphate

**Degradation pathway**:

- Thymine → DNA polymerase

DNA P

**Thymidine-PO₄**
NSCLC s/p Chemoradiotherapy: TdR is an Earlier Indicator of Response

<table>
<thead>
<tr>
<th></th>
<th>FDG</th>
<th>Thymidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Rx</td>
<td><img src="image1" alt="FDG Pre-Rx" /></td>
<td><img src="image2" alt="Thymidine Pre-Rx" /></td>
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<tr>
<td>3 wks Rx</td>
<td><img src="image3" alt="FDG 3 wks Rx" /></td>
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<td>10 wks Rx</td>
<td><img src="image5" alt="FDG 10 wks Rx" /></td>
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Shields A, Mankoff D, Krohn K, University of WA

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Challenges of [2-\(^{11}\text{C}\)]-TdR PET for Imaging Cellular Proliferation?

- Difficult radiochemical synthesis
- Short $T_{1/2}$
- Requires metabolite assays by HPLC
- Requires a parallel imaging study, $^{11}\text{CO}_2$ or $\text{H}_2^{15}\text{O}$
- Requires extensive modeling effort for data analysis
Characteristics of an Ideal Tracer for Clinical Tumor Proliferation Imaging

- Isotope half-life suitable for clinical studies (F-18 or longer)
- High tumor uptake
- Good tracer clearance
- No labeled metabolites
- Uptake reflects cellular proliferation
Thymidine Analogs

2-[C-11]Thymidine or [C-11]methyl-Thymidine

FU

FFUdR

2'-Deoxy-pseudo-thymidine

[C-11]methyl-FMAU or [F-18]FMAU

AZT

[2'-Deoxy pseudo-thymidine]
3’-deoxy-3’-[\(^{18}\text{F}\)]-fluorothymididine

[F-18]FLT is an investigational radiotracer not approved for routine clinical use

Salvage Pathway

Salvage pathway

Cell membrane

De novo pathway

DNA

Cell membrane

Grierson, Univ. of WA

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[F-18]FLT as a tracer

- FLT is monophosphorylated to FLT-MP by cytosolic thymidine kinase-1 (TK-1)
- TK-1 is upregulated just prior to and during the DNA synthesis phase of the cell cycle (Sherley, 1988)
- FLT-MP is unable to exit across the cell membrane (Schwartz, 2001)
- In A549 lung cancer cells FLT uptake correlates with S-phase and TK-1 activity (Rasey, 2002)
- In 22 asynchronously growing cell lines FLT uptake correlates with S-phase (r=0.76, p<0.0001) and [H-3]Thd uptake (r=0.88, p<0.0001) (Toyohara, 2002)
Imaging proliferation *in vivo* with [F-18]FLT and Positron Emission Tomography

In-Vivo Validation of 3’-deoxy-3’-\([^{18}F]\)fluorothymidine (\([^{18}F]\)FLT) as a Proliferation Imaging Tracer in Humans: Correlation of \([^{18}F]\)FLT Uptake by Positron Emission Tomography with Ki-67 Immunohistochemistry and Flow Cytometry in Human Lung Tumors


*Clin Ca Res 8:3315-3323, 2002*
[F-18]FLT PET Imaging

- 20-25min transmission scan over the lesion
- All 11 lesions imaged with 2-hr long dynamic PET starting 1 min prior to FLT injection
- 0.07mCi/kg FLT (5mCi max) injected IV over 1min.
- FLT uptake was quantitated by SUV & Patlak flux:
  - Max pixel SUV; all SUVs over 30-60min imaging segment
  - Partial volume corrected maxSUV (based on lesion dia.)
  - SUV and Patlak flux over an ROI including pixels > 50% max intensity
Lesion Histology

- 10 patients with 11 biopsy proven or suspected non-small cell lung cancer (NSCLC)
- 9 lesions resected; 1 open- and 1 core-biopsied
- 9 NSCLCs (2 Adenoca, 3SqC, 4LC); 2 benign inflammatory lesions
- Lesion size: 1.6cm to 7.7cm
- Ki-67 score = % of tumor cells stained with MIB-1 Ab per (4x) microscopic field (3mm diameter) or % of all cells stained in the inflammatory lesions
[F-18]FLT PET Imaging of lung lesions

Does FLT uptake by PET correlate with lesion proliferative rate assessed by Ki-67 immunohistochemistry?
Partial Volume Corrected FLT maxSUV and Ki-67 Score

Correlation: Spearman Rho = 0.86, P = 0.0013; Pearson r = 0.82, P = 0.004
Partial Volume and background Correction

- Max pixel recovery coefficients (RC) established for GE Advance PET scanner using spheres of known activity and size

<table>
<thead>
<tr>
<th>Sphere Diam. (cm)</th>
<th>Maximum Pixel RC</th>
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</thead>
<tbody>
<tr>
<td>3.3</td>
<td>1.01</td>
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<tr>
<td>2.8</td>
<td>1.01</td>
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<tr>
<td>2.2</td>
<td>0.88</td>
</tr>
<tr>
<td>1.6</td>
<td>0.57</td>
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<tr>
<td>1.2</td>
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<tr>
<td>1.0</td>
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</tr>
<tr>
<td>0.8</td>
<td>0.16</td>
</tr>
<tr>
<td>0.6</td>
<td>0.08</td>
</tr>
<tr>
<td>0.4</td>
<td>0.01</td>
</tr>
</tbody>
</table>

- Spheres imaged with same reconstruction parameters and filters used clinically (12mm Hanning filter, 55cm image diam., 128X128 array)
- corr maxSUV = backgrdSUV + [measured maxSUV - backgrdSUV]/RC
Patlak FLT Flux and Ki-67 Score

Correlation: Spearman Rho = 0.94, P < 0.0001; Pearson r = 0.86, P = 0.0007
<table>
<thead>
<tr>
<th>Size (cm)</th>
<th>PV-corr-maxSUV</th>
<th>Patlak</th>
<th>%Ki-67</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.9</td>
<td>1.30</td>
<td>0.82</td>
<td>2.5</td>
</tr>
<tr>
<td>2.1</td>
<td>3.54</td>
<td>1.97</td>
<td>20</td>
</tr>
<tr>
<td>2.6</td>
<td>4.44</td>
<td>2.59</td>
<td>60</td>
</tr>
<tr>
<td>1.6</td>
<td>6.99</td>
<td>5.44</td>
<td>90</td>
</tr>
</tbody>
</table>

H.Vesselle
UWMC
A correlation exists between proliferation (Ki-67 score) and FLT uptake in 11 lung lesions:

- FLT maxSUV: \( \text{Rho} = 0.76 \), \( P = 0.006 \)
- FLT PV-corrected maxSUV: \( \text{Rho} = 0.86 \), \( P = 0.0013 \)
- FLT Patlak flux: \( \text{Rho} = 0.94 \), \( P < 0.0001 \)
FLT Flux vs. Ki-67 Proliferation Score in Non-small Cell Lung Cancer

Spearman Rho = 0.88, P = 0.0001

3-Deoxy-3-[F-18]Fluorothymidine-PET for Noninvasive Assessment of Proliferation in Pulmonary Nodules


Cancer Research 62:3331-3334, 2002
30 SPNs evaluated 45 min after [F-18]FLT injection
8 benign and 22 malignant: 16 NSCLCs, 1SCLC, 1 NHL, 4 pulmonary metastases (1 colon, 2 RCCa, 1 osteosarcoma)
19 lesions resected, 11 biopsied
Ki-67 scores: 1-65% (malignant); <5% (benign)
Correlation between proliferation (Ki-67 score) and FLT uptake: r=0.87, p<0.0001
2 false (-): well diff NSCLC (Ki-67=10%), ca in situ
[F-18]FLT Uptake Correlates with Tumor Proliferation

- All studies of relatively small sample size (N≤30)

- Lung Tumors:
  - 4 studies found a correlation with proliferation (Vesselle, 2002; Buck, 2002; Yap, 2006; Yamamoto, 2007)

- Gliomas:
  - 2 studies demonstrated a strong correlation with Ki-67 score (Choi, 2005; Chen, 2005)
  - FLT uptake correlates with tumor grade (Choi, 2005)

- Lymphoma:
  - 1 Study found a correlation with Ki-67 score (Buck, 2006 (r = 0.84))
[F-18]FLT Uptake Correlates with Tumor Proliferation

- **Breast Cancer:**
  - 1 study found a correlation with Ki-67 proliferation score (Kenny, 2005)

- **Colorectal Cancer:**
  - 1 study demonstrated a correlation with proliferation score (Francis, 2003)

- **Head & Neck cancer:**
  - 1 Study found a correlation with Ki-67 score (Troost, 2007)

- **Sarcomas:**
  - 1 study found a correlation with IHC proliferation score and grade (Cobben, 2004)
Tumor Response Evaluation: FLT vs FDG

- Growth-arrested A549 cells placed 20hrs in fresh medium show a 6.4X increase in FLT uptake c/w 1.8X for DG (Rasey, *JNM*, 2002)

- FLT more sensitive than FDG for tumor response:
  - In mice bearing RIF-1 fibrosarcomas, 24 and 48 hrs after treatment with 5-FU, decreases in FLT uptake were larger than decreases in FDG uptake (Barthel; London, SNM2002)
  - Two weeks after 1st chemorx cycle FLT uptake of primary breast tumors decreased 23.9% c/w 4.6% for FDG (Silverman; UCLA, SNM2002)

- Contrary to FDG, FLT uptake is not affected by infection:
  - In rats bearing E. Coli infx, 24hr FLT T/N=1.14  c/w FDG T/N=3.13 (Carter et al, SNM2002)
Tumor Response Evaluation: Early Clinical Results with FLT PET

- **Lymphoma (NHL):** In 22 pts, mean of 68% decrease in FLT uptake just 2 days after one cycle of CHOP, 77% decrease at 7 days, and 85% at 40 days after a second cycle (Herrmann, 2007)
  - FLT uptake after one week following the 1st cycle correlated with eventual clinical response at 3 months (Herrmann, 2007)

- **Breast Cancer:**
  - In 14 patients with metastatic breast cancer, the percent decrease in FLT uptake over the first 2 weeks of therapy correlated with CA 27.29 marker levels at 5.8 months later (r = 0.79) (Pio, 2006)
  - Another study in 13 patients found a greater decrease in FLT uptake after 1 week of chemotherapy for those that had a subsequent complete or partial response compared with those who developed stable disease (Kenny, 2007)

- **NSCLC:** Work in progress
Roles of Proliferation Imaging: The case of NSCLC

- **Stages I, II and T3N1M0:** Prognostic evaluation of early stage resectable NSCLC to identify those patients at highest risk of recurrence who would benefit from adjuvant chemotherapy.

- **Stage IIIA-N2 and IIIB:**
  - Define areas of proliferation for optimal selection of RT fields.
  - Optimize chemo regimen selection by demonstrating early response.
  - Improve assessment of tumor and mediastinal nodes response to therapy at end of therapy.
  - Identify areas of residual tumor proliferation to define fields for RT boost therapy.

- **Stage IV:** Optimize doublet chemo regimen selection by demonstrating early response.
NSCLC with Mediastinal Adenopathy

FDG

FLT
FLT PET
Pre- and Post-Chemotherapy

Pre-chemotherapy

Post-chemotherapy

FLT maxSUV = 3.0

FLT maxSUV = 2.6
FLT PET
Pre- and Post-Chemoradiotherapy

45.12 MAX SUV 15.4

83.Q9 MAX SUV 12.1

FLT maxSUV = 4.3

FLT maxSUV = 1.27

FLT PET Pre-Rx

FLT PET Pre-chemoRT

FLT PET Post-chemoRT

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<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
</tr>
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<tbody>
<tr>
<td><strong>FDG</strong></td>
<td>![Pre-FDG]</td>
<td>![Post-FDG]</td>
</tr>
<tr>
<td><strong>FLT</strong></td>
<td>![Pre-FLT]</td>
<td>![Post-FLT]</td>
</tr>
<tr>
<td><strong>Rx</strong></td>
<td>Cisplatin + etoposide + 6100 cGy</td>
<td>2 months Post-chemoRT</td>
</tr>
</tbody>
</table>
Effect of Radiation on FLT Uptake

After 1000 rads

After 5000 rads

Differential uptake of FLT in proliferating bone marrow
FDG uptake in inflammation, no uptake of FLT

FDG post-therapy (5940 rads)

FLT post-therapy
FDG PET and FLT PET Pre- and Post-ChemoRT

Pre: Post-obstructive Pneumonitis

FDG

Rx: Cisplatin + etoposide + 4200 cGy

Post

2 months Post-chemoRT
Esophagitis post-Radiotherapy

FDG

FLT
A strong correlation exists between proliferation (Ki-67 score) and FLT uptake in several cancer types.

FLT uptake may help distinguish benign from malignant lesions in the lung.

FLT uptake may improve grading for certain tumor types.

FLT uptake will likely be of prognostic value in resectable NSCLC.

FLT PET is still being investigated in tumor response to therapy.

FLT has advantages over FDG in inflammation/infection.
Contributors

Dept of Surgery
Eric Vallières, Michael Mulligan
Doug E. Wood

Dept. of Pathology
Rodney A. Schmidt
Diana Jordan

Division of Medical Oncology
Renato Martins and Keith Eaton

Division of Nuclear Medicine
John Grierson
Kenneth A. Krohn
Jeanne Link
Mark Muzi
Lanell M. Peterson
Jeffrey M. Pugsley
Alex Salskov
Linda Wiens