PET Imaging of HYPOXIA
What questions can hypoxia imaging help us answer?
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Associate Director of Cancer Imaging Program, Fred Hutchinson Cancer Research Center
Professor of Diagnostic Radiology, Oregon Health & Science University

Why is O₂ important?
What happens when it is absent?
Acute hypoxia results in cell death via necrosis.
Chronic hypoxia leads to adaptive changes to promote survival.
Cycling hypoxia: intermittent spatial and temporal fluctuations. Increased ROS

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The need for a fast-reacting O₂ Sensor
Hypoxia Inducible Factor: HIF

HIFs lead to up regulation:
Angiogenesis
Metabolism
Motility/Mets
Inflammation
pH regulation
Proliferation
100+ genes

Thinking ahead: What do these results suggest about the anticipated contrast in images of tissue oxygenation?


PO₂ Histograms and Microvascular of Normal Tissues and Malignant Tumors

Thinking ahead: What do these results suggest about the anticipated contrast in images of tissue oxygenation?

Strategies for Imaging Hypoxia (including preclinical evaluation)

- No unique $O_2$Hb level, %Hb sat’, tissue $PO_2$
- Gradient from supply (normoxic) to site of consumption is highly regulated
- Hypoxia depends on demand of individual cells / tissues—substantial heterogeneity, spatial & temporal
- Identification of hypoxia has implications in many medical settings

Hypoxia is a phenomenologic concept

The Problem: Identifying regional hypoxia in vivo

Tumors are characterized by being hypoxic, and hypoxia is known to cause resistance to photon radiotherapy and some chemotherapies.

- Directly measure $PO_2$ by image-guided electrodes
- Image nitroimidazoles with PET
- Endogenous markers expressed under hypoxic conditions

Hypoxia also leads to disease progression in tumors with high genomic instability

How can we use hypoxia information to select the best therapy and improve our patient’s outcome?

Desirable Characteristics of a Measurement Technique for Hypoxia

- Distinguish normoxia / hypoxia
- At a $PO_2$ relevant to tumors
- Evaluate chronic & transient hypoxia
- Reflect cellular $PO_2$, not vascular
- Applicable to any tumor site
- Complete loco-regional evaluation
- Simple, non-toxic, repeatable
- Easily comparable between labs

Now there are many other hypoxia imaging agents: FAZA, FETA, EF5

Pre-clinical Studies of FMISO: In Vitro

- Numerous cell lines
  - EMT6, RIF1, V79 fibroblasts, 36B10
- Grown in chambers with controlled and measured $O_2$ partial pressures
- Grown as tumor spheroids

18FMISO Images

Non-small cell lung cancer
Reoxygenation

Tissue: Blood Ratio

- 2.8
- 2.1
- 1.4
- 0.7

Wui-jin Koh
Patient was continued with photon therapy.
Post neutron image shows no reoxygenation.
Treated with 15 Gy neutrons, 3/ wk.
MRI, FDG, FMISO pre therapy.

Hypoxia Imaging in Brain Tumors

MRI

PET Scan: 20 min SUV, 2 hrs after injection.
VOIs: VOIs from registered MRI, CT, PET.
Blood: 3 venous blood samples, SUV units.
Analysis: Tissue-to-blood ratio (TB).
Maximum TB value (TBmax) = 1.2.

Color-coded PET FMISO images reveal hypoxic pixels above TB = 1.2.

Spence & Muzi

FMISO Imaging Methods

Hubert Vesselle

Hypoxia Imaging: Glioma

53 yo man
Post resection
HV=129 cc
T/Bmax=3.0
HV=129 cc
T/Bmax=3.0

Patients with the greatest amount of hypoxia?
Which patient has the more clinically significant hypoxia?

Representative FMISO Images in Brain Tumors

Low uptake
53 yo man
Post resection
HV=27 cc
T/Bmax=1.5

FMISO PET Images provide additional insight

Eppendorf pO2 Imaging Methods

Spence & J Douglas

University of Washington, Seattle

49 yo F with rt frontal GBM resected 9/19
MRI, FDG, FMISO pre therapy.
Treated with 15 Gy neutrons, 3/ wk.
Post neutron image shows no reoxygenation.
Patient was continued with photon therapy.

FMISO Images

Representative FMISO Images in Brain Tumors

High uptake
55 yo woman
Post bx
HV=53 cc
T/Bmax=1.5

FMISO (134-154m)

FMISO (144-164m)

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FMISO (144-164m)

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**Spatial Correlation: FMISO and FDG**

- Little brain/tumor overlap for FMISO, significant for FDG. Tracers provide different information.
- Usually hypoxia results in increased glycolysis (FDG), but hypoxic pixels cover a wide range of FDG SUV.

**Data Analysis for $^{18}$FMISO**

Advantages of a simple uptake mechanism

- Early biodistribution reflects BF; late reflects tissue/plasma partition coefficient
- Penetrates BBB freely with no protein binding
- Any metabolites do not enter cells and clear rapidly
- RESULT: FMISO is imaged without a need to normalize for delivery or injected dose (SUV)—just blood level at time of imaging.
- Quantify as hypoxia volume: volume of pixels with T/B > cut-off which is shown to be robust.

**MESSAGE:** Kinetic models of dynamic FMISO are not likely to be clinically significant.

**Hypoxia Imaging to Predict Outcome**

Because hypoxia is a common phenotype for tumors, it should be useful in a wide range of cancers.

- Head & neck squamous cell carcinoma
- Sarcoma (soft tissue and bone)
- Primary brain tumors
- Breast cancer

**Imaging Hypoxia in Head & Neck Cancer**

Does FMISO give additional prognostic value over that of FDG?

**Cox Regression Univariate Statistics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Node</td>
<td>1.84</td>
<td>.01</td>
</tr>
<tr>
<td>Stage</td>
<td>1.33</td>
<td>.17</td>
</tr>
<tr>
<td>Age</td>
<td>1.10</td>
<td>.55</td>
</tr>
<tr>
<td>FDG SUV</td>
<td>1.44</td>
<td>.14</td>
</tr>
<tr>
<td>T/B$_{max}$</td>
<td>1.68</td>
<td>.002</td>
</tr>
<tr>
<td>HV (sqrt)</td>
<td>1.46</td>
<td>.04</td>
</tr>
</tbody>
</table>

**FMISO PET Predicts Outcome for GBM Patients**

(Spence, Clin Cancer Res 14:2623, 2008)

FMISO not hot

Hypoxic

HV $<12.8$ cm$^3$

T/B $<2.06$

Not Hypoxic

Classification not dependent on cutoff parameter.
Hypoxia in Prostate Cancer
Impact on disease recurrence after RT

- Single-center prospective trial of 279 pts with histologically confirmed adenocarcinoma of the prostate, median F/U 6.6 yrs
- T1&T2, Gleason 6-9, median 7; PSA 7.8 (range 0.9-33) ng/mL
- PO2 measured using US-guided trans-rectal needle electrodes, 40-80 readings, 2-4 trucks
- Therapy: 76-80 Gy (89%), NO hormonal treatment (79%)
- Time to biopsy: 34 mo (range 20-107)
- Correlate with HP(PO2<10 mmHg)
- Primary endpoint: biochemical failure
- Biochemical recurrence in 79 pts
- Secondary: local recurrence by US/MRI
- Tested HP(PO2) as independent predictor

![Grand median PO2, n=279](image)


Hypoxia Imaging to Test Important Hypotheses

Because hypoxia is a common phenotype for tumors, it should impact treatment in a wide range of cancers.

Improving oxygenation
Radiation Therapy boost for glioblastoma
Image-guided radiation treatment planning (HNSCC)
Selecting pts for hypoxia-selective cytotoxins
Hypoxia and anti-angiogenic therapies

Survival for patients with advanced head & neck tumors divided by median Eppendorf PO2≤2.5 mmHg

CONCLUSIONS

- Pretreatment tumor PO2 was a significant prognostic factor for survival after RT alone or in combination with surgery, chemotherapy or radiosensitizers.
- Analysis of PO2≤2.5 mmHg as covariate supports a monotonic relationship between oxygenation and prognosis rather than a threshold effect.
- Limitations: meta anal, did not assess local control
- What are therapeutic strategies to target or overcome hypoxia in the clinic?

![Survival](image)


Steps Toward Validating PET for Image-Guided Radiation Therapy

- Select imaging procedure mechanically coupled to therapy
- Plan both conventional therapy and IGRT
- Carry out conventional treatment plan
- Test Hypothesis: Local recurrence is more frequent in regions that would have been treated more aggressively, had the IGRT plan been used.
- Work is in progress for FMISO images of HNSCC pts treated with radiosensitizers, Hypoxia cytotoxins

This is a general strategy toward integrating experimental diagnostics with experimental therapeutics.
FMISO Imaging and IMRT Can be Used to Plan a 10 Gy Boost Volume: Will this make a difference?

- Experiment: Plan dose escalation based on FMISO-PET (HV 0 - 23 cm²)
  - Boost from 70 Gy to 80 Gy to hypoxic sub-volumes
  - No increase in dose to normal critical tissues
  - Pinnacle to build 7 equiangular beam profiles
- Calculate Tumor Control Prob using LQ Model
  - Using clinically determined α=0.47 Gy⁻¹, width 0.8 Gy⁻¹
  - OER ~1.5, α/β=12 (from Radiat Ther Oncol 69:267, 2003)
- ~40% improved TCP when a 10 Gy boost is applied to FMISO volume
- Normal tissue complication probability increased by only ~0.5%
- Co-registered FMISO-PET/CT can be used to develop clinically feasible RTPs with higher doses to hypoxic regions, increasing the predicted TCPs.

Significant results from MSKCC (84 Gy): NY Lee, IJROBP 70:2, 2008.

Hypoxia-Selective Treatment for HNSCC

- Hypoxia is a common tumor phenotype.
- Tirapazamine, a hypoxic cytotoxic, potentiates RT and cisplatin.
- HNSCC, stage III / IV, no distant metastases, no prior therapy
- RT 70 Gy/7wks plus cisplatin + 5FU or cisplatin + tirapazamine
- Outcome: Clinically, radiologically, metabolically free of disease at 2 yrs, with good salivary function

Tirapazamine

Loco-Regional Failures

FDG

FMISO

Treatment

(n=45)

RT/cis/5FU

RT/TPZ/cis

T2N2b squamous cell ca of the pyriform fossa / left node mass

FDG Baseline

FMISO Baseline

FDG 12 wks after Rx

CR in non-hypoxic 1°

No response in hypoxic node

Impact of FMISO-PET Imaging on DVH

Prescription: 70 Gy to primary PTV, 50 Gy nodes, 10 Gy boost FMISO

Will a 10 Gy boost improve outcome?

<table>
<thead>
<tr>
<th>Tumor control probability</th>
<th>Normal tissue complication</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCP (70 Gy)</td>
<td>NTCP (70Gy)</td>
</tr>
<tr>
<td>68.8</td>
<td>1.95</td>
</tr>
<tr>
<td>45.7 to 81</td>
<td>0 to 6.2</td>
</tr>
<tr>
<td>TCP (80 Gy)</td>
<td>NTCP (80Gy)</td>
</tr>
<tr>
<td>87.9</td>
<td>4.7</td>
</tr>
<tr>
<td>76 to 99</td>
<td>0.1 to 19</td>
</tr>
<tr>
<td>% Increase</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>17 - 104</td>
</tr>
</tbody>
</table>

Is local progression spatially correlated with focal pre-treatment FMISO PET?

- Pre-treatment FMISO uptake is spatially correlated with the location of subsequent tumor recurrence.
- Thus, radiation boost to the FMISO volume may improve local control.

These results support a Phase II trial to investigate the impact of a sub-volume boost in a prospective manner.
Survival by treatment & hypoxia
Stage III & IV HNSCC

| FMISO-PET imaging | 70 Gy / 7 wks | PLUS CIS and either Tirapazamine or 5-FU |

Rischin D, Hicks RJ, Fisher R, et al., J Clin Oncol 2006; 24:2098-2104

Phase II Study of a Radiotherapy Total Dose Increase in Hypoxic Lesions Identified by 18F-Misonidazole PET/CT in Patients with Non–Small Cell Lung Carcinoma (RTEPS Study)

- Consortium of 15 academic PET centers in France
- FMISO-PET for RT boost in patients with NSCLC
- Locally advanced, no contraindication to chemorads (cis-platin plus either etoposide or vinorelbine)
- Protocol: escalate RT to hypoxic sub-regions
- Followed dose-limiting constraints for spinal cord (46 Gy) and lungs (<30% of volume with ~20 Gy)
- Isotrop expansion based on FDG PET/CT
- Planned dose 66 Gy, escalate to 86 Gy if FMISO pos
- Response at 3 mo assessed by RECIST 1.1 criteria


Study Details

- 54 pts based on intent to treat: 7 women, 47 men
- Histology: 26 squamous, 21 aden, 7 undifferentiated
- Most were IIIA or IIIB
- FMISO results: 34 positive, 20 negative
- Boost given to 24 of the 34 positive patients. Ten pts not escalated because of organ-at-risk constraints.
- Response rate (3 mo): 57% (95% confid int 43-71%)
- Planned dose 66 Gy, escalate to 86 Gy if FMISO pos
- Response at 3 mo assessed by RECIST 1.1 criteria
- Secondary endpoints: toxicity, DFS, OS at 1 yr
- Did not attribute death to local recurrence or distant failures


Results: RT boost in NSCLC

- FMISO uptake strongly associated with poor prognosis
- Poor prognosis could not be reversed by RT doses up to 86 Gy
- DFS was longer in FMISO negative pts (RED)
- RT boost was not associated with DFS when adjusted for FMISO status—boost BLUE, standard GREEN


Future Role of Hypoxia Imaging

Hypoxia is a common phenotype for tumors and it can be useful in guiding treatment for a wide range of cancers. So what’s next?

- Much stronger synergism between Radiation Medicine and molecularly targeted therapy
- Precision medicine

Looking to the Future of Radiation Oncology
NCI Workshop for Preclinical and Clinical Development of Radiosensitizers, August 2012

“Although there continue to be new advances in how treatment is planned and delivered, advances in the field of radiation oncology have gradually met a plateau where the therapeutic index cannot be further improved because of physical limitations and dose-limiting structures.” JNCI 2013; 105(10):686-93.

What does this mean for the future of our discipline?
What does this imply for your career?
Looking to the Future of Radiation Oncology

“Although there continue to be new advances in how treatment is planned and delivered, advances in the field of radiation oncology have gradually met a plateau where the therapeutic index cannot be further improved because of physical limitations and dose-limiting structures.”

“For additional progress to be made, technological innovations must be complemented by biological innovations, such as development of novel radiosensitizing agents and biology-driven patient selection.”


Physics is ahead of biology. It’s time to help the biologists.

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**Clinical Development of Cancer Drugs in Combination With External Beam Radiation Therapy: US Food and Drug Administration Perspective**

Amanda J. Walker, MD, Hyun Kim, MD, Halah Saber, PhD,
Paul G. Kluetz, MD, Geoffrey Kim, MD, and Richard Pazdur, MD

Office of Hematology and Oncology Products, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, Maryland

- Half of pts with cancer who are cured will have RT as part of their care.
- Addition of systemic therapies to RT may afford additional radiosensitization or synergistic benefit.
- Since 2006 more than 250 new drug applications have been approved by OHOP/FDA.
- Number approved for use with radiation: ONE.
- Why? Limited regulatory precedence and challenges in clinical trial design that includes radiation. Imaging makes it even more challenging.


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**Summary: Future role of hypoxia imaging**

- Radiation boost protocols
  - Target the worst disease; boost T/Bmax from imaging
  - Possibility of reducing margins in some cases
  - Hypoxic cells may or may not be treatment limiting
- Combination RT and drugs
  - Hypoxia mediates differential response to anti-EGFR Rx
  - Hypoxia signaling through mTOR and unfolded protein resp
  - HIF links to activation of PI3K, MEK, AKT, other pathways
  - Anti-angiogenesis treatment may exacerbate hypoxia
- Era of PRECISION MEDICINE
  - Determine the points within pathways that make hypoxic tumors vulnerable to molecular agents added to radiation


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**Hypoxia as a target for synergistic therapies**

- Hypoxia is a well-known factor in response to rads
  - High or low LET, fractionation, internal emitters
  - Hypoxia activated pro-drugs
  - Molecular targets: HIF, ARNT, PHD enzymes, UPR (SCD1 inhibition), mTOR, ER stress
  - Inhibitors of glutaminase, fatty acid synthase
  - Hypoxia is a well-known factor in response to numerous chemotherapeutics
  - Checkpoint immunotherapy can radiosensitize RT
  - Imaging methods are available for selecting an appropriate patient cohort for such trials.

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**Opportunities for combining molecularly-targeted therapies with radiation**

- Single-agent activity that is additive to rads
  - Targets: EGFR, ALK/ROS1, TP53
  - Radiation enhancement by direct sensitization
    - Targets: PARPi, cell-cycle regulators, repair inhibitors
  - Antiangiogenic agents, REDOX manipulation
  - Determining sequencing of drug/rads combos
    - Rads most effective at G2/M; most resistant at G1/S

Each of these approaches needs a complementary biomarker

Requires understanding the complexity of human cancers.