PET Imaging of HYPOXIA

What questions can hypoxia imaging help us answer?

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No Disclosures to report
FMISO is used under an IND

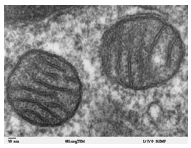
Why is O_2 important?

What happens when it is absent?

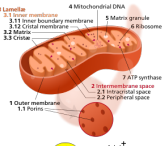
Acute hypoxia results in cell death via necrosis.

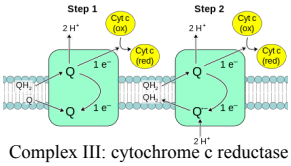
Chronic hypoxia leads to adaptive changes to promote survival.

Cycling hypoxia: intermittent spatial and temporal fluctuations. Increased ROS

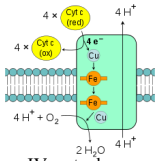


Chemistry in the MITOCHONDRIA produce electrons and intermembrane H^+ gradient to make ATP





Complex III: cytochrome c reductase

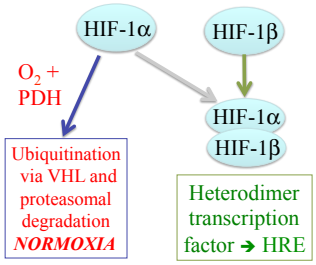


IV: cytochrome c oxidase

$$O_2 \xrightarrow{e^-} \bullet O_2^- \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} \bullet OH \xrightarrow{e^-} H_2O$$

The need for a fast-reacting O_2 Sensor

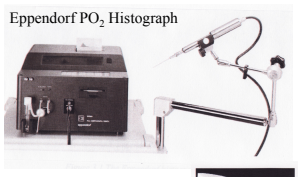
Hypoxia Inducible Factor: HIF



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

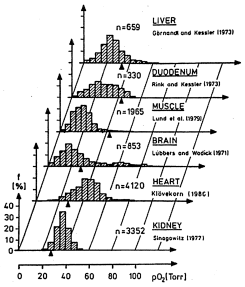
Direct Measurement of PO_2 by Electrode

O_2 partial pressure (mm Hg)



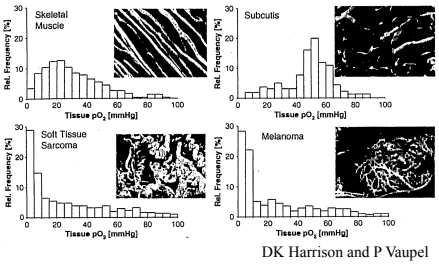
The Au electrode is in a Pt needle. Electrolysis of O_2 gives a current proportional to PO_2.

0.17 µm Au



DK Harrison and P Vaupel, *Oxygen Transport to Tissues* 2014:812;25-31.

PO_2 Histograms and Microvascular of Normal Tissues and Malignant Tumors



DK Harrison and P Vaupel

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_2Hb level, %Hb sat'n, 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 phenomenolgic 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.

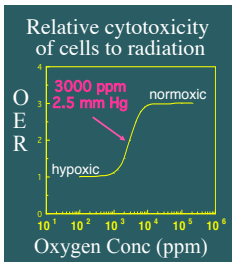
- Directly measure PO_2 by image-guided electrodes
- Image nitroimidazoles with PET
- Endogenous markers expressed under hypoxic conditions

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

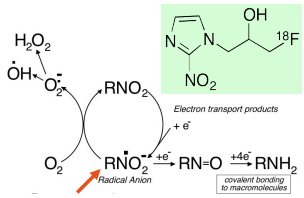
How can we use hypoxia information to select the best therapy and improve our patient's outcome?

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
- Provide complete loco-regional evaluation
- Simple, non-toxic, repeatable
- Easily comparable between labs



A Positive Image of the Absence of O_2



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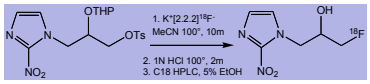
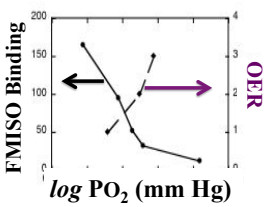


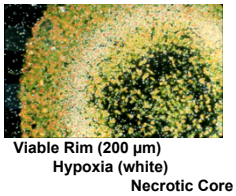
Simple "box" synthesis
20% RCY EOS
5 Ci/μmol

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

Pre-clinical Studies of FMISO: *In Vitro*

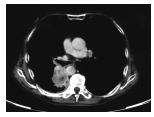
- Numerous cell lines
  - EMT6, RIF1, V79 fibroblasts, 36B10
- Grown in chambers with controlled and measured O_2 partial pressures
- Grown as tumor spheroids

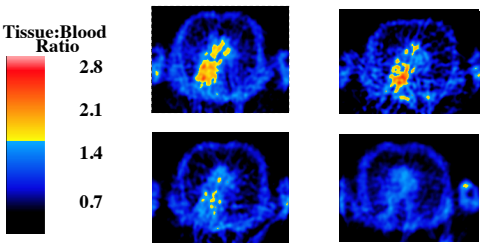




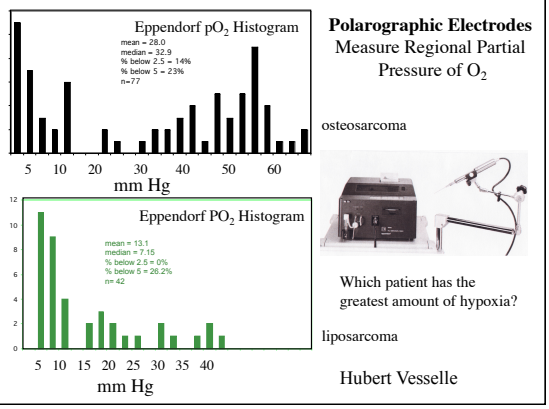
^{18}FMISO Images

Non-small cell lung cancer
Reoxygenation





Wui-jin Koh



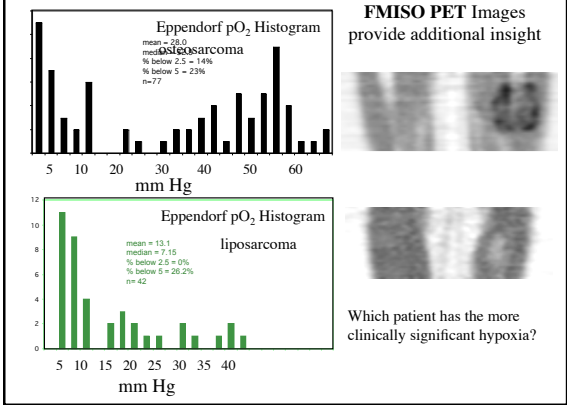
Polarographic Electrodes Measure Regional Partial Pressure of O_2

osteosarcoma

Which patient has the greatest amount of hypoxia?

liposarcoma

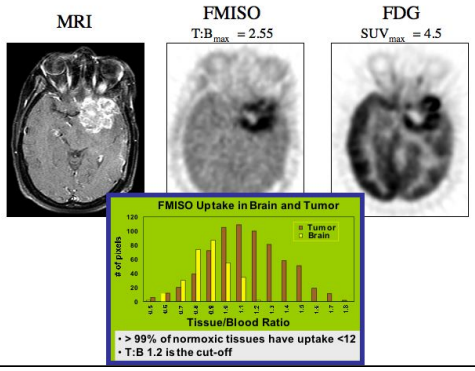
Hubert Vesselle



FMISO PET Images provide additional insight

Which patient has the more clinically significant hypoxia?

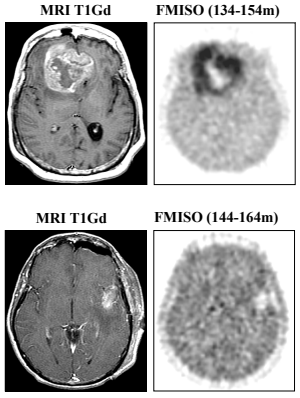
Hypoxia Imaging: Glioma



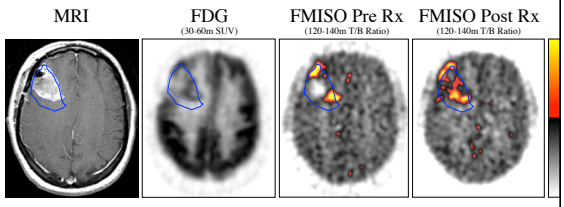
High uptake
55 yo woman
PostBx
HV=129cc
T/Bmax=3.0

Representative FMISO Images in Brain Tumors

Low uptake
53 yo man
Post resection
HV=2.7cc
T/Bmax=1.5



Hypoxia Imaging in Brain Tumors



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

The thermometer is B/W in the normal range (< 1.2) and changes to color above the hypoxia threshold of 1.2.

Spence & J Douglas

University of Washington, Seattle

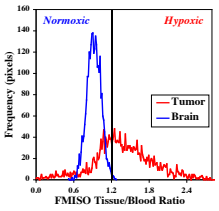
FMISO Imaging Methods

PET Scan: 20 min SUV, 2 hrs after injection

VOIs: VOIs from registered MRI, CT, PET

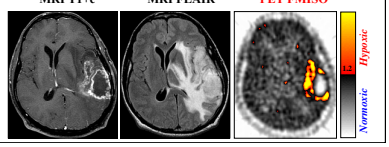
Blood: 3 venous blood samples, SUV units

Analysis: Tissue-to-blood ratio (TB)
Maximum TB value (TBmax)
hypoxic volume (HV) TB pixels>1.2

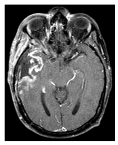


Color-coded PET FMISO images reveal hypoxic pixels above T:B = 1.2

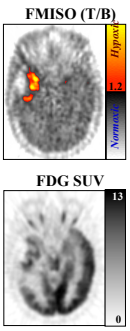
Spence & Muzi

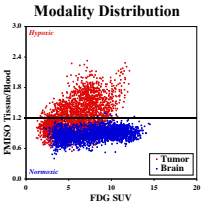


Spatial Correlation: FMISO and FDG



Patient: 34 yo male, no RT, frontal GBM, Post Surg





- 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.

Muzi & Spence

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

Variable	Hazard Ratio	p-value
Node	1.84	.01
Stage	1.33	.17
Age	1.10	.55
FDG SUV	1.44	.14
T:Bmax	1.68	.002
HV (sqrt)	1.46	.04

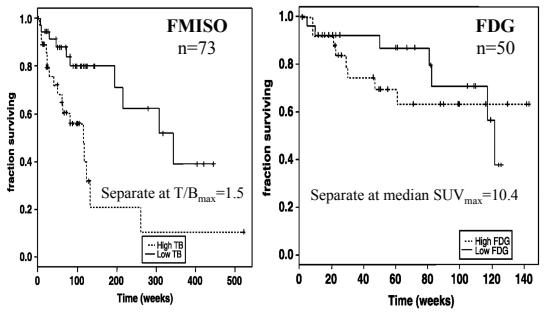
FMISO
$T:B_{max}$ = 1.51

FDG
SUV_{max} = 10.2

Evaluation of pre-therapy FMISO scans:
73 patients, previously untreated
28 deaths

Advanced disease: biopsy-proven SCC
59% T3 & T4
63% N2 & N3
79% showed significant hypoxia

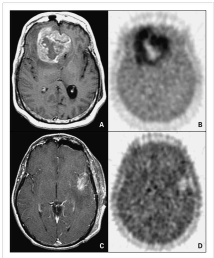
Kaplan-Meier Overall Survival HNSCC



Rajendran, *Clin Cancer Res* **12**:5435, 2006.

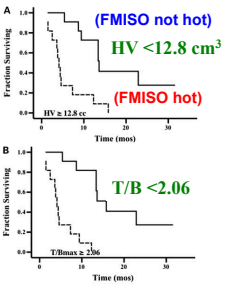
FMISO PET Predicts Outcome for GBM Patients

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



Hypoxic

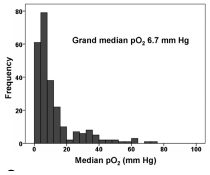
Not Hypoxic



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
- PO_2 measured using US-guided trans-rectal needle electrodes, 40-80 readings, 2-4 tracks
- Therapy: 76-80 Gy (89%), NO hormonal treatment (79%)
- Time to biopsy: 34 mo (range 20-107)
- Correlate with HP_{10}, PO_2 ≤10 mmHg
- Primary endpoint: biochemical failure
- Biochemical recurrence in 79 pts
- Secondary: bRFR at site of O_2 measure
- Secondary: local recurrence by US/MRI
- Tested HP_{10} as independent predictor



Milosevic M, Warde P, Menard C, et al. Clin Can Res 2012; 18(7):2108-14

Hypoxia in Prostate Cancer
Results and Conclusions

Multivariate predictive models of relapse-free rate (RFR)
b=biochemical; L=local

Variable	HR	P
Entire cohort of 247 patients with prostate cancer, bRFR		
Gleason score[a]	2.66	0.015
PSA	1.075	<0.001
HP_{10}[b]	1.023	0.019
HP_{10} with time[c]	0.9995	0.001
142 patients with bulk[d] tumor at the site of the oxygen measurements, bRFR		
Age	1.073	0.021
PSA	1.085	<0.001
HP_{10}[b]	1.036	0.004
HP_{10} with time[c]	0.9992	<0.001
70 patients with prostate biopsies for local control, LRFR		
HP_{10}[b]	1.037	0.043
HP_{10} with time[c]	0.9991	0.032

- Hypoxia influences outcome of pts after high-dose RT
- Independent of neoadjuvant or concurrent hormonal therapy
- Effect of hypoxia on bRFR was maximal early in F/U; diminished with time.
- Early clinical behavior is driven by the dominant focus of disease
- Impact on risk of LN or bone mets not examined because hormonal therapy started before imaging.
- Anticipate new treatments for high-risk patients with hypoxia
- Hence need for widely available ways to assess hypoxia

Milosevic M, Warde P, Menard C, et al. Clin Can Res 2012; 18(7):2108-14

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

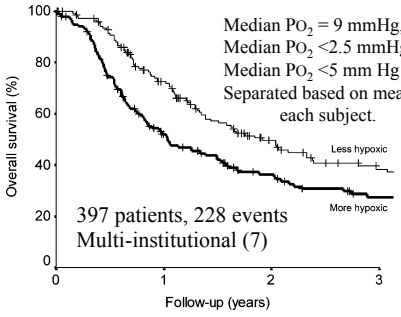
Radiation Therapy boost for glioblastoma

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 PO_2≤2.5 mmHg



Median PO_2 = 9 mmHg, range 0-62
Median PO_2 <2.5 mmHg =19%
Median PO_2 <5 mm Hg =38%
Separated based on mean PO_2<2.5 for each subject.

397 patients, 228 events
Multi-institutional (7)

Dominated by:
- Males (333)
- Smokers (201)
- Stage IV (359)
- Pharynx (277)

Nordsmark M, Bentzen SM, Rudat V, et al. Radiother Oncol 2005; 77:18-24.

Survival for patients with advanced head & neck tumors divided by median Eppendorf PO_2≤2.5 mmHg
CONCLUSIONS

- Pretreatment tumor PO_2 was a significant prognostic factor for survival after RT alone or in combination with surgery, chemotherapy or radiosensitizers.
- Analysis of PO_2 ≤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?
  Increase PO_2, Radiosensitizers, Hypoxia cytotoxins

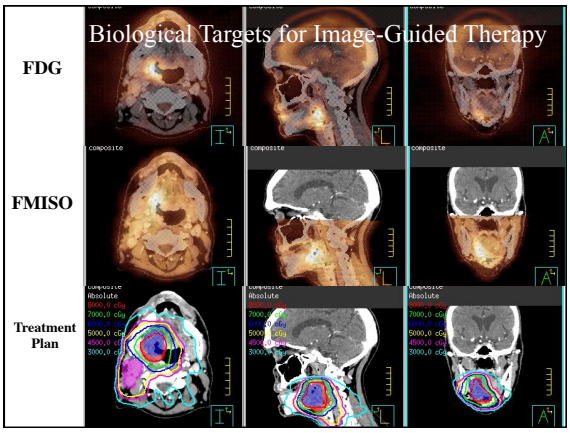
Nordsmark M, Bentzen SM, Rudat V, et al. Radiother Oncol 2005; 77:18-24.

Steps Toward Validating PET for Image-Guided Radiation Therapy

- Select imaging procedure mechanistically 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 radiation ± chemotherapy.

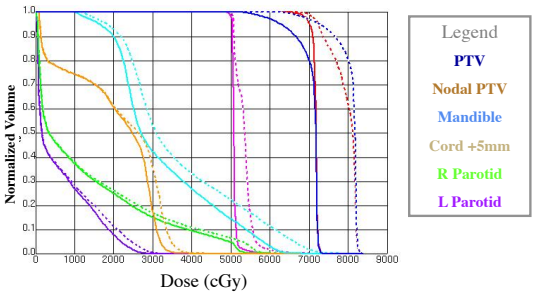
This is a general strategy toward integrating experimental diagnostics with experimental therapeutics.

Biological Targets for Image-Guided Therapy



Impact of FMISO-PET Imaging on DVH

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



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 cm^3)
  - 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 *Radiotherap 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.

KRG Hendrickson, MH Philips, WP Smith, UW Seattle

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

Will a 10 Gy boost improve outcome?

Tumor control probability		
TCP (70 Gy)	65.8	37 to 81
TCP (80 Gy)	87.9	76 to 95
% Increase	39	17 - 104

Normal tissue complication		
NTCP (70Gy)	1.95	0 to 6.2
NTCP (80Gy)	4.7	0.1 to 19

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

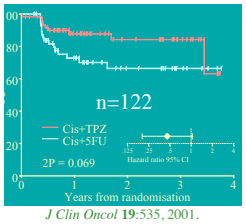
- Pre-treatment FMISO uptake is spatially correlated with the location of subsequent tumor recurrence.
- Thus, radiation boost to the FMISO volume may improve local control.

Wendy Gao, et al. ASTRO 2012.

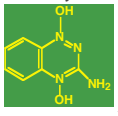
These results support a Phase II trial to investigate the impact of a sub-volume boost in a prospective manner.

Hypoxia-Selective Treatment for HNSCC

- Hypoxia is a common tumor phenotype.
- Tirapazamine, a hypoxic cytotoxin, 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



J Clin Oncol **19**:535, 2001.



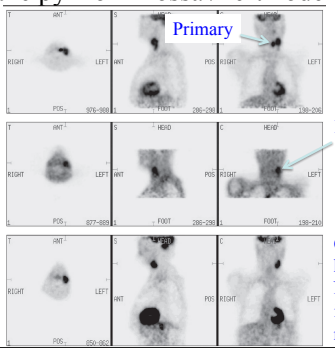
Tirapazamine

Loco-Regional Failures

Treatment (n=45)	FMISO neg	FMISO pos
RT/cis/5FU	**1/10**	8/13
RT/TPZ/cis	2/3	**1/19**

Peter MacCallum Cancer Centre, Melbourne

Images for a patient with T2N2b squamous cell ca of the pyriform fossa / left node mass



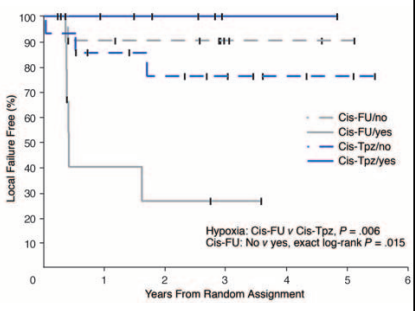
Rischin, JCO 2006; 13:2098-2104

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 ^{18}F-Misonidazole PET/CT in Patients with Non–Small Cell Lung Carcinoma (RTEP5 Study)

- Consortium of 15 academic PET centers in France
- FMISO-PET for RT boost in patients with NSCLC
- Locally advanced, no contraindication to chemrads (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)
- Isotropic 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

Vera P, Thureau S, Chaumet-Riffaud P, et al. *J Nucl Med* 2017; 58:1045-1053.

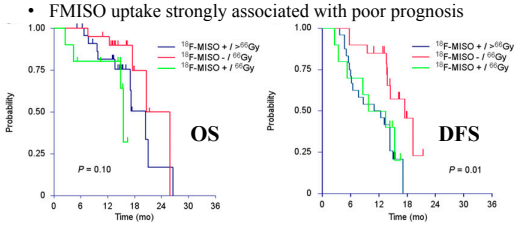
Study Details

- 54 pts based on intent to treat: 7 women, 47 men
- Histology: 26 squamous, 21 adeno, 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

Vera P, Thureau S, Chaumet-Riffaud P, et al. *J Nucl Med* 2017; 58:1045-1053.

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

Vera P, Thureau S, Chaumet-Riffaud P, et al. *J Nucl Med* 2017; 58:1045-1053.

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."

JNCI 2013; 105(10):686-93.

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

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, Haleh 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: ??

Int J Radiation Oncol Biol Phys 2017; 98(1):5-7.

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- 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.

Int J Radiation Oncol Biol Phys 2017; 98(1):5-7.

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

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.

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.

Lin SH,George TJ, Ben-Josef E, et al. JNCI 2013; 105(10):686-93.