UT Southwestern Medical Center

Radiology

Hypoxia-Inducible Factor 2α Targeted PET Radiotracers for Precision Medicine in RCC

Sashi Debnath, PhD

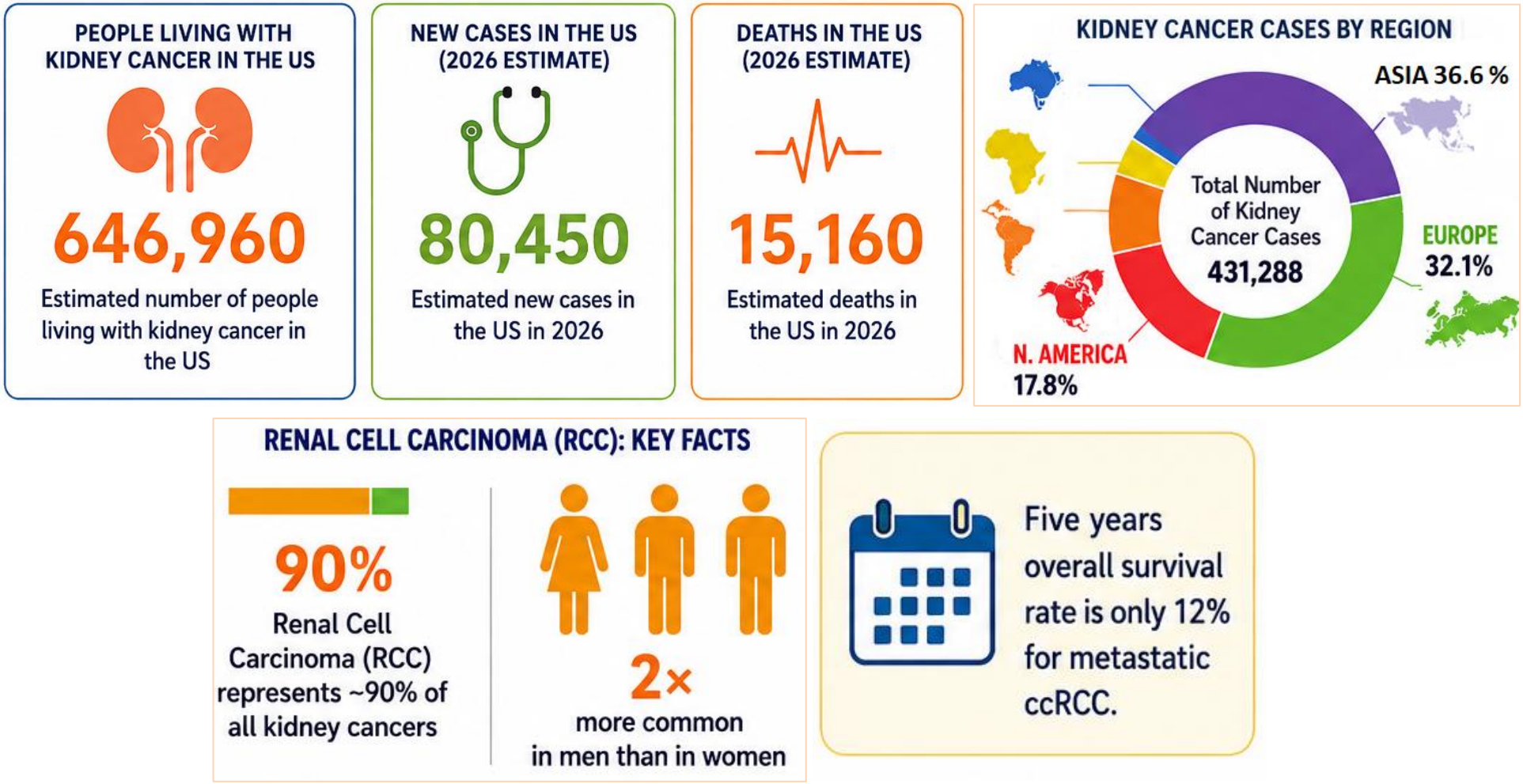
MHSRS 2026

Session: Precision Readiness: Integrating Pharmacogenomics to Enhance Warfighter Health
August 5, from 3:30 PM to 5:30 PM

Non-Disclosure

- Sashi Debnath, PhD has no conflicts to report.

Background: Kidney Cancer Key Facts and Trends

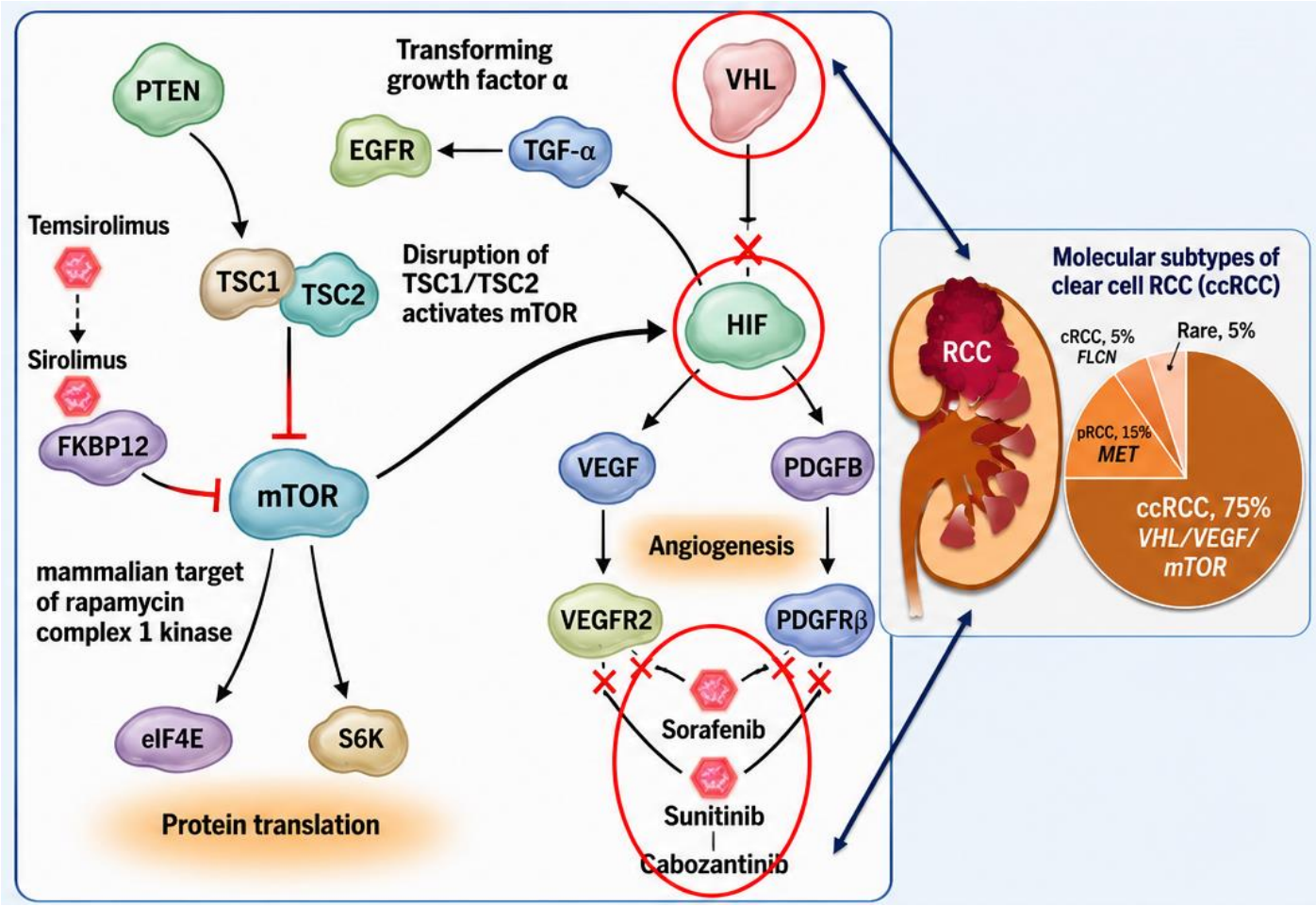


- European Urology, 82(5), pp.529-542, 2022.
- https://www.kidneycancer.org/fast-facts/

Background

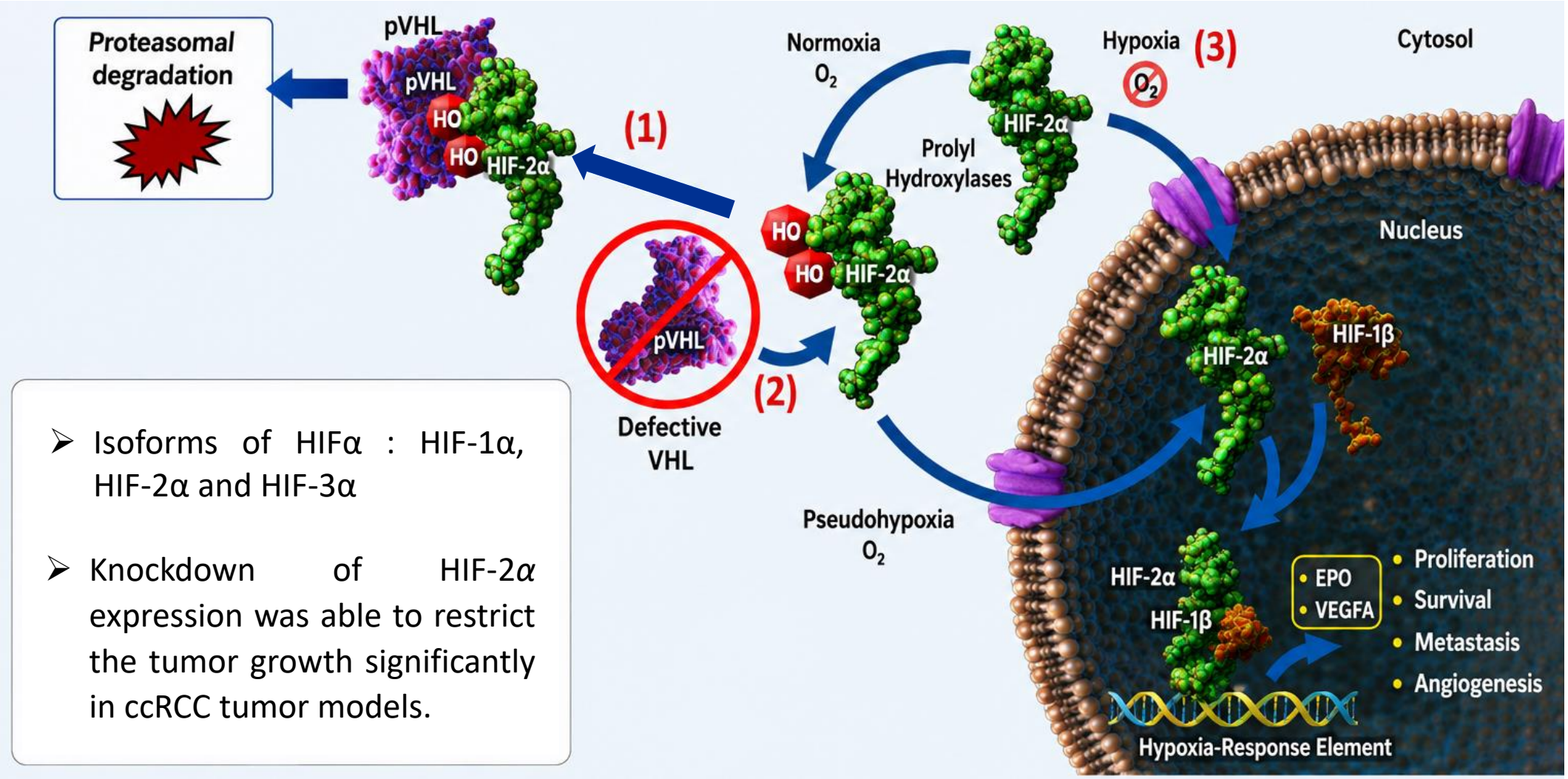
❑ Molecular pathways and targeted therapies in RCC

- Critical tumor suppressors protein pVHL recognized to be inactive in more than 90 % of ccRCC patients.
- HIF2 inhibition are more direct and potentially more selective than angiogenesis, mTOR and EGFR inhibition.



- N Engl J Med 2007, 356(2): 185 – 187.
- Genes & development, 1997. 11(1): p. 72-82.

pVHL Deficiency Results in HIF-2α Activation

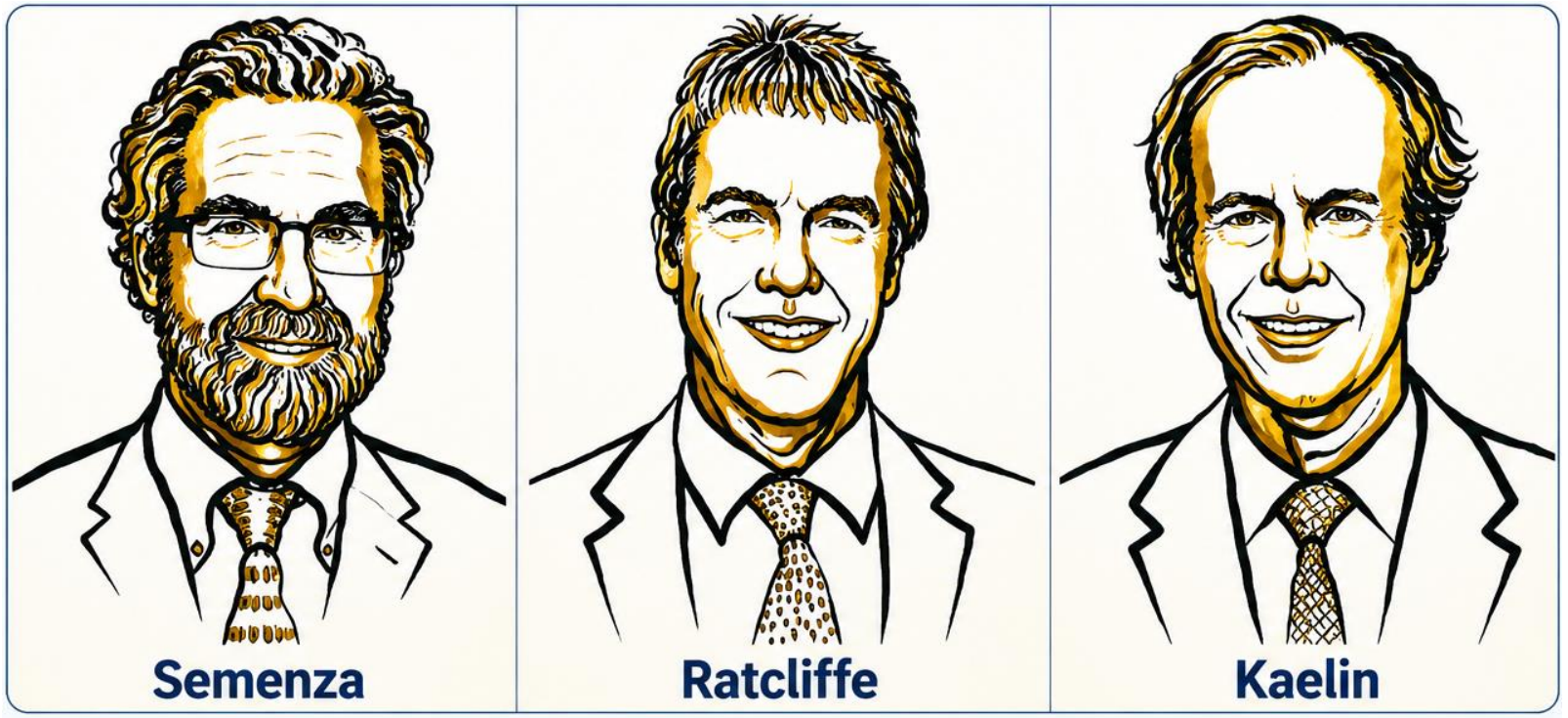


- Isoforms of HIFα : HIF-1α, HIF-2α and HIF-3α
- Knockdown of HIF-2*α* expression was able to restrict the tumor growth significantly in ccRCC tumor models.

- https://www.sec.gov/Archives/edgar/data/1525145/000119312519122479/d693812ds1.htm

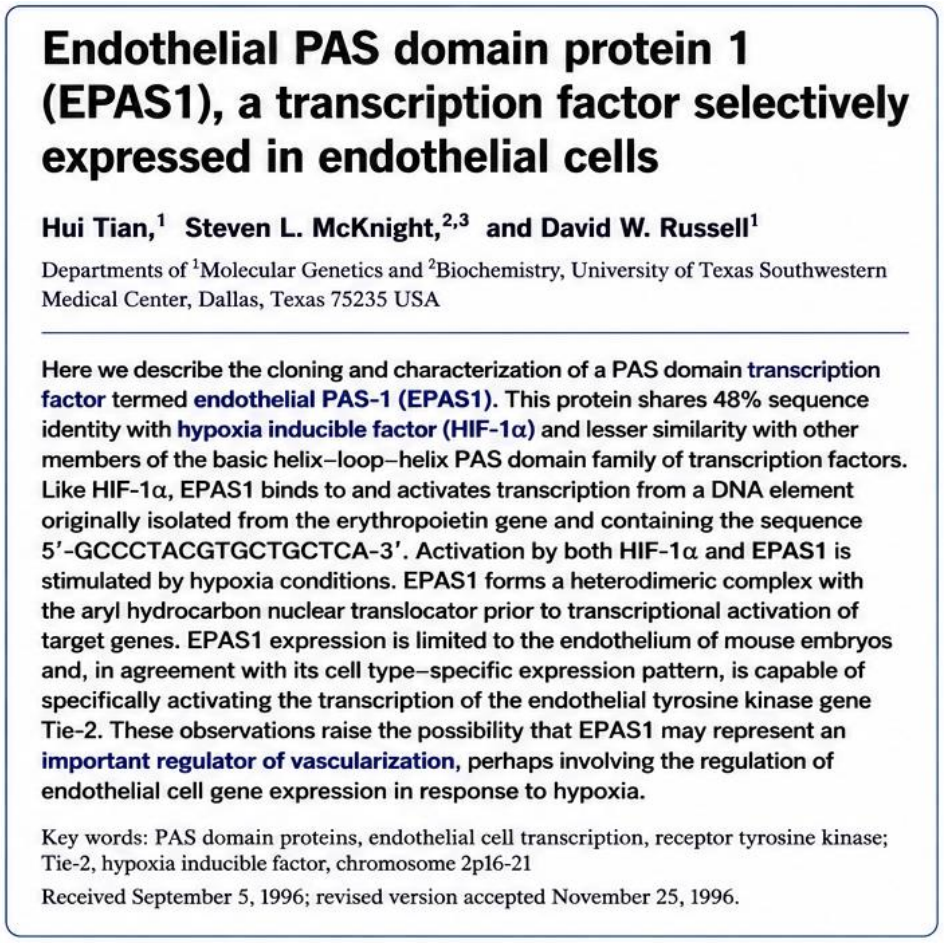
2019 Nobel Prize in Physiology or Medicine

How cells sense and adapt to oxygen availability



- VHL regulated HIF-1α post-translational and oxygen-sensitive degradation. Regulation of HIF-1α by VHL depends on hydroxylation of HIF1α, a covalent modification that is itself dependent on oxygen.

Original Discoveries at UT Southwestern to Bedside



Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells

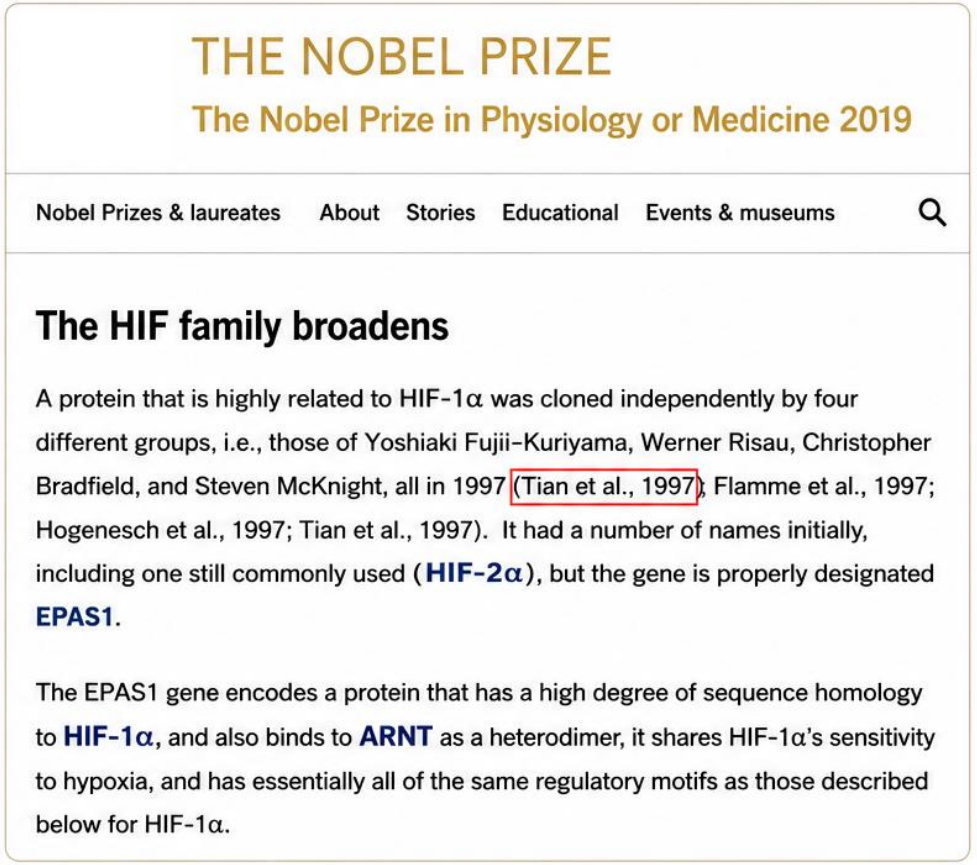
Hui Tian,[1] Steven L. McKnight,[2,3] and David W. Russell[1]

Departments of [1]Molecular Genetics and [2]Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75235 USA

Here we describe the cloning and characterization of a PAS domain transcription factor termed endothelial PAS-1 (EPAS1). This protein shares 48% sequence identity with hypoxia inducible factor (HIF-1α) and lesser similarity with other members of the basic helix–loop–helix PAS domain family of transcription factors. Like HIF-1α, EPAS1 binds to and activates transcription from a DNA element originally isolated from the erythropoietin gene and containing the sequence 5′-GCCCTACGTGCTGCTCA-3′. Activation by both HIF-1α and EPAS1 is stimulated by hypoxia conditions. EPAS1 forms a heterodimeric complex with the aryl hydrocarbon nuclear translocator prior to transcriptional activation of target genes. EPAS1 expression is limited to the endothelium of mouse embryos and, in agreement with its cell type–specific expression pattern, is capable of specifically activating the transcription of the endothelial tyrosine kinase gene Tie-2. These observations raise the possibility that EPAS1 may represent an important regulator of vascularization, perhaps involving the regulation of endothelial cell gene expression in response to hypoxia.

Key words: PAS domain proteins, endothelial cell transcription, receptor tyrosine kinase; Tie-2, hypoxia inducible factor, chromosome 2p16-21

Received September 5, 1996; revised version accepted November 25, 1996.



THE NOBEL PRIZE

The Nobel Prize in Physiology or Medicine 2019

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The HIF family broadens

A protein that is highly related to HIF-1α was cloned independently by four different groups, i.e., those of Yoshiaki Fujii-Kuriyama, Werner Risau, Christopher Bradfield, and Steven McKnight, all in 1997 (Tian et al., 1997; Flamme et al., 1997; Hogenesch et al., 1997; Tian et al., 1997). It had a number of names initially, including one still commonly used (**HIF-2α**), but the gene is properly designated **EPAS1**.

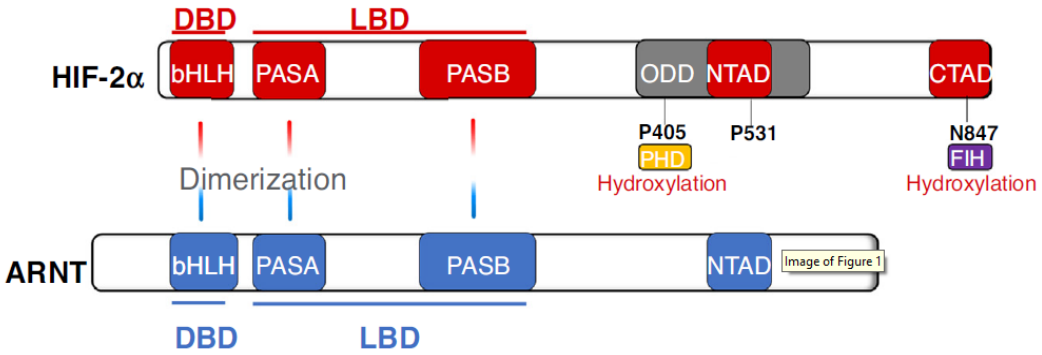
The EPAS1 gene encodes a protein that has a high degree of sequence homology to **HIF-1α**, and also binds to **ARNT** as a heterodimer, it shares HIF-1α's sensitivity to hypoxia, and has essentially all of the same regulatory motifs as those described below for HIF-1α.

- *EPAS-1 is now commonly called as "HIF-2α".*

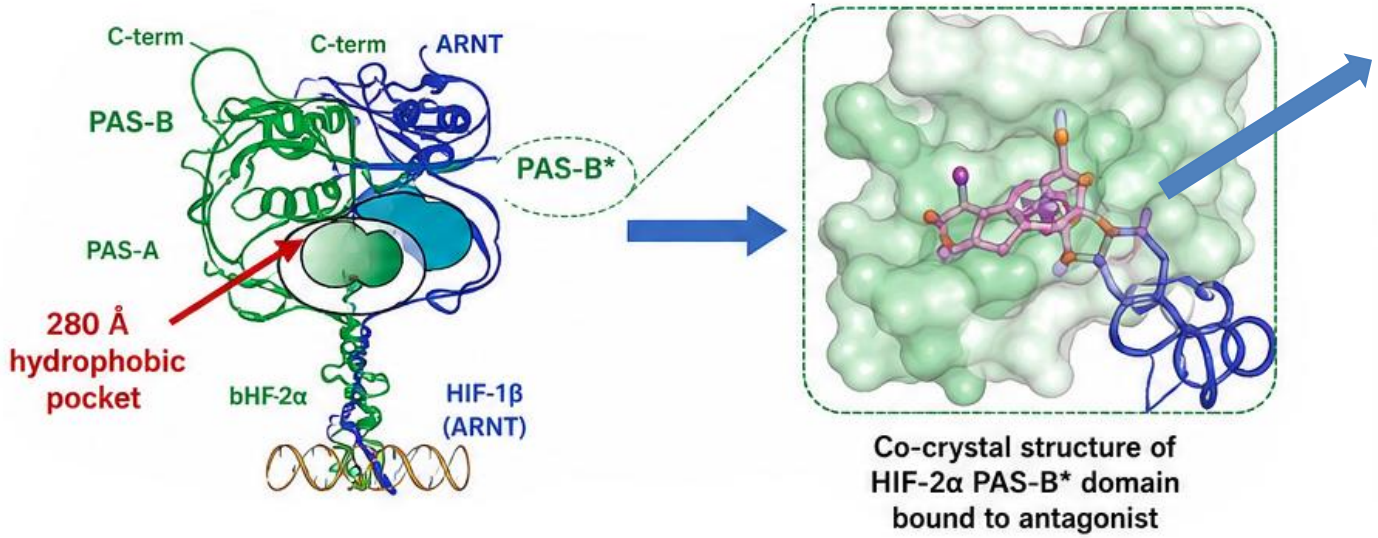
- PAS domain named after the three proteins where it was discovered: Per (period circadian protein), ARNT, and Sim (single-minded protein)

Structure-Activity Relationship Design of HIF-2α Inhibitors

- HIF-2α domain architecture

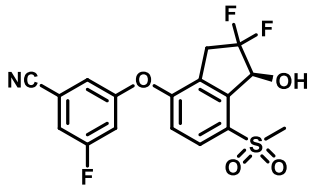


- HIF-2α antagonist binds to PAS-B domain



Co-crystal structure of HIF-2α PAS-B* domain bound to antagonist

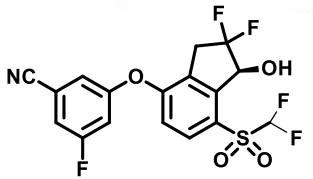
- HIF-2α Inhibitor development pipeline



PT2385

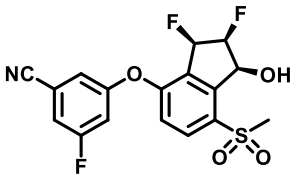
1st Generation HIF-2α Inhibitor

Phase I, II, Treatment



PT2399

Phase I, Treatment



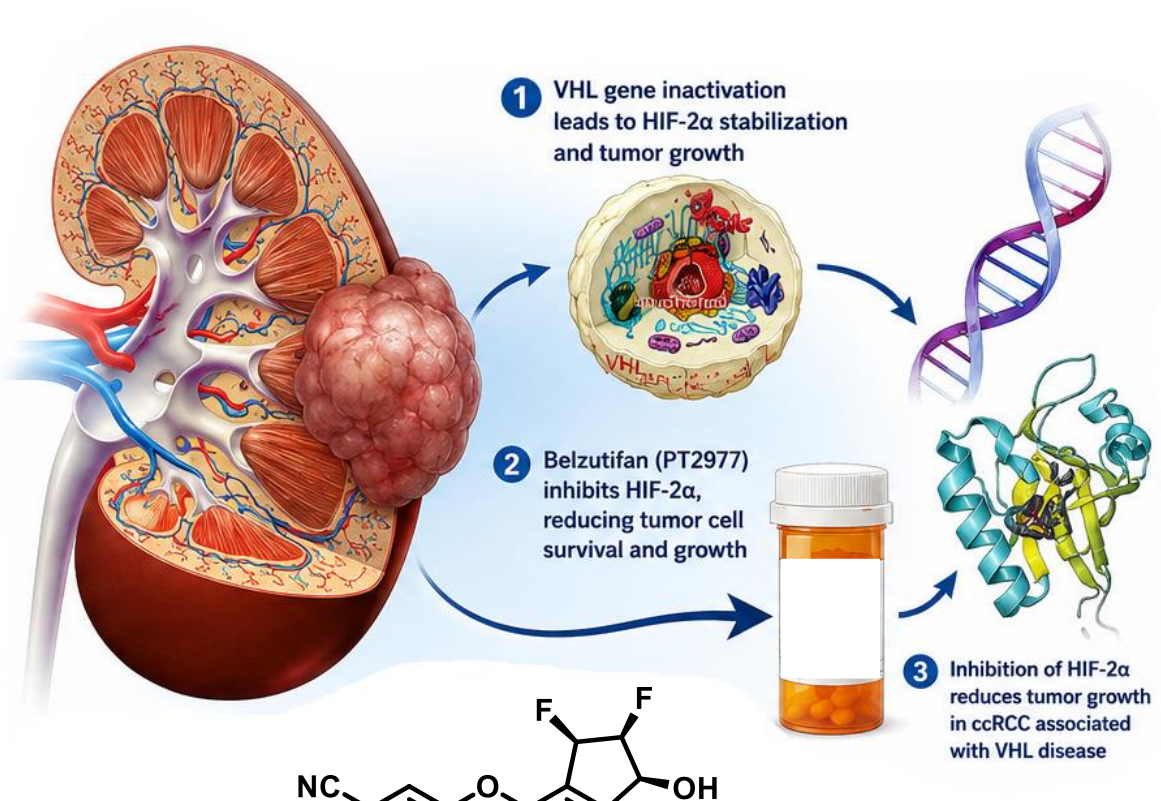
PT2977

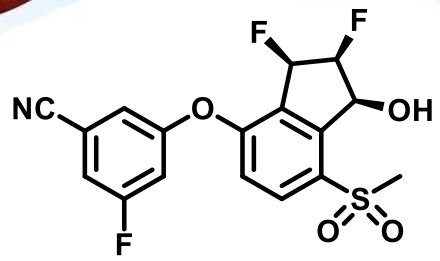
Phase I, II, III, Treatment

Peloton Therapeutics, Inc.

- Kevin H. Gardner, Proceedings of the National Academy of Sciences, 106(2), pp.450-455, 2009.
- Kevin H. Gardner, J. Am. Chem. Soc. 2009, 131, 48, 17647–17654

FDA Approval of Belzutifan (PT2977)





PT2977/belzutifan/MK-6482

BREAKTHROUGH APPROVAL

The U.S. Food and Drug Administration has approved belzutifan (PT2977) for the treatment of VHL disease–associated renal cell carcinoma.

FEATURED COVERAGE

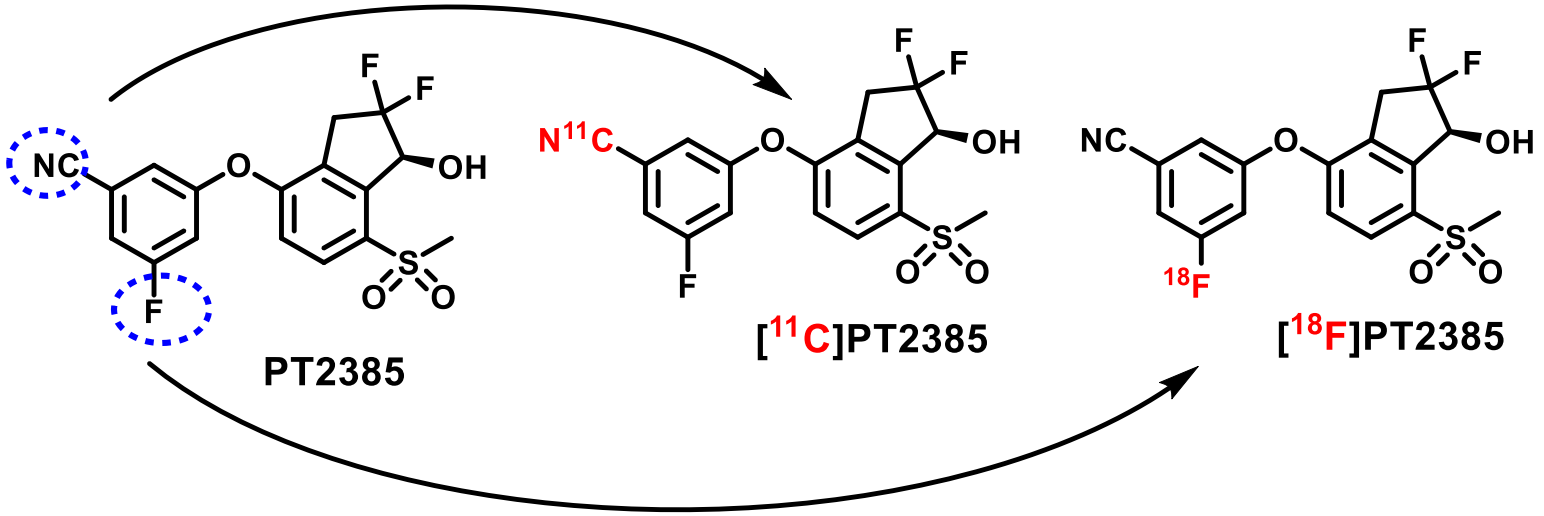
BIOTECH FINANCES	Peloton Therapeutics Initiates Phase 2 Trial of Oral HIF-2 Inhibitor PT2977 for Treatment of von Hippel–Lindau Disease–associated Kidney Cancer
UTSouthwestern Medical Center	FDA approval of belzutifan culminates 25-year journey at UTSW from gene discovery to a first-in-class drug

- Merck acquired Peloton Therapeutics in 2019 for $2.2 billion
- Approval in 2021 for therapeutic action on HIF-2α positive ccRCC

- https://www.utsouthwestern.edu/newsroom/articles/year-2021/fda-approval-belzutifan.html

Can We Leverage HIF-2α Inhibition Pathway to Develop PET Tracer?

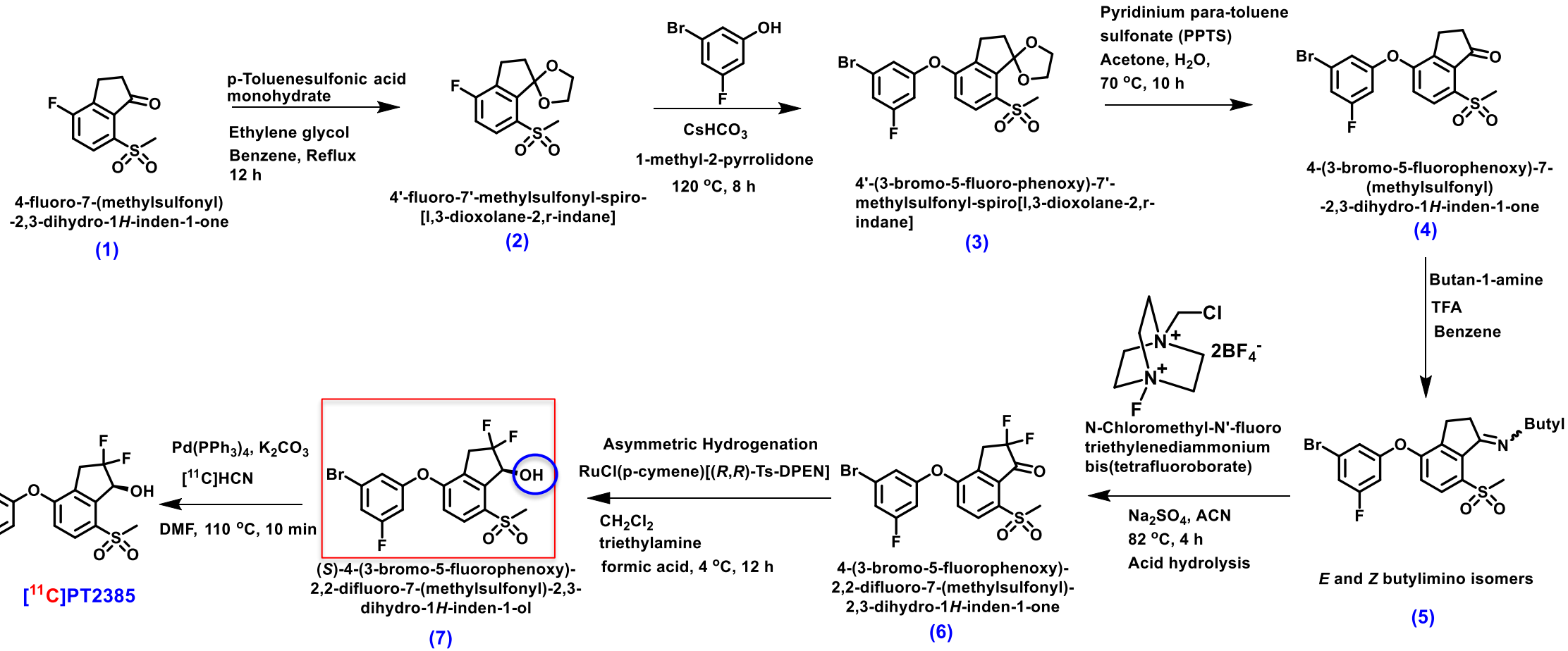
❑ Radiolabeling Strategy of PT2385 for PET Imaging of HIF-2α



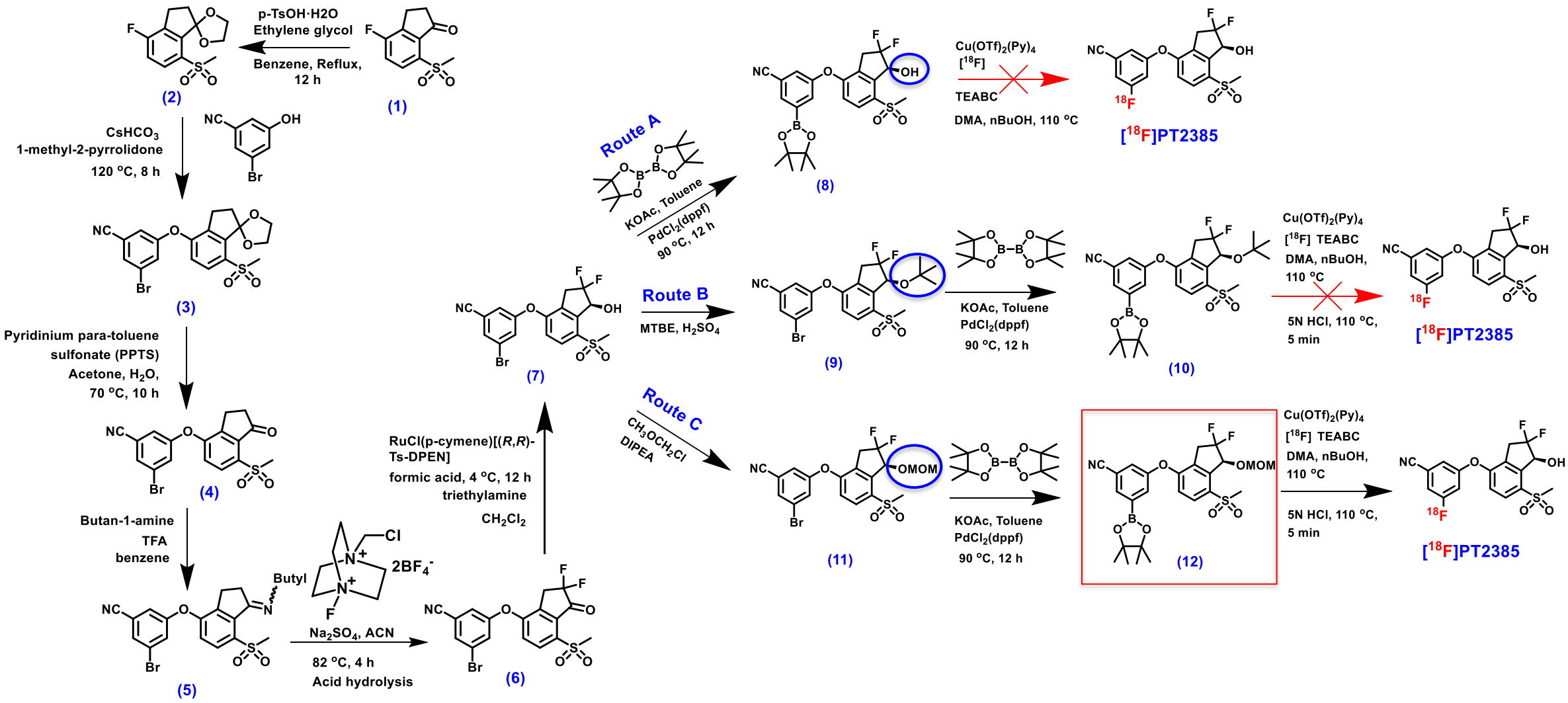
- Other structures
- First in class HIF-2α Inhibitor
- Toxicology data available

- https://patents.google.com/patent/US20210187130A1/en

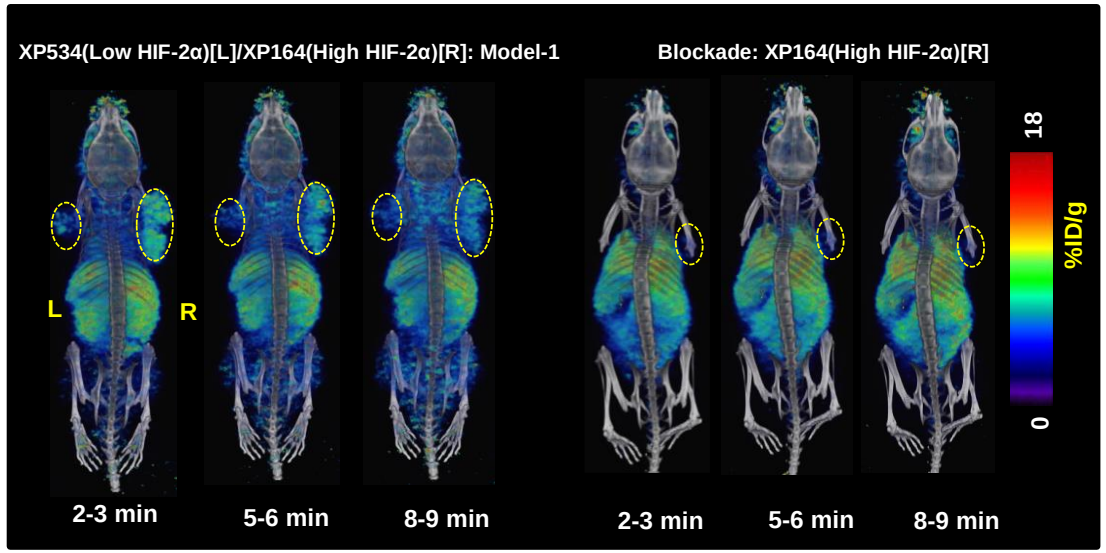
Synthetic Routes to [^{11}C]PT2385

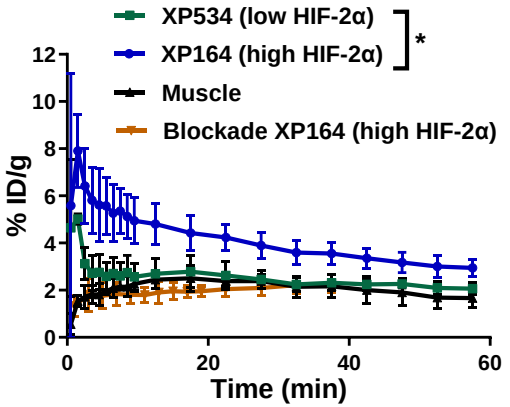


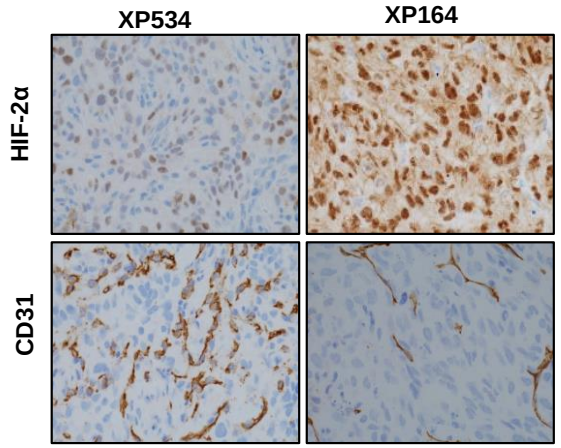
Synthetic Routes to [^{18}F]PT2385



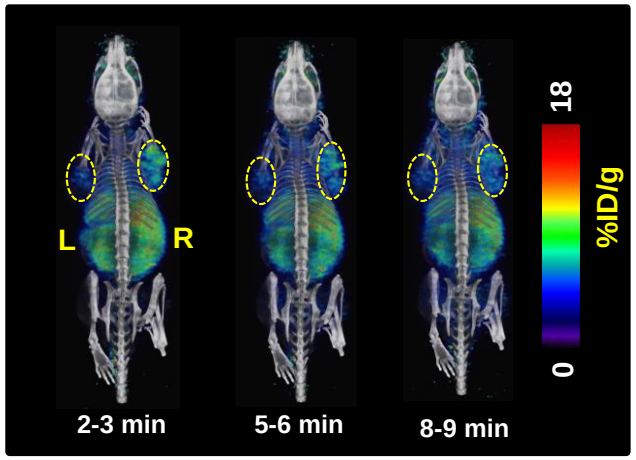
PET with [^{18}F]PT2385 is Capable of Differentiating Tumoral HIF-2α Levels

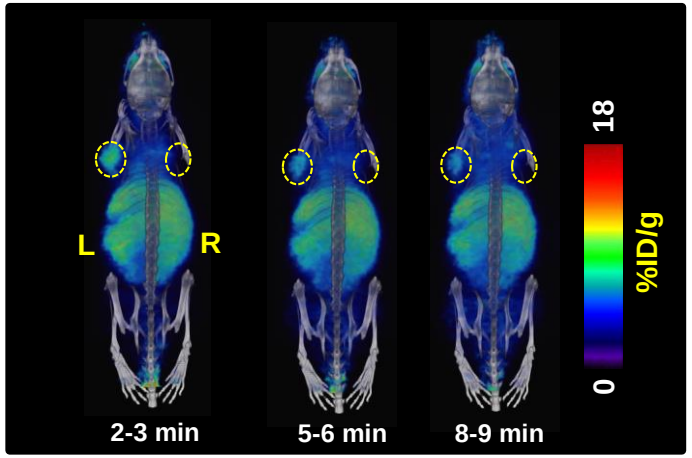


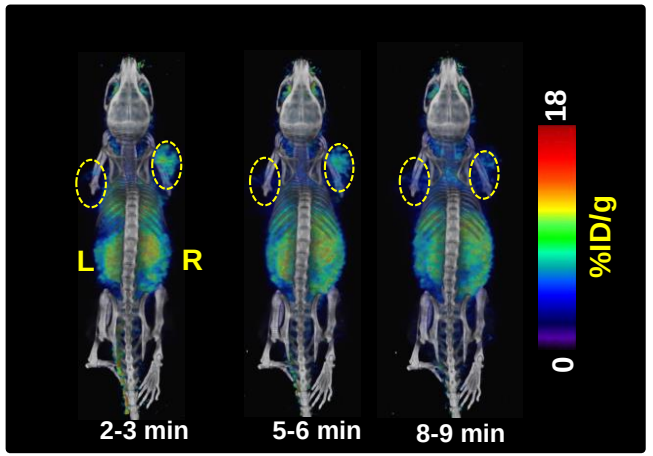


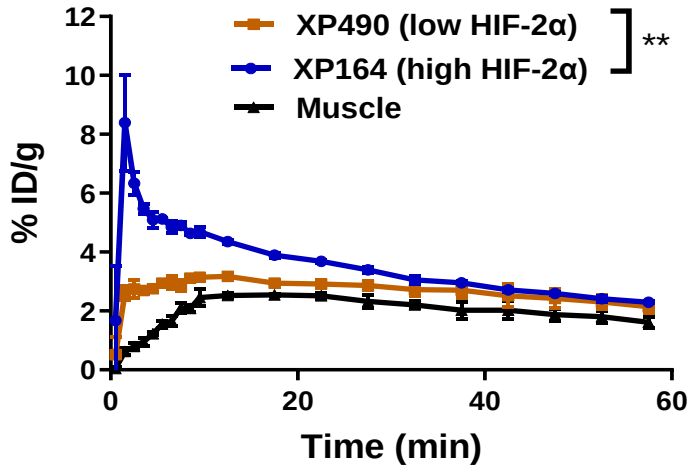


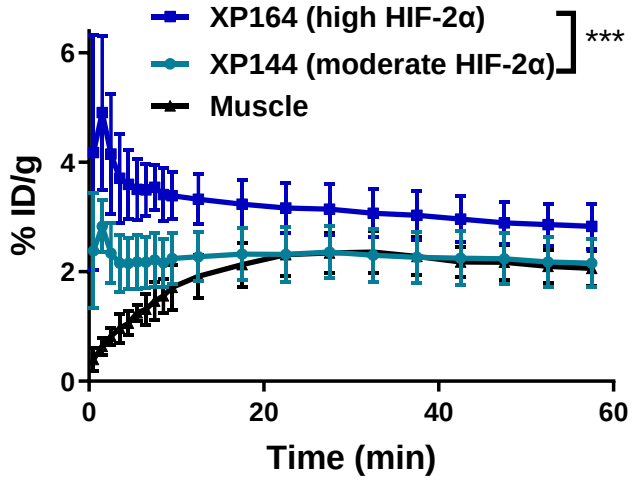
PET with [^{18}F]PT2385 is Capable of Differentiating Tumoral HIF-2α Levels

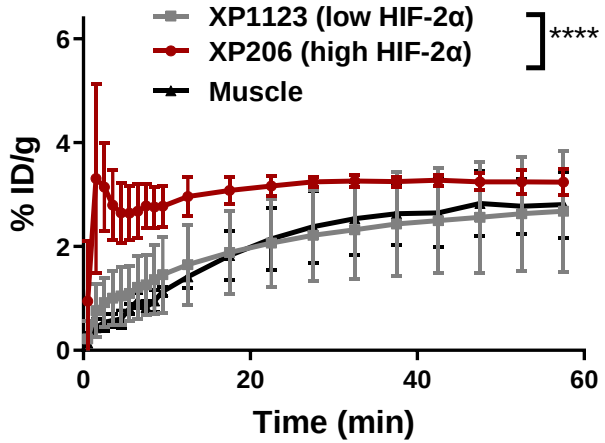




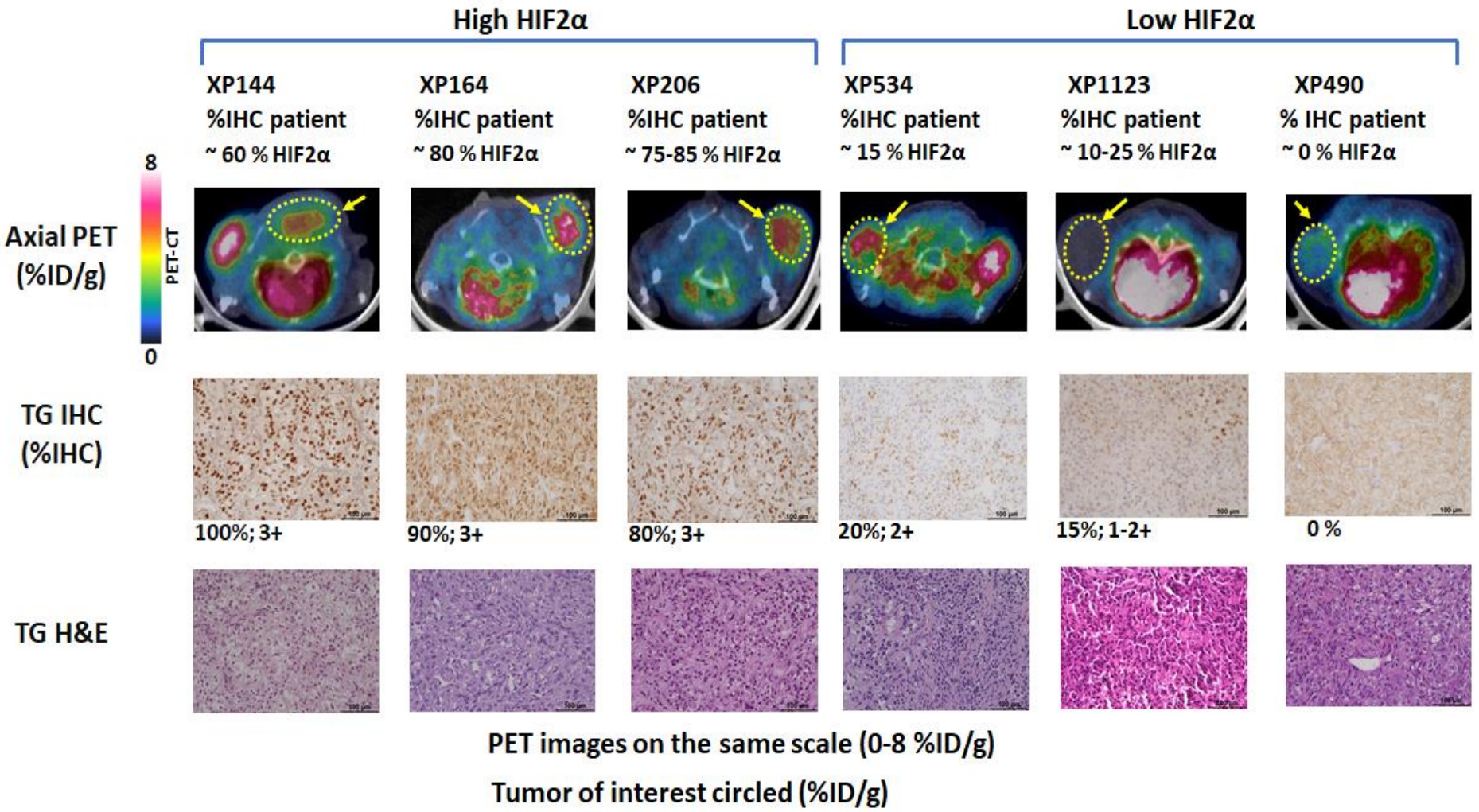








Immunohistochemistry (IHC) Staining to Quantify HIF-2α Expression



Dosimetry estimates for [^{18}F]PT2385

Preclinical Dosimetry in Mice

3 ♀ and 4 ♂ mice

~0.37 MBq [^{18}F]PT2385 i.v.

Dynamic PET/CT (0–2 h)
+ Static PET/CT (5 h p.i.)

Data Processing & Scaling

Quantify organ uptake
(%ID/g in mice)

Scale to humans
(%ID/g)

Scaling factor =
$\frac{\text{Human body weight}}{\text{Mouse body weight}}$

Fit time–activity curves
(Exponential models)

$$A_1 e^{-a_1 t} + A_2 e^{-a_2 t}$$

or $A_1 e^{-a_1 t}$

Human Dosimetry Estimation

Apply OLINDA/EXM
(standard organ masses)

Calculate absorbed dose
(mSv/MBq)

Organ absorbed dose (mSv/MBq)

	♂	♀
Urinary bladder	0.068	0.100
Kidneys	0.040	0.060
Liver	0.020	0.030
Lungs	0.015	0.025
All other organs (each)	~0.01–0.10	

Most organs: ~0.01–0.1 mSv/MBq

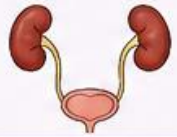
Effective Dose

Effective dose
(mSv/MBq)

♂	♀
0.0114	0.0160

Key Finding

Urinary bladder receives the highest absorbed dose, consistent with predominant urinary excretion.



First PET Radiotracer Developed by CRP from Benchtop to Bedside

1st-in-Human Clinical Trial

- Preclinical Preparation towards Clinical Trials
- Regulatory Filing for Investigational New Drug (IND) Application to US-FDA for 1st-in-human Clinical Trials with [^{18}F]PT2385
- Study Protocol Approved by the Institutional Research Board (IRB)
- To date, we have scanned 10 human subjects.
- This strategy may be extendable to additional HIF-2α–driven diseases.

U.S. Food and Drug Administration

IND 156933

STUDY MAY PROCEED

Dr. Neil M. Rofsky, MD, MHA, FACR
UT Southwestern Medical Center – Dept of Radiology
5323 Harry Hines Blvd
Dallas, TX 75390-8896

Dear Dr. Rofsky:[1]

Please refer to the Investigational New Drug application (IND) 156933, submission dated June 3, 2021, submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act (FDCA) for [F-18] PT2385.

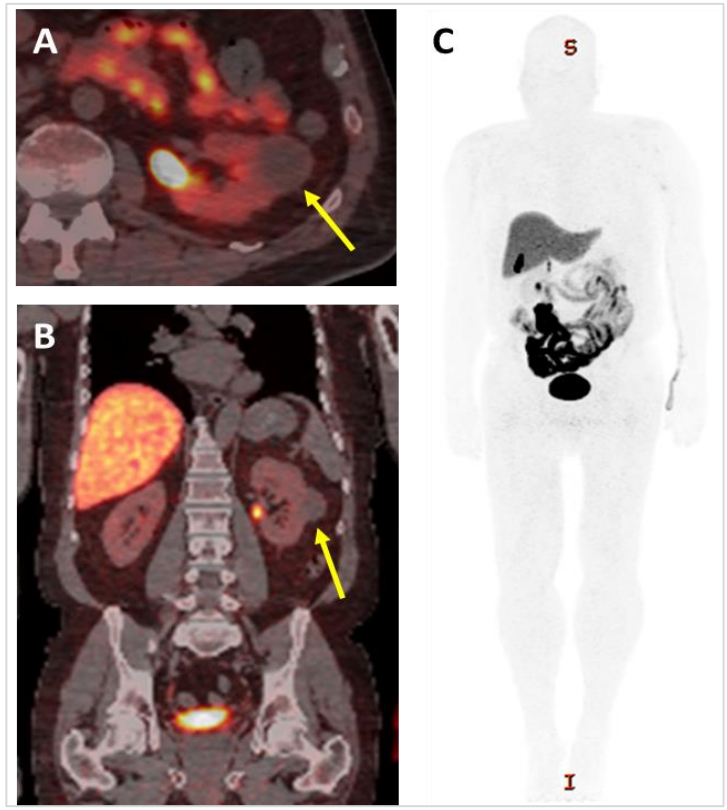
We have completed our safety review and your chemistry responses of June 22 and 23 and clinical response of June 30, 2021, and we have concluded that you may proceed with your proposed clinical investigation as of this letter date.

THUY M NGUYEN
07/07/2021 03:00:06 PM

LIBERO L MARZELLA
07/07/2021 04:44:16 PM

Phase I trial [^{18}F]PT2385

- The first human data



Axial (**A**) and coronal (**B**) PET/CT images at 30 min p.i. of 5-mCi of [^{18}F]PT2385 (**C**) at 4 h post injection.

Kinetic Modeling and Semi-quantitative Uptake Measures of [^{18}F]PT2385 in Patients with RCC

	Tumor to Kidney Ratio (Unit)			
Subject	SUV (5 min. PI)	Net Influx (K_i)	Distribution Vol.	HIF2α Staining
1	0.4	0.5	0.4	No
2	0.4	0.6	0.3	High
3	1.3	3.6	1.2	Present
4	0.4	17.6	0.4	High

Interestingly and encouragingly, the Net Influx (K_i) was substantially higher in tumor than in healthy kidneys in two of three patients with HIF2α staining, suggesting that [^{18}F]PT2385 indeed binds to tumoral HIF2α.

- https://clinicaltrials.gov/ct2/show/NCT04989959

Summary

- Radiotracer with identical structure to PT2385, [^{11}C]PT2385 and [^{18}F]PT2385 have been judiciously designed and successfully synthesized for noninvasive imaging of HIF-2α.
- These data set the foundation for an investigational new drug (IND) approval from the FDA, and a clinical trial that is currently accruing patients (NCT04989959).
- This approach may be applicable to other HIF-2α–dependent diseases.

Acknowledgements

- Professor Xiankai Sun
- Professor James Brugarolas
- Professor Orhan K. Öz
- Dr. Payal Kapur
- Dr. Spencer L. Bowen
- Christina Stevens
- Vanina Toffessi Tcheuyap
- UTSW Radiology Cyclotron Team

Financial Support

- Cancer Center's Developmental Funds for Clinical Oncology Trials (Brugarolas/Sun)
- Cancer Prevention and Research Institute of Texas (RP110771 & RP 170638, Sun)
- UT Southwestern Kidney Cancer SPORE, (5 P50 CA196516-03 – Project 1 (Brugarolas/Sun) & Translational Imaging Core (Pedrosa/Sun)
- DOD-KCRP Translational Research Partnership Award (HT94252310903, Sun/Brugarolas)