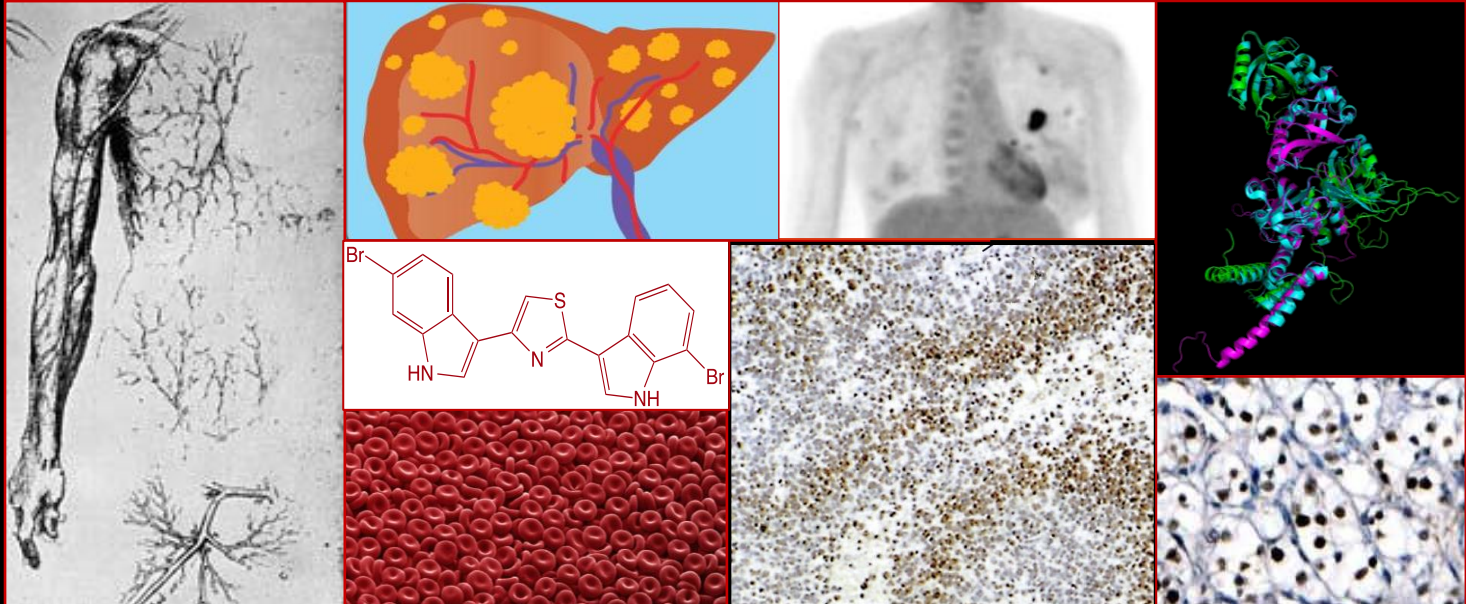


Hypoxia-Inducible Factors in Physiology and Medicine

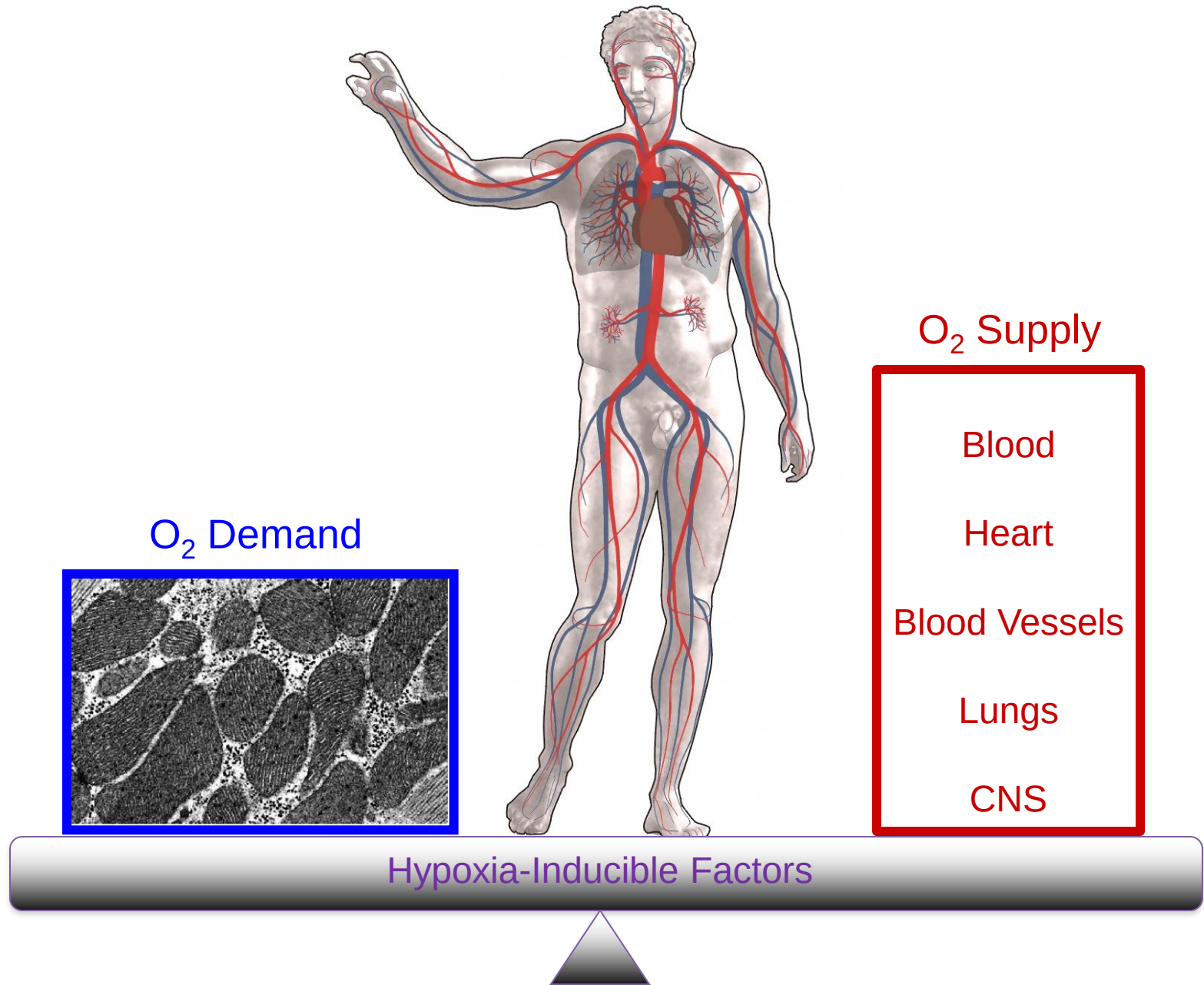


Gregg L. Semenza, M.D., Ph.D.
Vascular Program, Institute for Cell Engineering
Department of Genetic Medicine
Johns Hopkins University School of Medicine
Baltimore, Maryland USA



Armstrong Oxygen Biology
Research Center at Johns Hopkins

Oxygen Homeostasis: A Balancing Act



Nobel Prize Ceremony - Stockholm 12-10-2019



Nobel Prize Ceremony - Carl XVI Gustaf, King of Sweden



2019 Nobel Prize in Physiology or Medicine



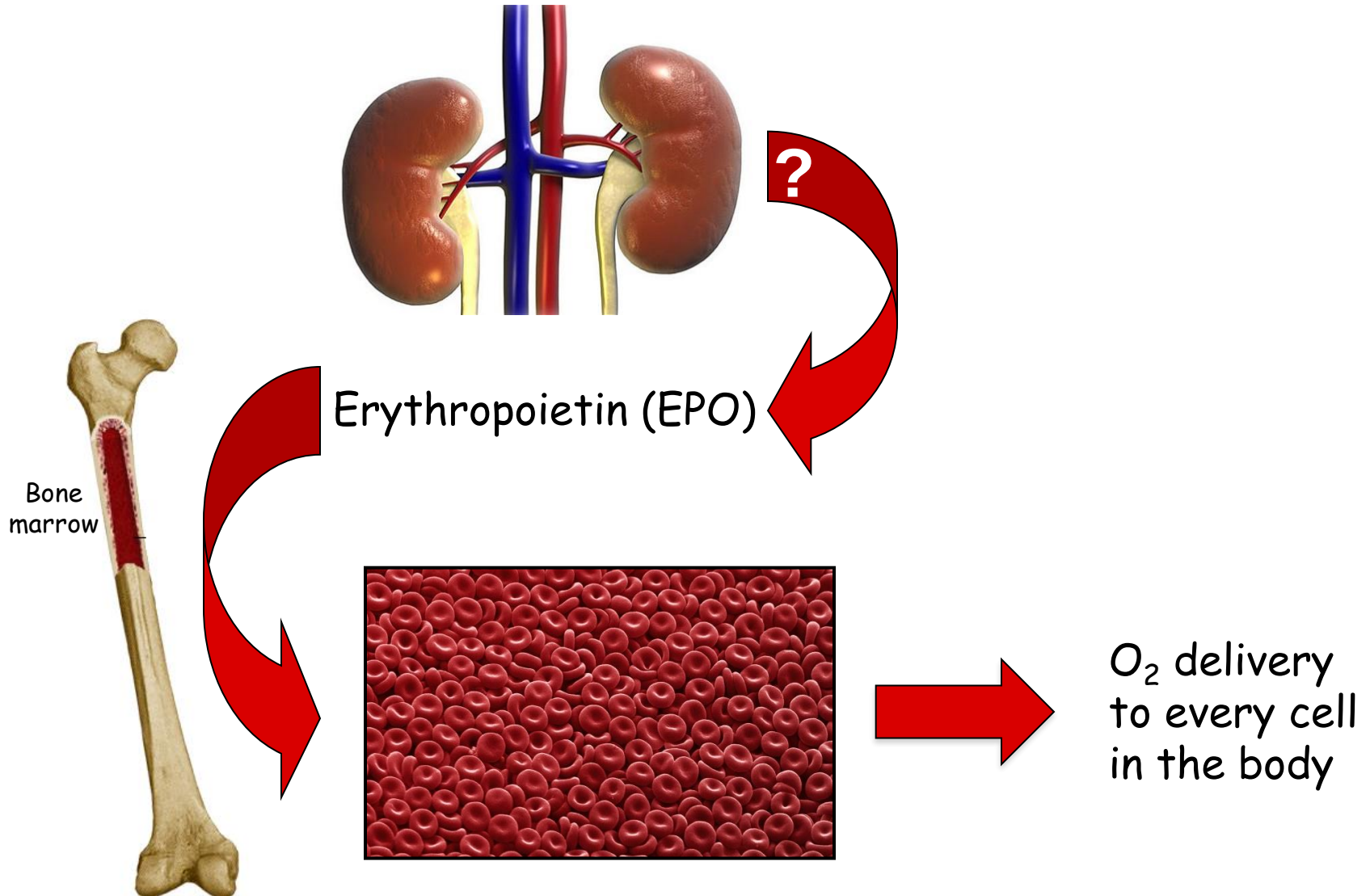
Alfred Nobel died
12-10-1896



Let us improve life through
science and art.
-- Virgil, *Aeneid*, Book 6

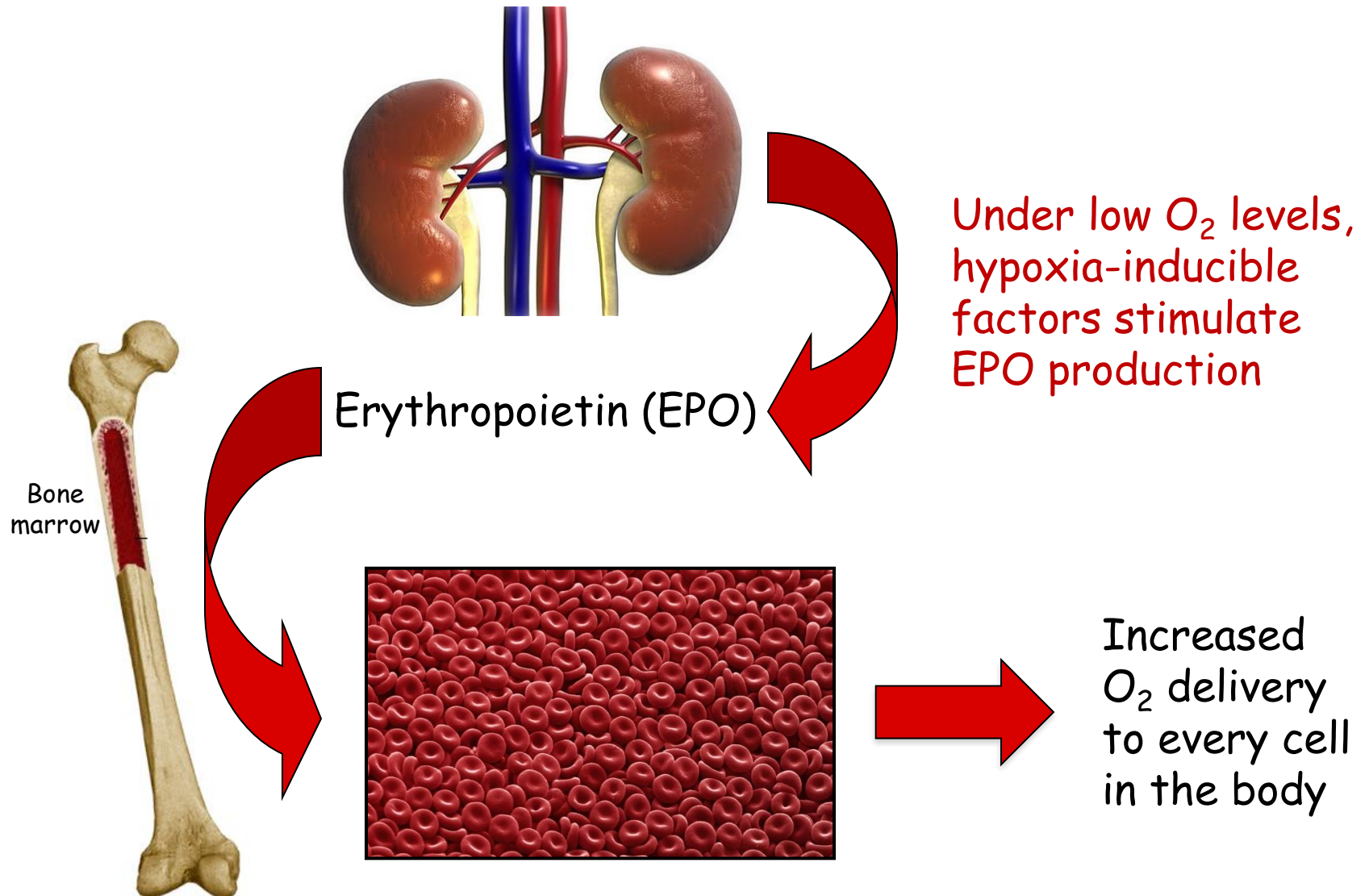
Erythropoietin Controls Red Blood Cell Production

What Controls Erythropoietin Production?

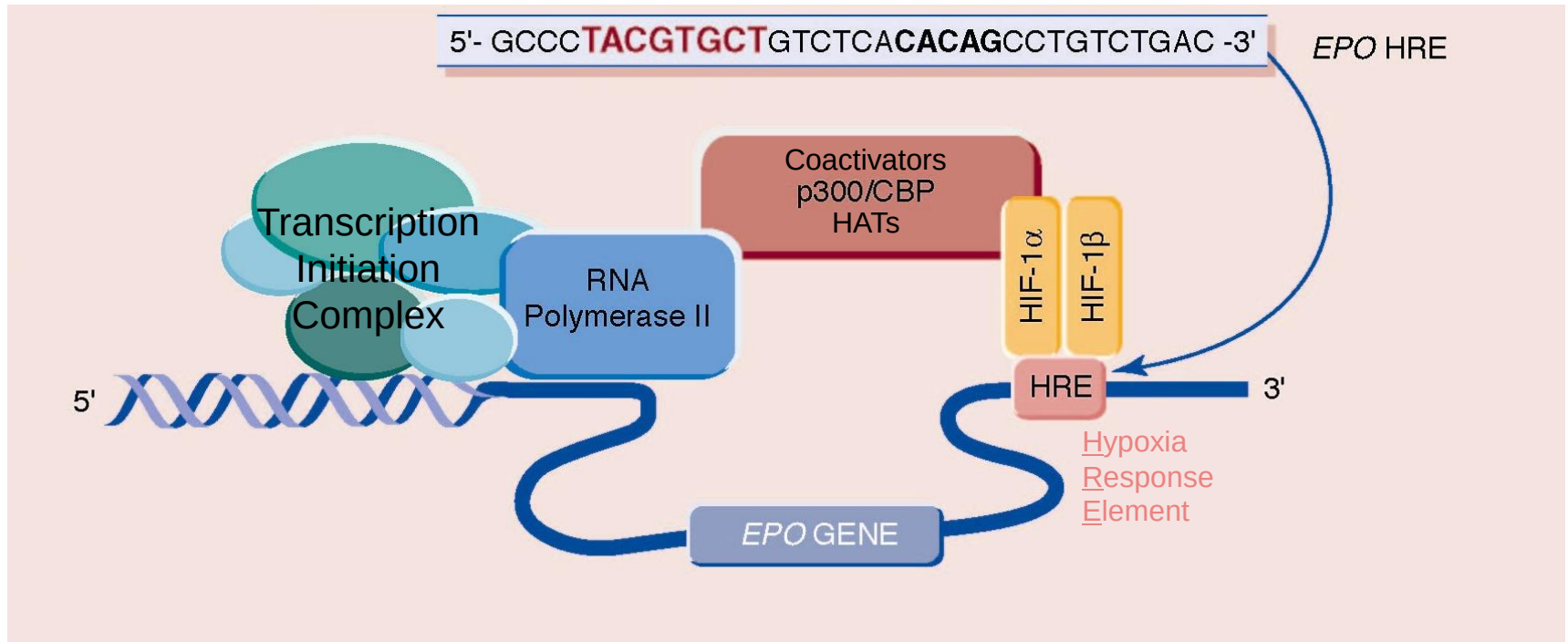


Erythropoietin Controls Red Blood Cell Production

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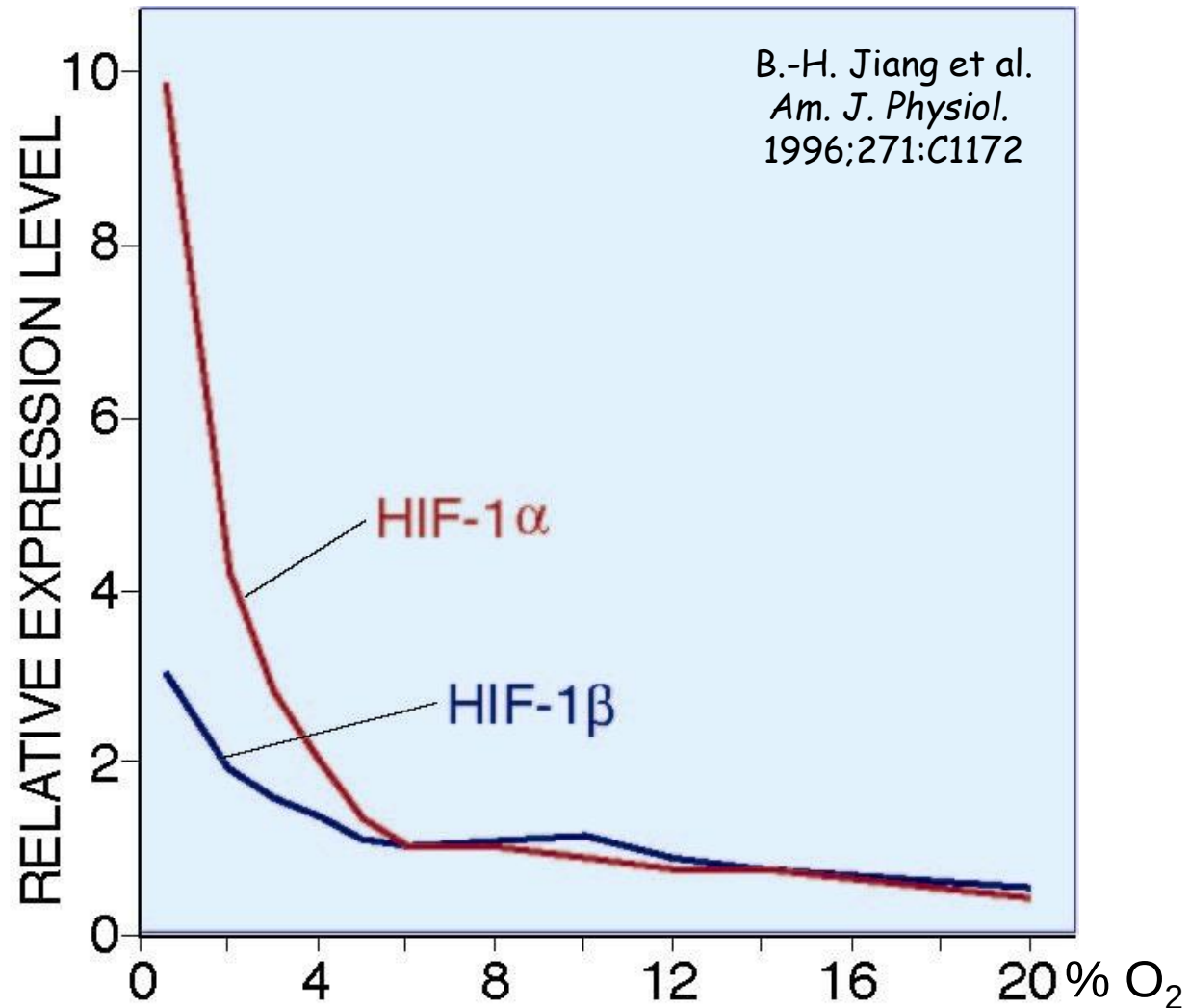


Hypoxia-Inducible Factor 1 (HIF-1) Activates *EPO* Gene Transcription in Response to Decreased O₂ Availability



G. L. Semenza and G. L. Wang, *Mol. Cell. Biol.* 1992;12:5447
G. L. Wang and G. L. Semenza, *J. Biol. Chem.* 1995;270:1230
G. L. Wang et al., *Proc. Natl. Acad. Sci. USA* 1995;92:5510

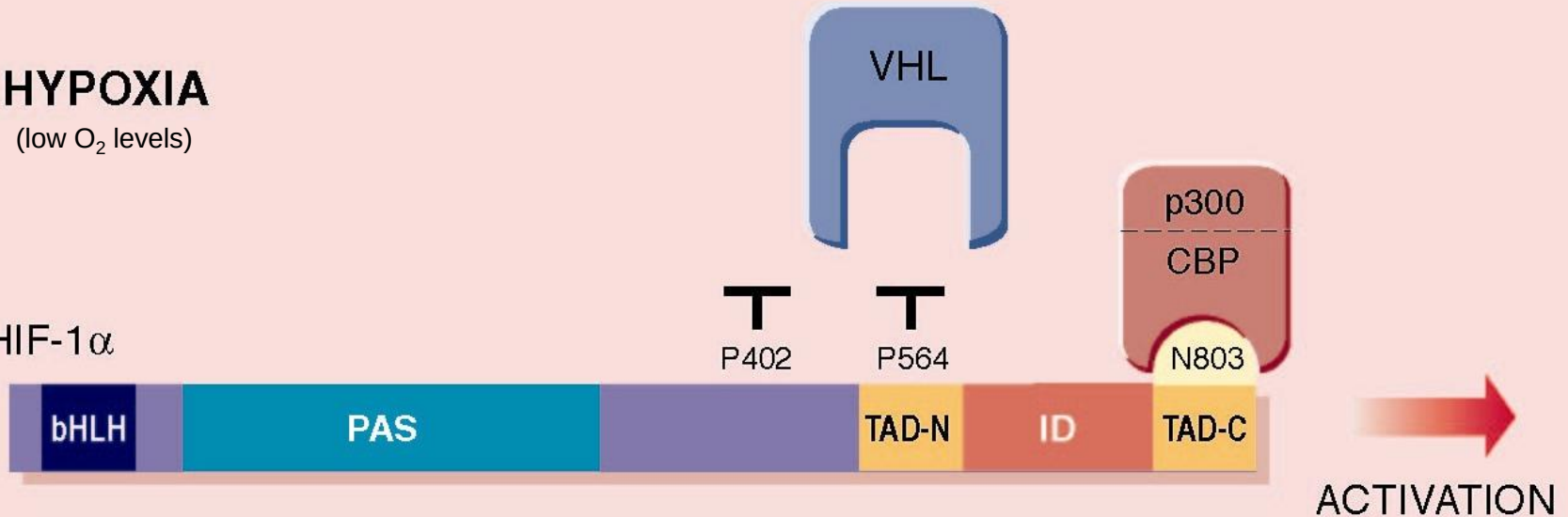
HIF-1 α Protein Accumulates in a Graded Response to Hypoxia



HYPOXIA

(low O_2 levels)

HIF-1 α

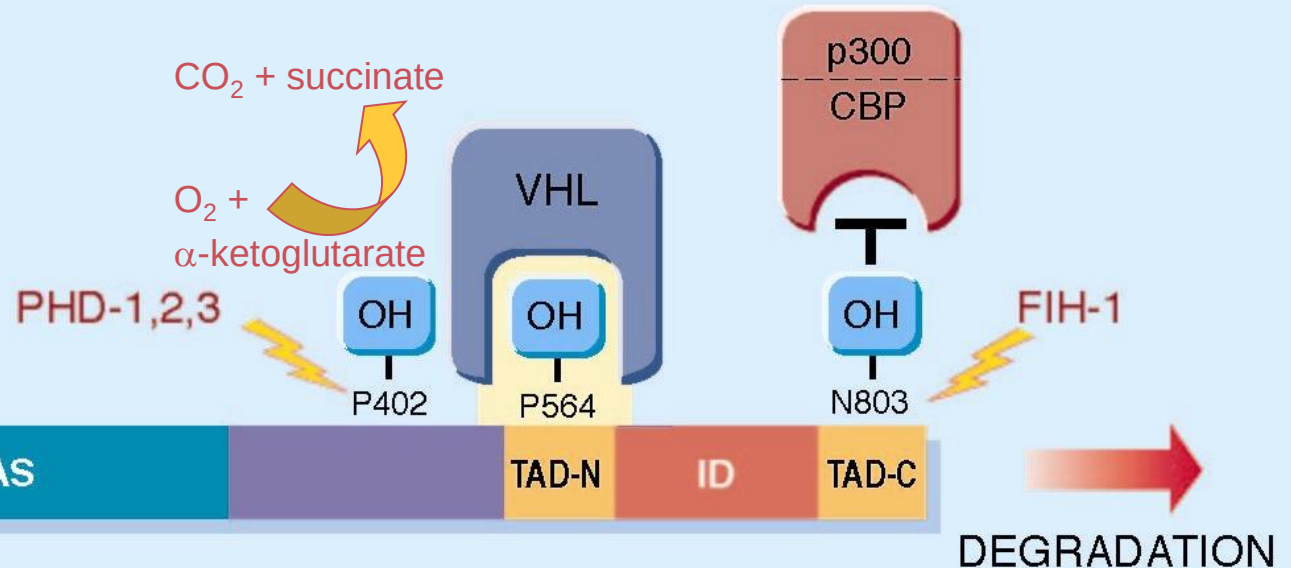


HIF-1 α is Regulated by Oxygen-dependent Hydroxylation

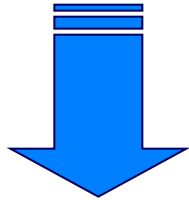
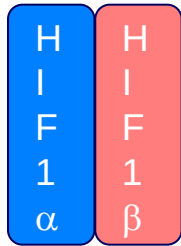
NORMOXIA

(normal O_2 levels)

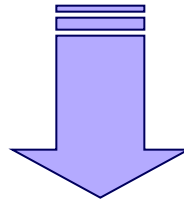
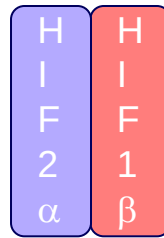
HIF-1 α



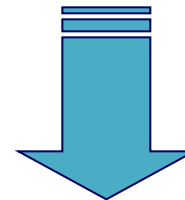
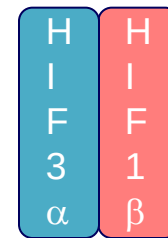
HIF-1 α , HIF-2 α and HIF-3 α Heterodimerize with HIF-1 β and Activate Gene Transcription



All nucleated
cell types of
~all metazoan
species

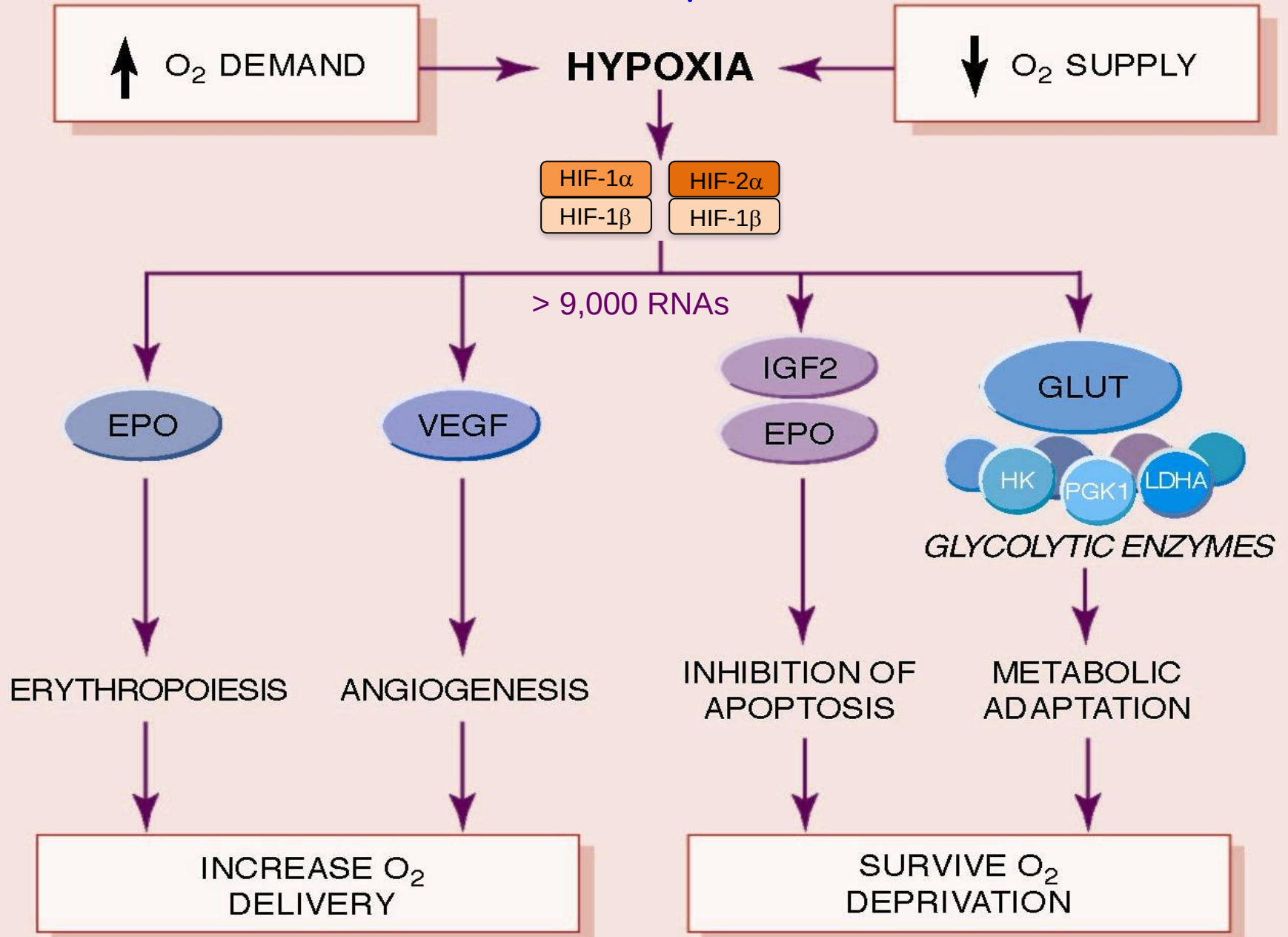


Certain cell
types of
vertebrate
species

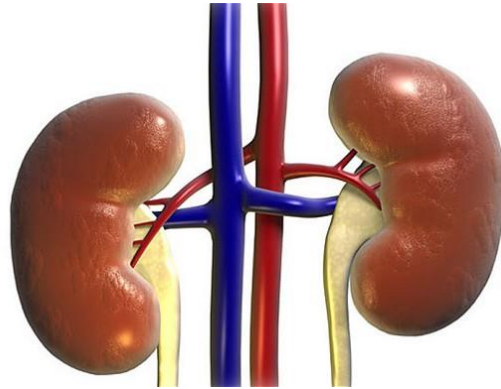


Certain cell
types of
vertebrate
species

HIFs Mediate Homeostatic Responses to Reduced O₂ Levels

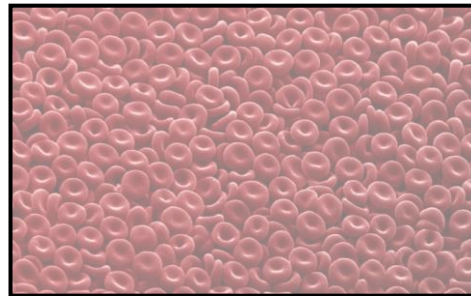


Patients with Chronic Kidney Disease are Anemic Because Their Kidneys Stop Making EPO

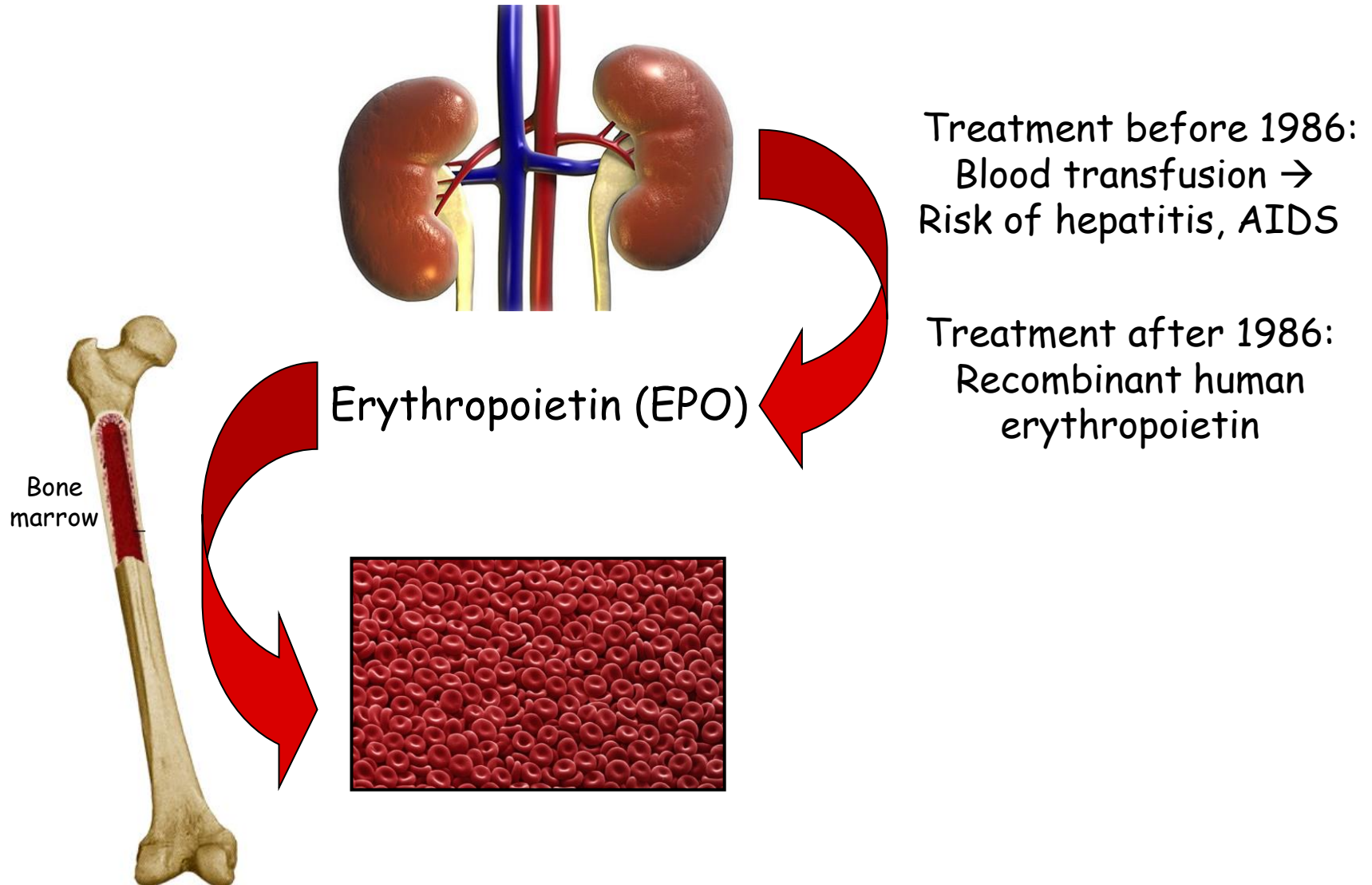


Treatment before 1986:
Blood transfusion →
Risk of hepatitis, AIDS

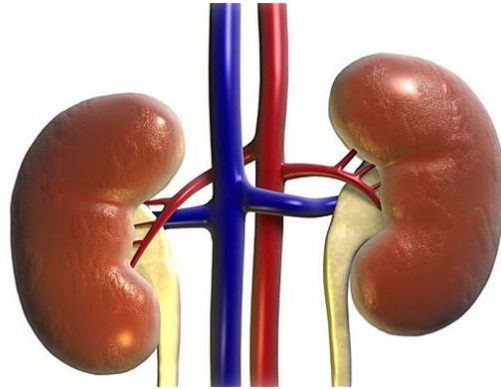
Erythropoietin (EPO)



Patients with Chronic Kidney Disease are Anemic Because Their Kidneys Stop Making EPO



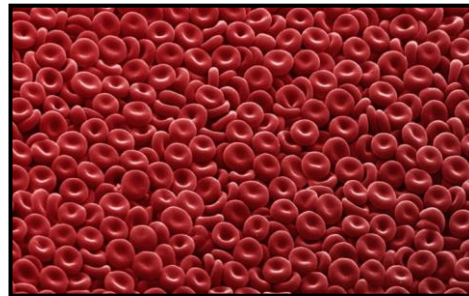
Patients with Chronic Kidney Disease are Anemic Because Their Kidneys Stop Making EPO



Treatment before 1986:
Blood transfusion →
Risk of hepatitis, AIDS

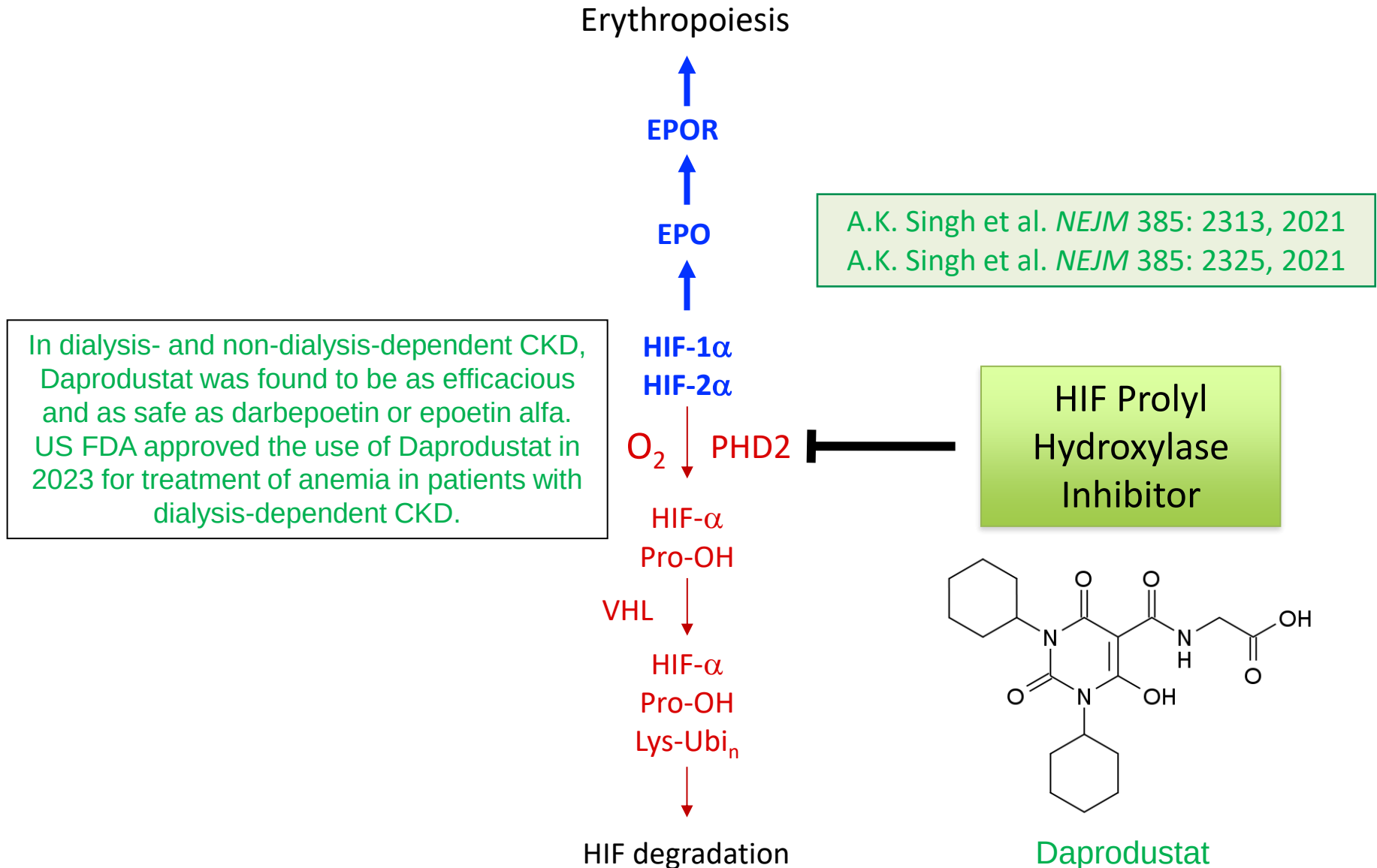
Treatment after 1986:
Recombinant human
erythropoietin

Erythropoietin (EPO)

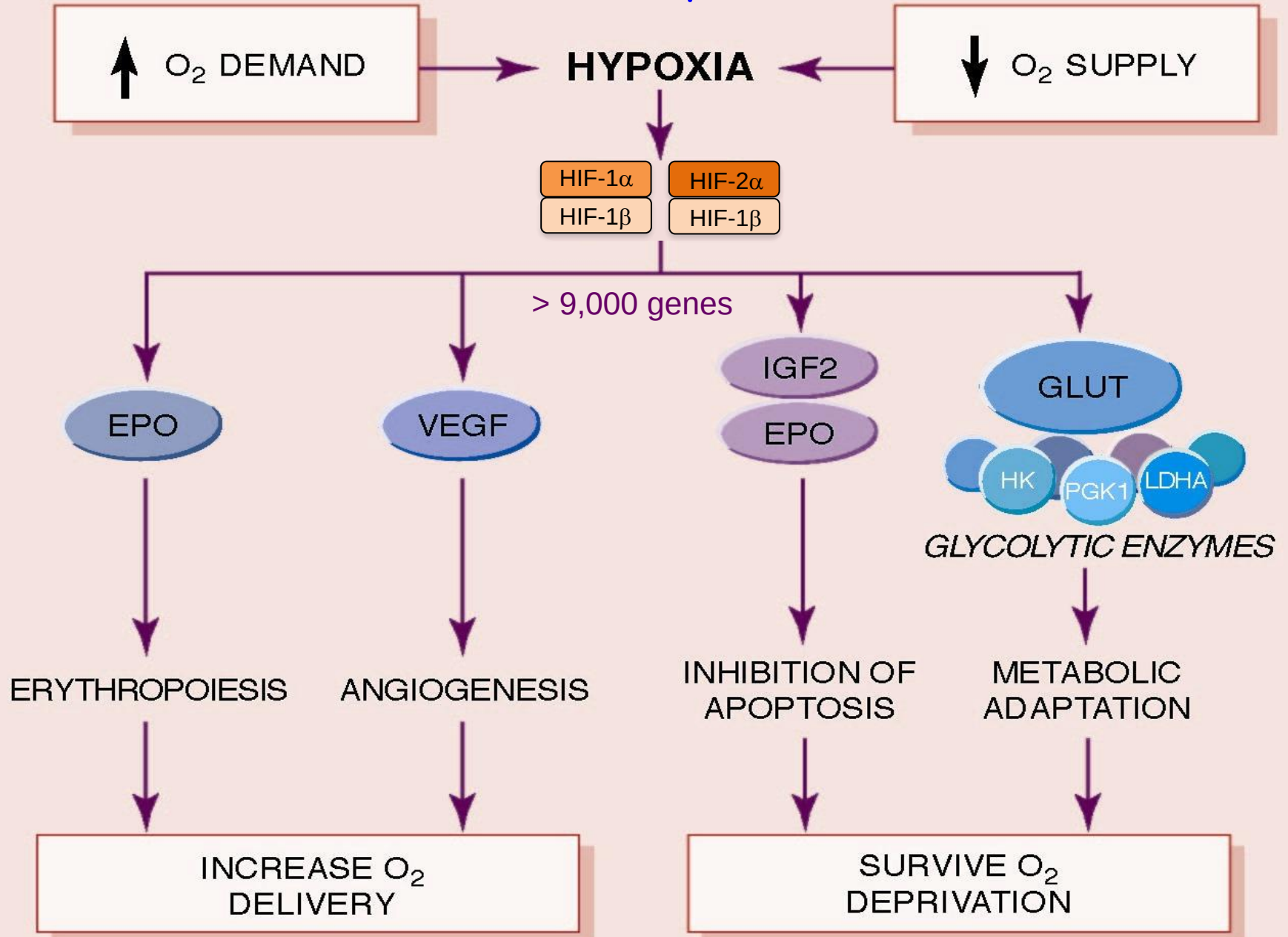


Drug:	<u>HIF-PHI</u>	<u>EPO</u>
Route:	Pill	Injection
Iron:	Stimulates uptake	Injection
Inflammation:	Effective	Ineffective

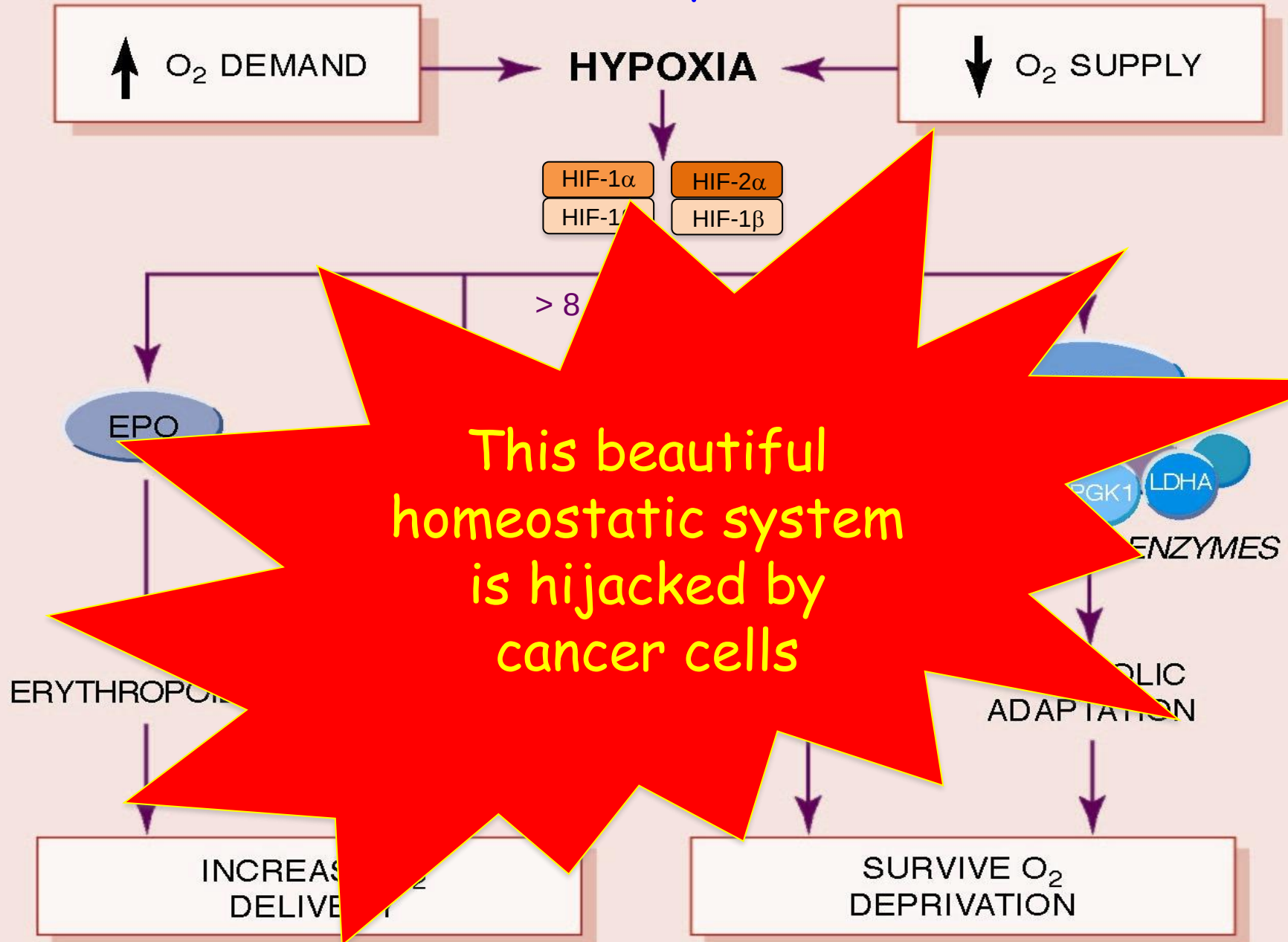
Prolyl Hydroxylase Inhibitor Stimulates Erythropoiesis In Patients with Chronic Kidney Disease



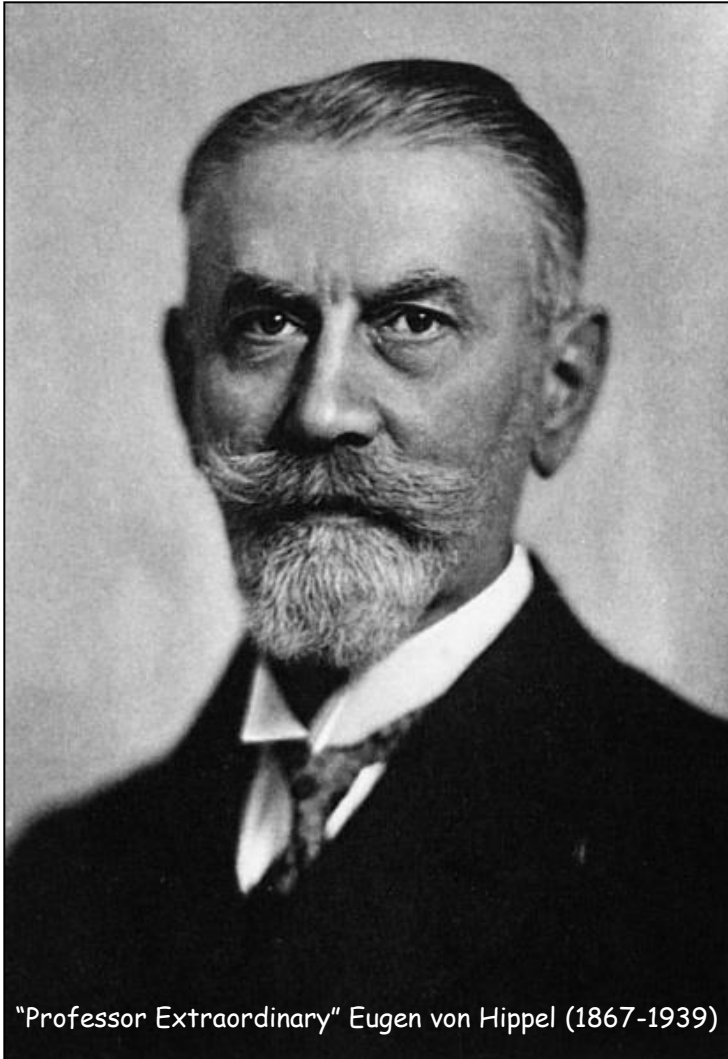
HIFs Mediate Homeostatic Responses to Reduced O₂ Levels



HIFs Mediate Homeostatic Responses to Reduced O₂ Levels



"About a Very Rare Disease of the Retina": The von Hippel-Lindau Syndrome



"Professor Extraordinary" Eugen von Hippel (1867-1939)

(Aus der Universitäts-Augenklinik zu Heidelberg.)

Über eine sehr seltene Erkrankung der Netzhaut.

Klinische Beobachtungen.

Von

Prof. Eugen v. Hippel
in Heidelberg.

Mit Tafel III—VI, Fig. 1—5.

Im Jahre 1895 stellte ich in der Demonstrationssitzung des Heidelberger Kongresses einen Patienten mit einer sehr ungewöhnlichen Erkrankung der Netzhaut vor, in der Hoffnung eine Belehrung darüber zu erhalten, wie der Fall zu deuten sei. Von den zahlreichen Untersuchern schien niemand einen analogen gesehen zu haben, eine Ansicht über das Wesen der Sache wurde nur von v. Michel geäußert, der sich zuerst für die Annahme eines Tumors, dann für Tuberkulose aussprach. Der Fall wurde von mir verfolgt, bis das Auftreten von Katarakt eine weitere ophthalmoskopische Beobachtung unmöglich machte. Am 15. VIII. 1896 sah ich einen zweiten vollkommen analogen Fall; ich konnte damals nur ein paar kurze Notizen machen, da der Patient, den ich zum Zeichnen des Befundes bestellt hatte, ausblieb und erst fünf Jahre später wieder erschien.

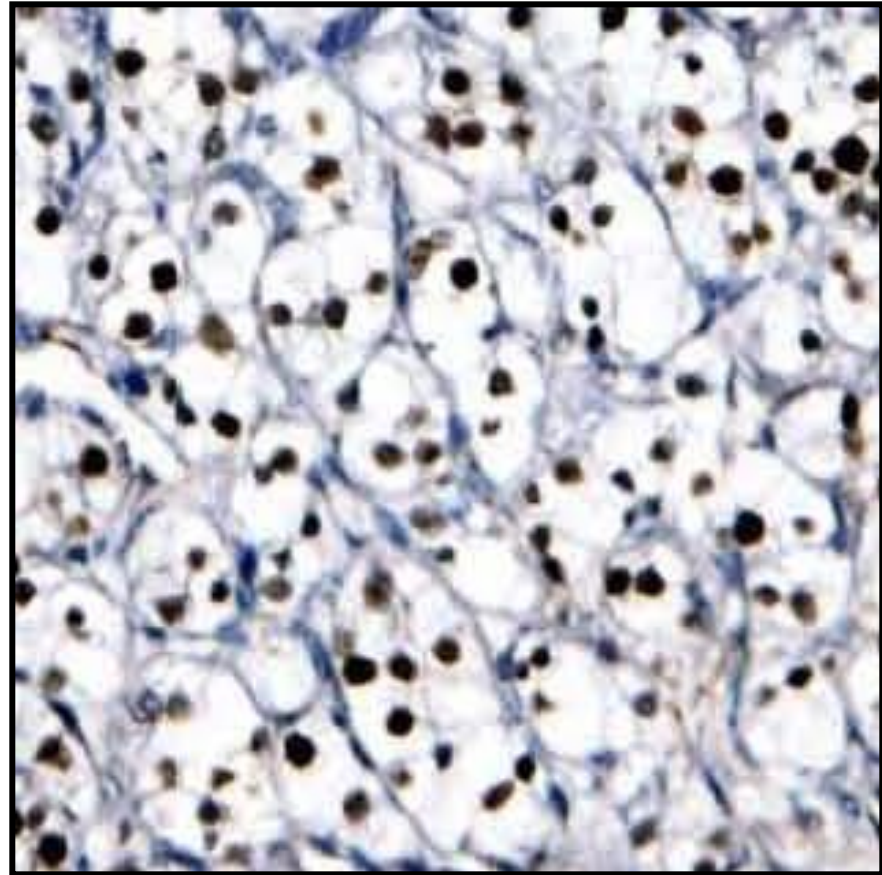
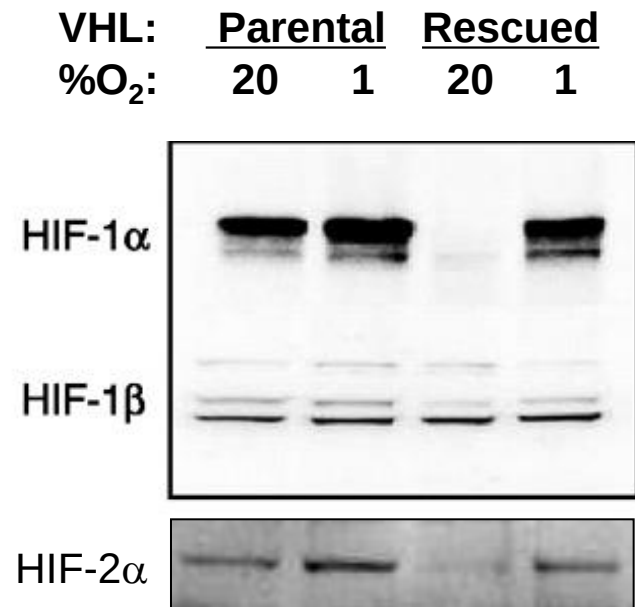
Die beiden Beobachtungen betreffen ein offenbar sehr seltenes Krankheitsbild, ich fand zunächst in der Literatur nur einen analogen Fall. Als ich beim vorjährigen Heidelberger Kongress meine Abbildungen zeigte und kurz erläuterte, wurde ich noch auf drei Veröffentlichungen¹⁾ aufmerksam gemacht, in welchen ähnliche Beobachtungen mitgeteilt wurden, ausserdem führten Sattler, Wagenmann und Herzog je einen derartigen Fall an, den sie zu untersuchen und behandeln Gelegenheit hatten.

¹⁾ Fall Leplat, v. Dzialowski und Goldzieher. (Vgl. die spätern epikritischen Bemerkungen.)

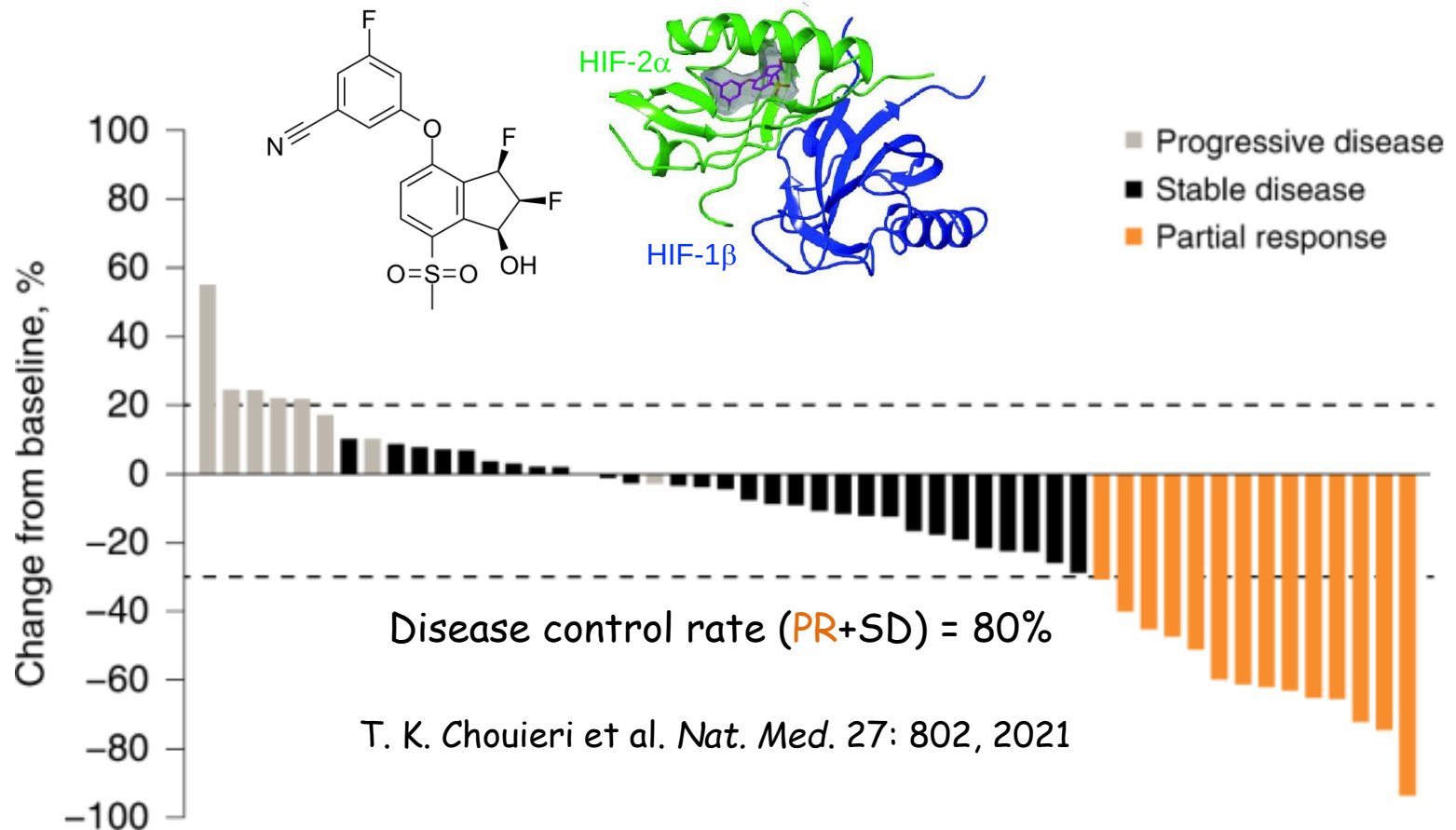
von Hippel E. Über eine sehr self seltene Erkrankung der Netzhaut. Klinische Beobachtungen Archive fur Ophthalmologie 1904;59:83-106.

Lindau A. Studien ber Kleinbirncysten Bau. Pathogenese und Beziehungen zur Angiomatosis Rentinae. Acta Radiol et Microbiol Scandinavica Suppl 1926;1:1- 128.

Von Hippel Lindau Syndrome: VHL Loss-of-Function Dysregulates HIF- α Expression in Renal Cell Carcinoma

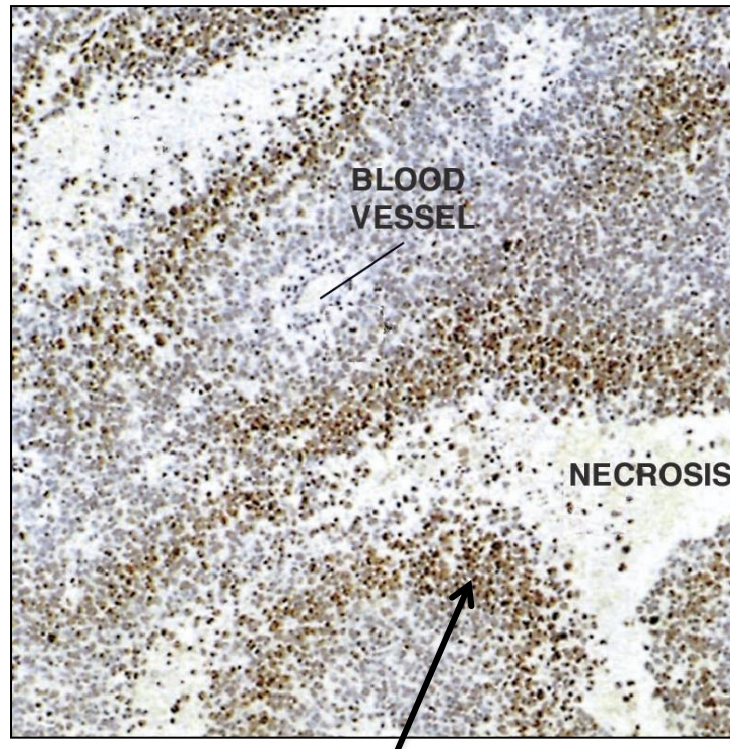


Phase I Trial of HIF-2 Inhibitor Belzutifan in Patients with Previously Treated Advanced Renal Cell Carcinoma



Approved by US FDA for: treatment of RCC in VHL patients (2021)
treatment (3rd line) all other RCC (2023)

Advanced Cancers Are Hypoxic and Express High Levels of Hypoxia-Inducible Factors

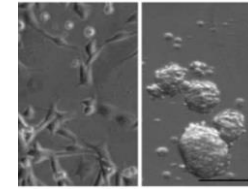


HIF-1 α expression by hypoxic cancer cells

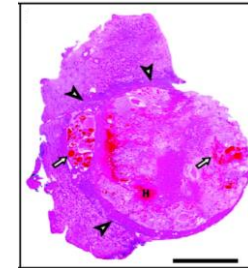
Increased HIF-1 α expression in the primary tumor biopsy is associated with decreased survival in bladder, brain, breast, cervical, colorectal, endometrial, gastric, hepatocellular, lung, oropharyngeal, ovarian, pancreatic, and prostate cancer.

Consequences of HIF Activation in Cancer Cells

1. Maintenance of Cancer Stem Cells



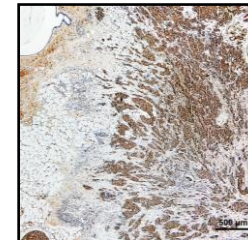
2. Autocrine Growth Factor Signaling



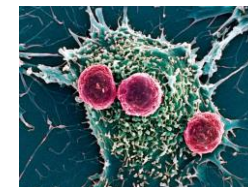
3. Vascularization



4. Glucose/Energy Metabolism



5. Invasion and Metastasis

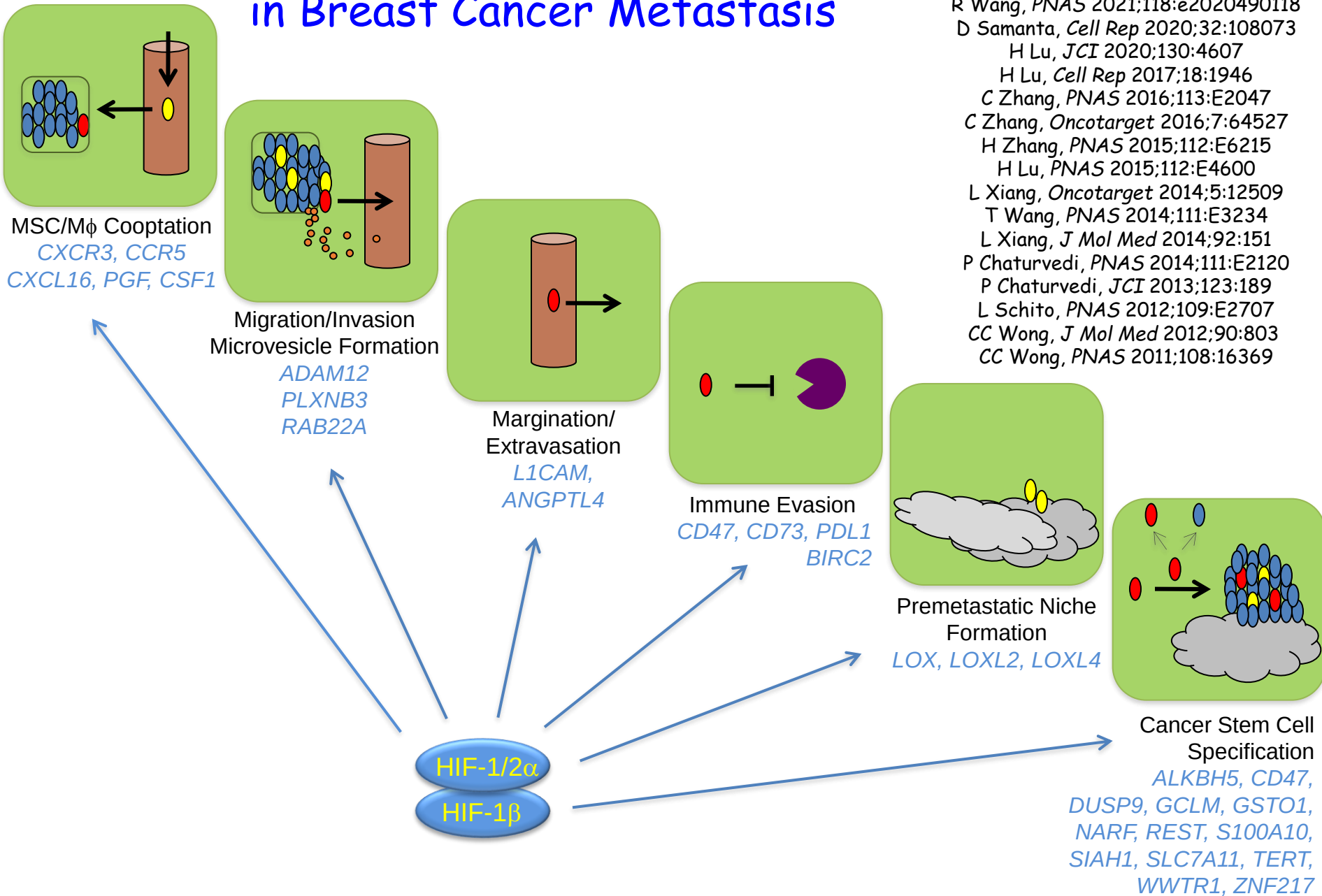


6. Immune Evasion

7. Chemotherapy and Radiation Resistance

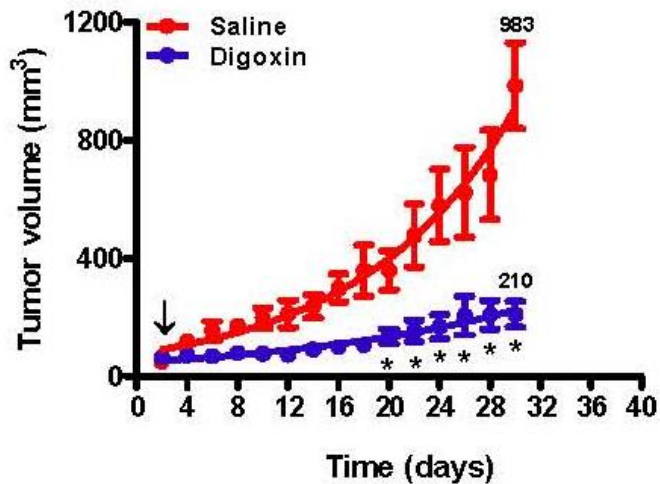
Deconvoluting the Role of HIFs in Breast Cancer Metastasis

Q Zuo, *Cell Rep* 2023;42:112164
 Y. Yang, *Sci Adv* 2022;8:eabo5000
 H Lu, *Cell Rep* 2021;36:109757
 R Wang, *PNAS* 2021;118:e2020490118
 D Samanta, *Cell Rep* 2020;32:108073
 H Lu, *JCI* 2020;130:4607
 H Lu, *Cell Rep* 2017;18:1946
 C Zhang, *PNAS* 2016;113:E2047
 C Zhang, *Oncotarget* 2016;7:64527
 H Zhang, *PNAS* 2015;112:E6215
 H Lu, *PNAS* 2015;112:E4600
 L Xiang, *Oncotarget* 2014;5:12509
 T Wang, *PNAS* 2014;111:E3234
 L Xiang, *J Mol Med* 2014;92:151
 P Chaturvedi, *PNAS* 2014;111:E2120
 P Chaturvedi, *JCI* 2013;123:189
 L Schito, *PNAS* 2012;109:E2707
 CC Wong, *J Mol Med* 2012;90:803
 CC Wong, *PNAS* 2011;108:16369

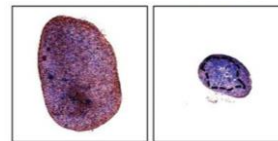
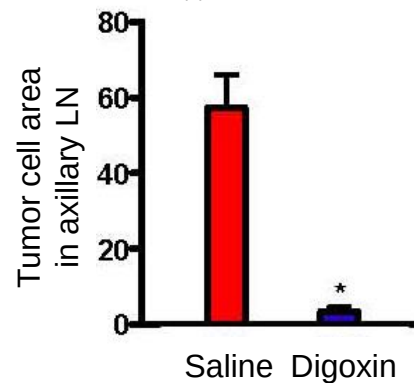


HIF-1 Inhibitor Digoxin Decreases Primary Tumor Growth and Metastasis in a Model of Triple-Negative Breast Cancer

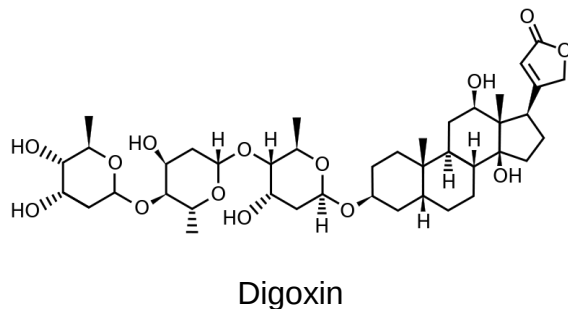
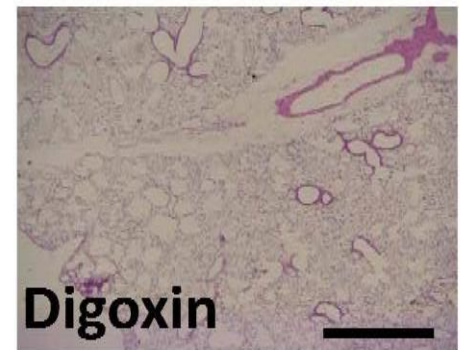
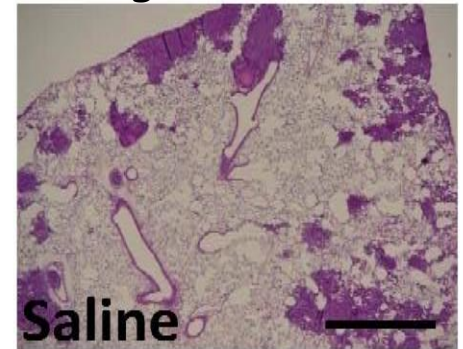
Primary tumor growth



Lymph node metastasis



Lung metastasis

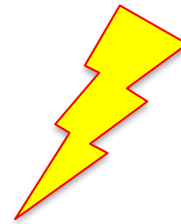


L. Schito et al. *PNAS* 2012;109:E2707
C.C. Wong et al. *J. Mol. Med.* 2012;90:803

T Cells are Programmed To Kill Cancer Cells

*Adaptive
immunity*

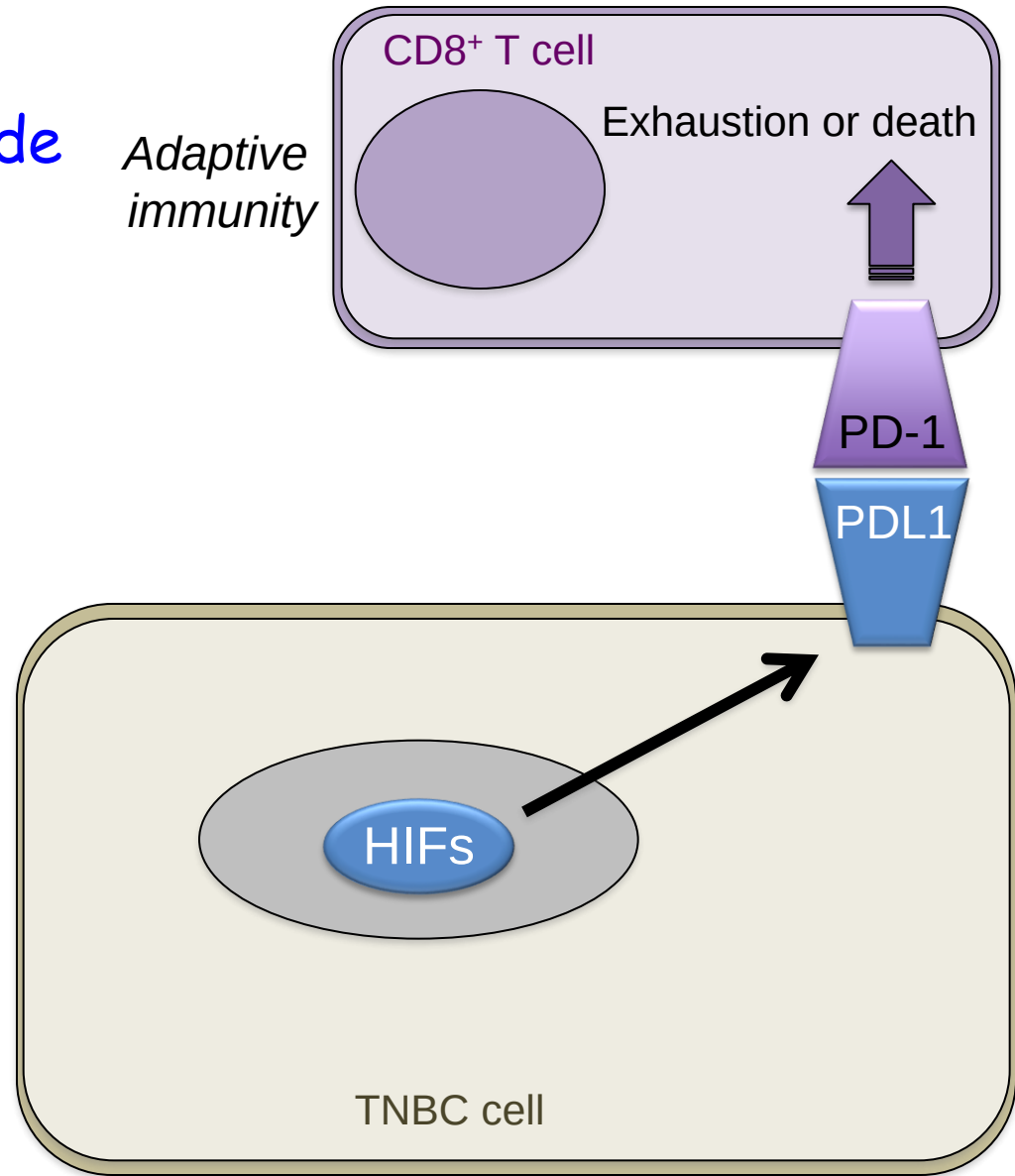
CD8⁺ T cell



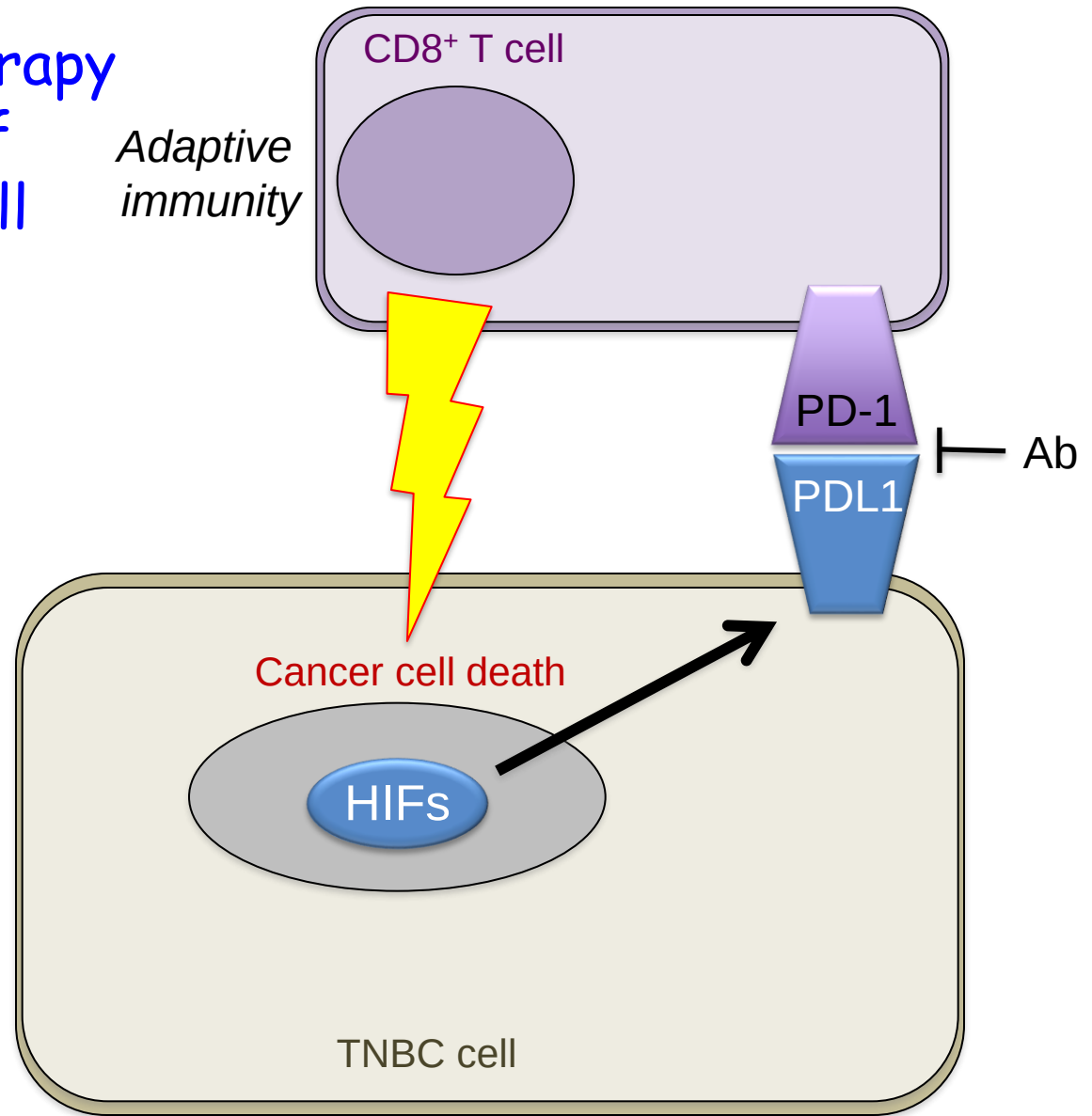
Cancer cell death

TNBC cell

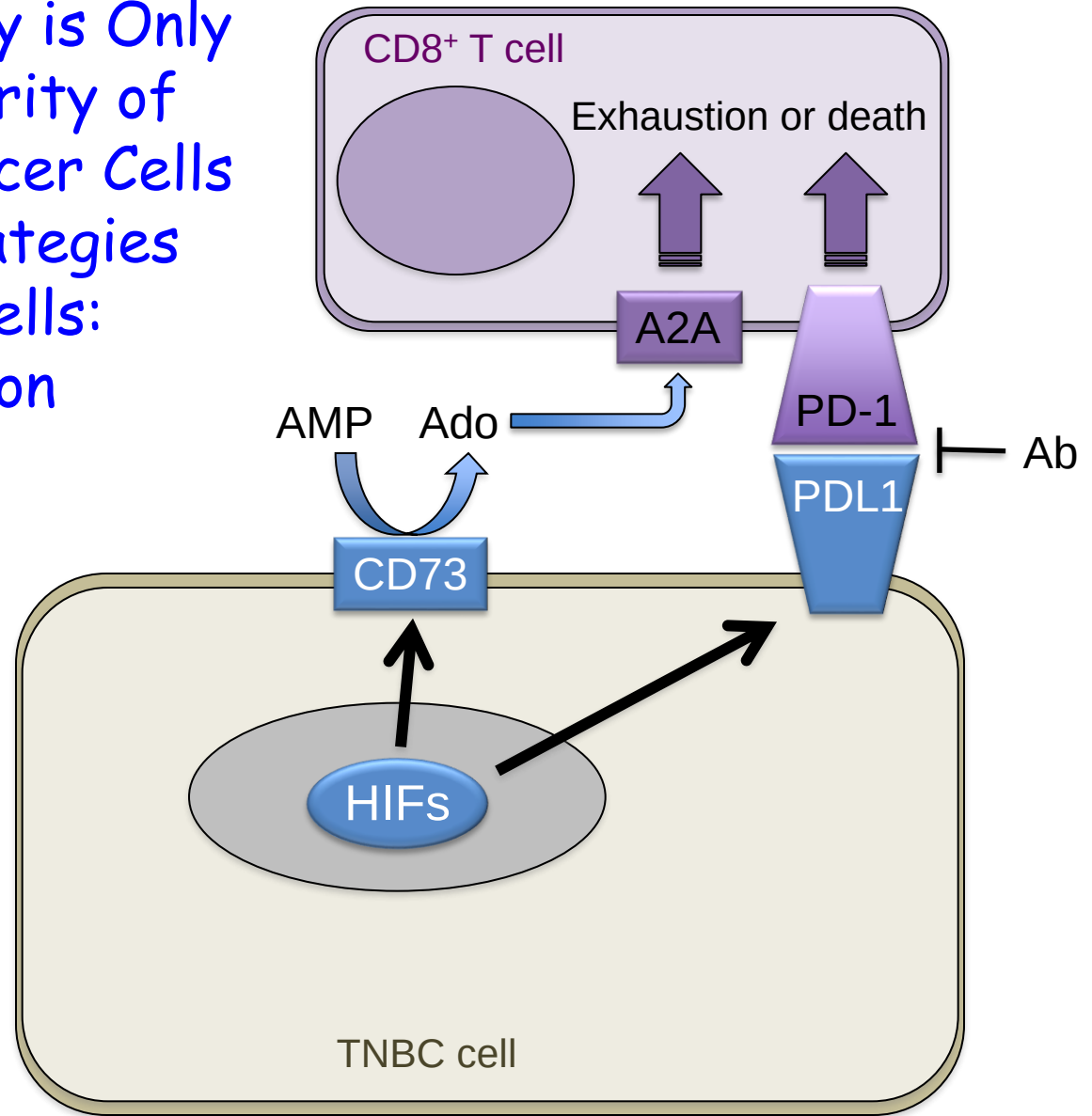
But Cancer Cells Evade Killing by T Cells Through PD-L1



Anti-PD-1 Immunotherapy Restores Ability of Immune Cells to Kill Cancer Cells



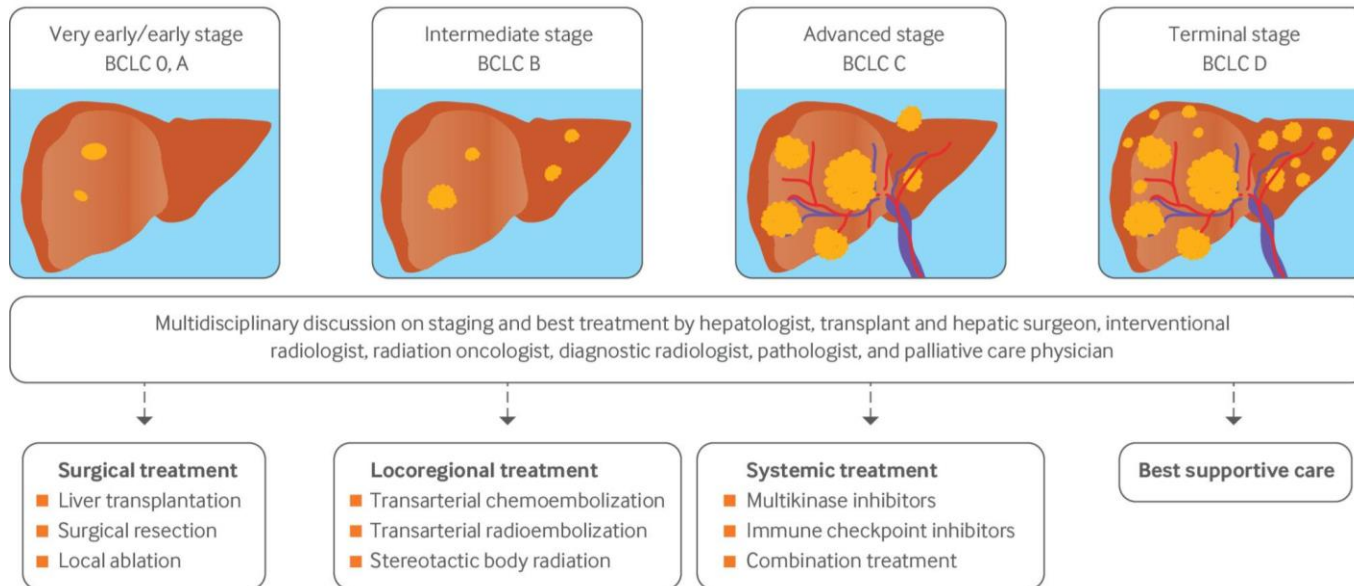
But Immunotherapy is Only
Effective in a Minority of
Patients Because Cancer Cells
Employ Other Strategies
For Evading T Cells:
CD73 Expression



Targeting HIFs in Hepatocellular Carcinoma

Hepatocellular Carcinoma (HCC) is the third leading cause of cancer death worldwide and the most rapidly growing cancer diagnosis in the U.S. over the last two decades

Most HCC patients have advanced disease at Dx with limited options: 5-yr survival < 15%.

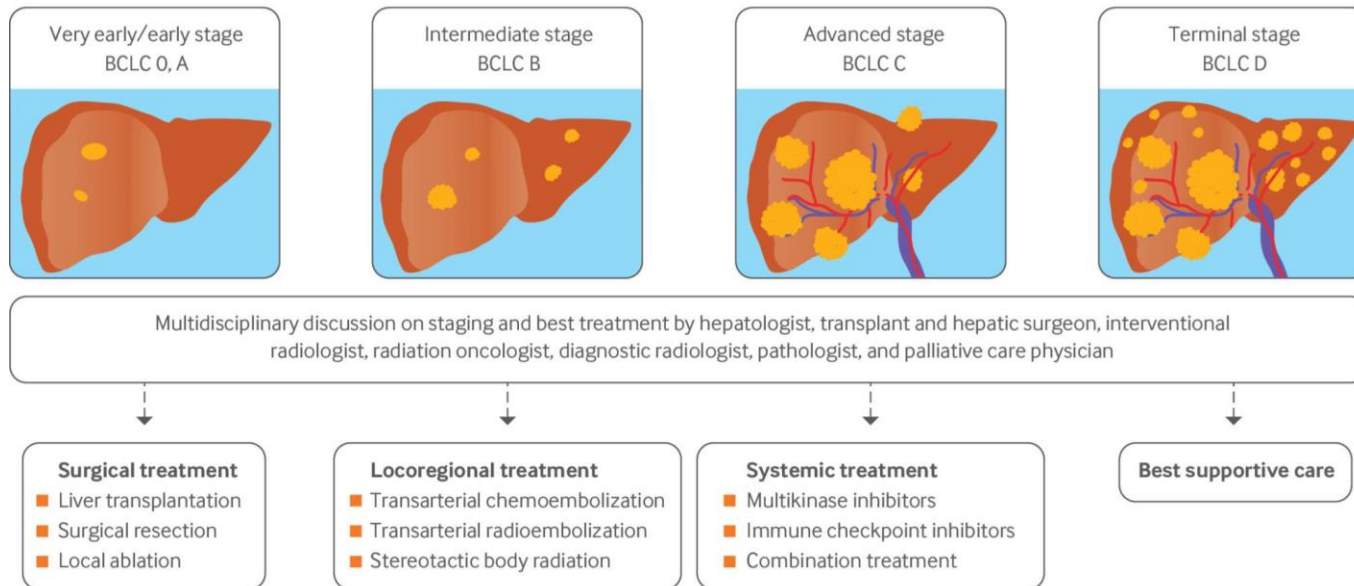


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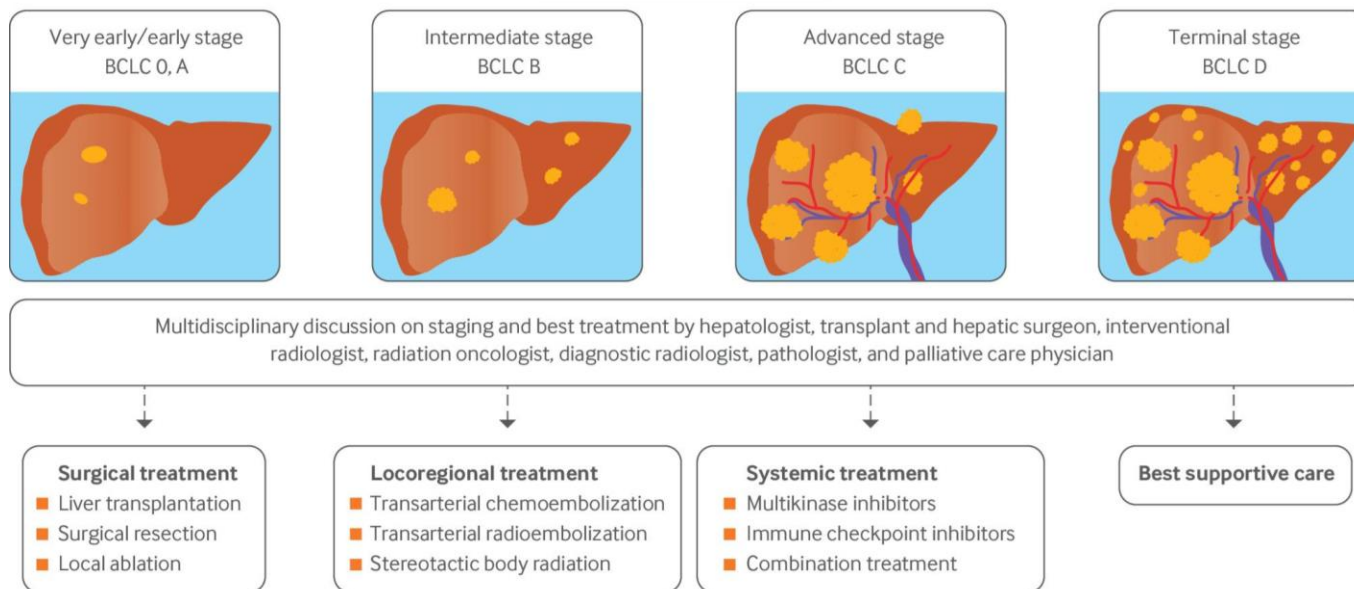
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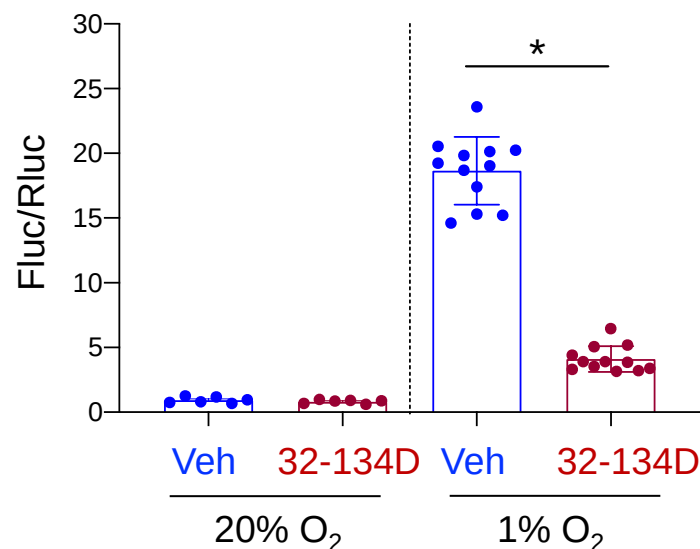
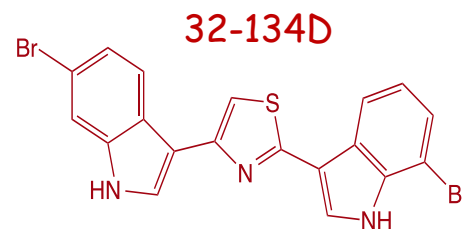
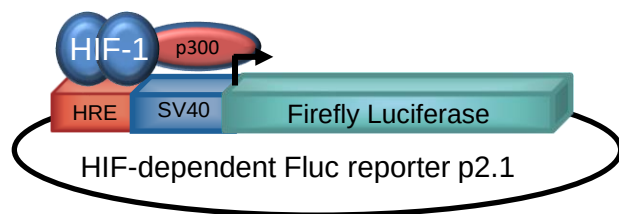
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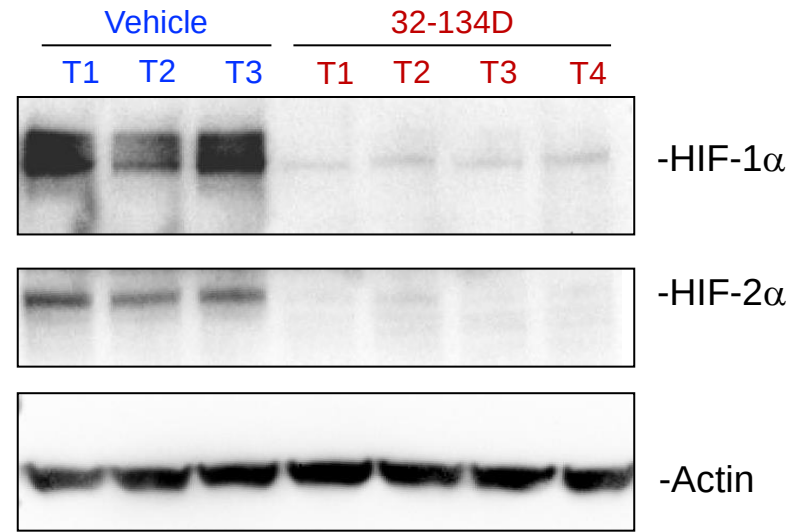
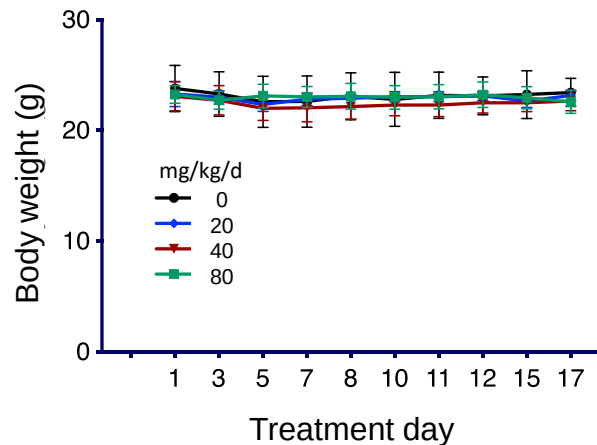
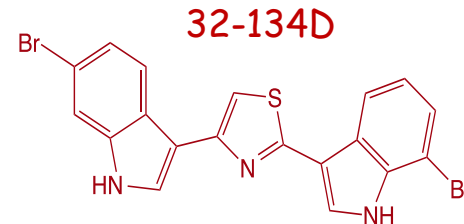
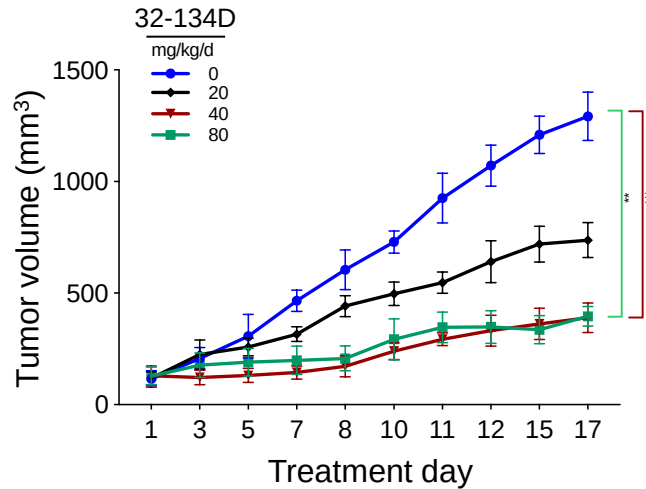
HIF-1 α expression is increased in 60% of cases and is associated with increased risk of recurrence after radiation therapy or surgery and increased patient mortality.



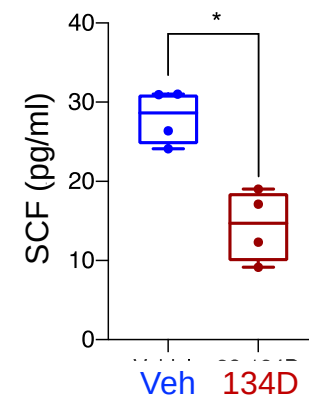
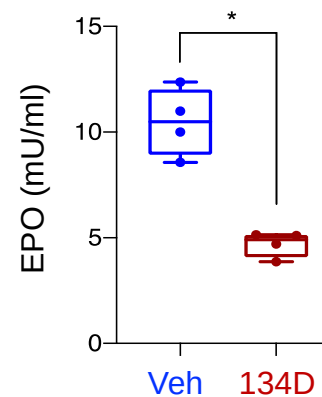
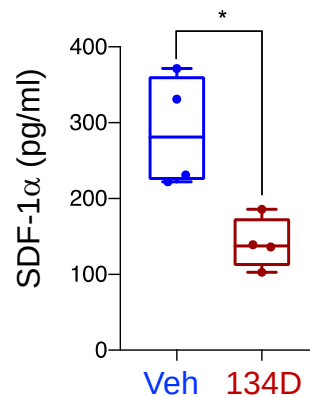
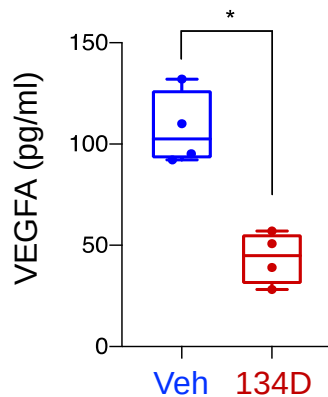
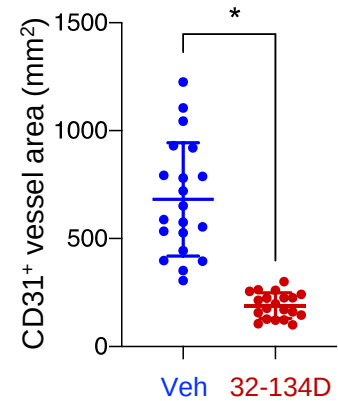
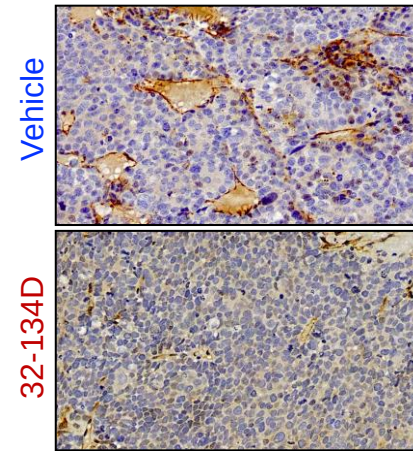
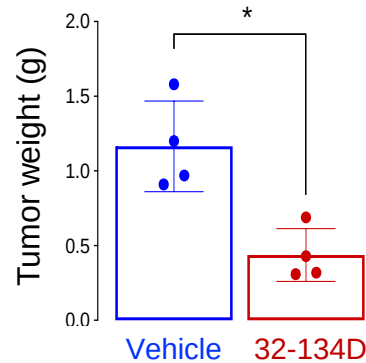
Identification of a Small Molecule Inhibitor Of HIF Transcriptional Activity in Hep3B Cells



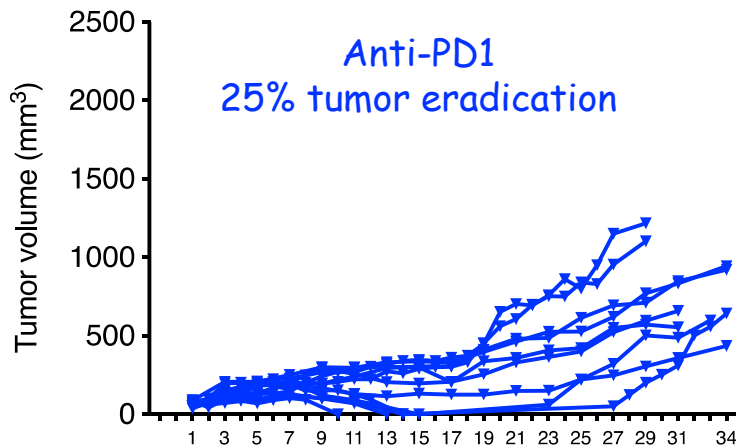
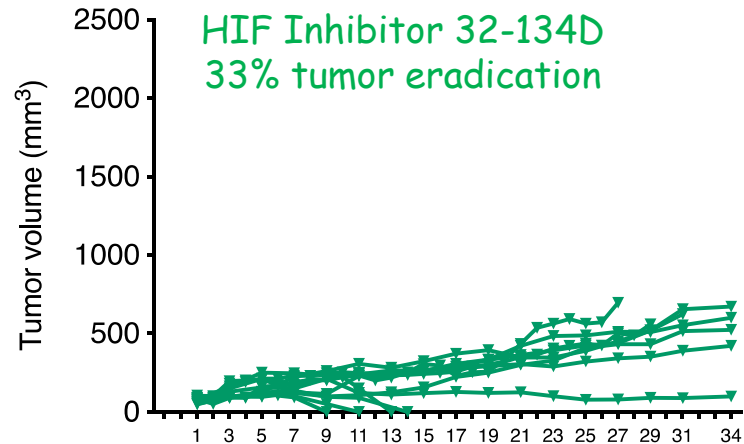
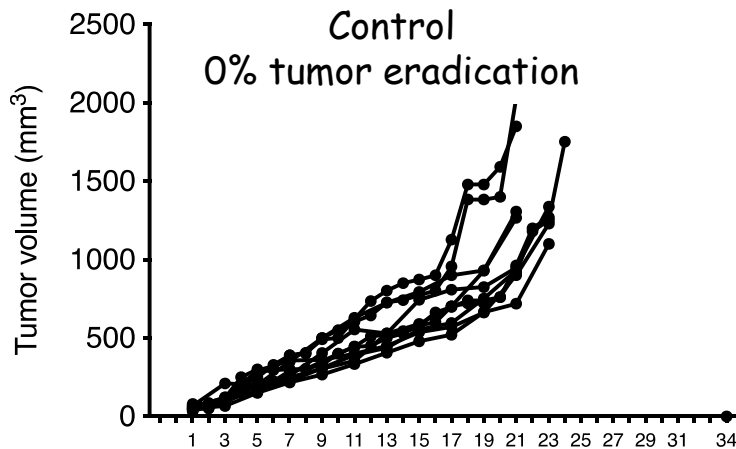
32-134D Inhibits Hep3B Tumor Xenograft Growth By Triggering Degradation of HIF-1 α and HIF-2 α



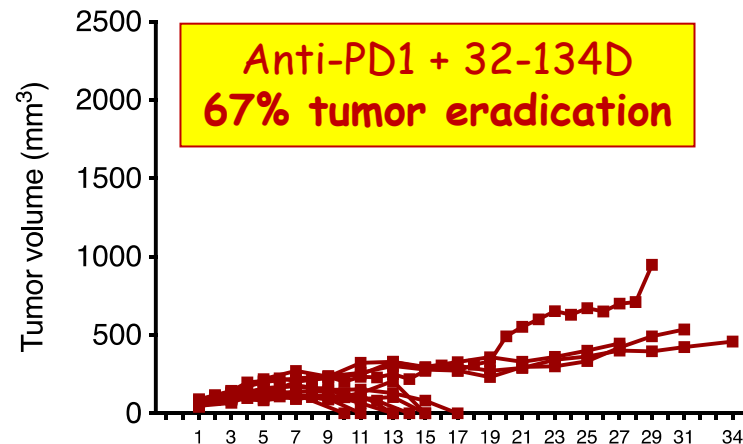
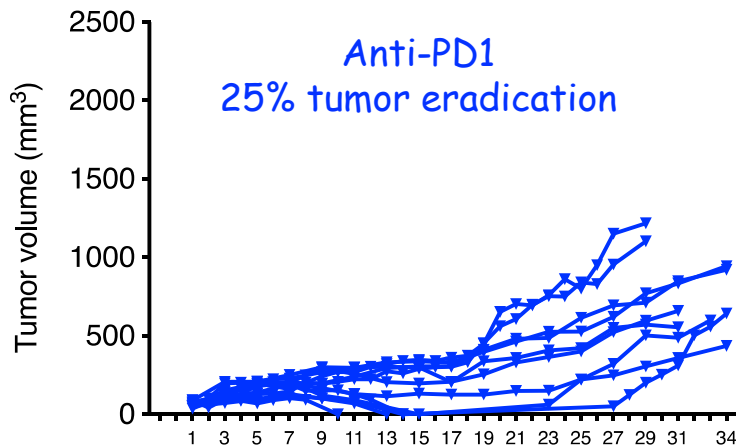
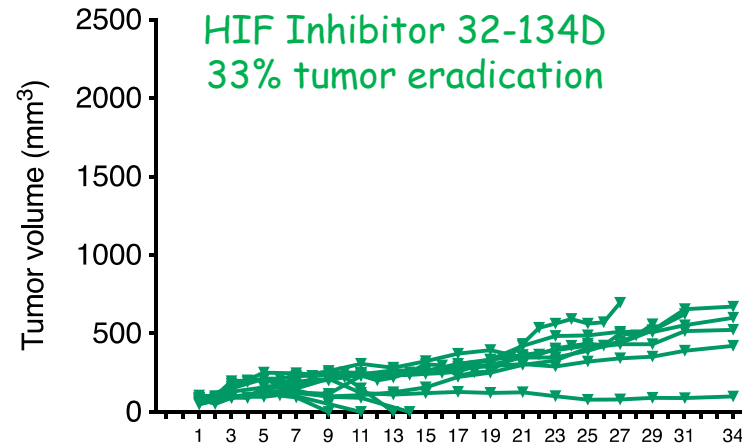
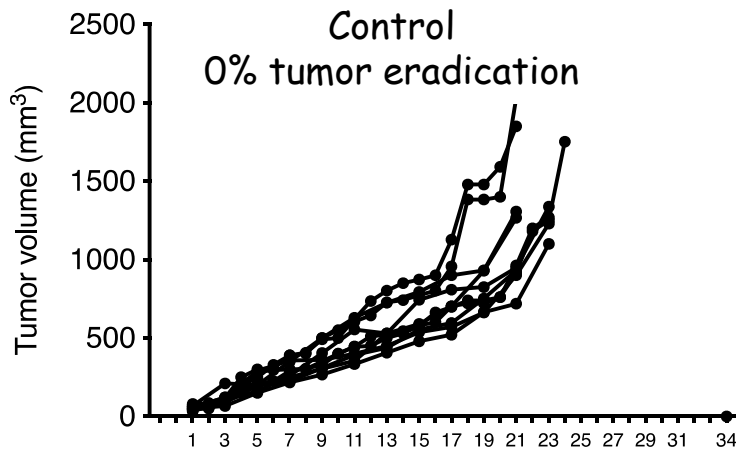
32-134D Blocks Hep3B Tumor Xenograft Vascularization By Inhibiting Expression of Angiogenic Growth Factors



Effect of Anti-PD1 or 32-134D Administration on Hepa1-6 Tumor Growth in Syngeneic C57L Mice

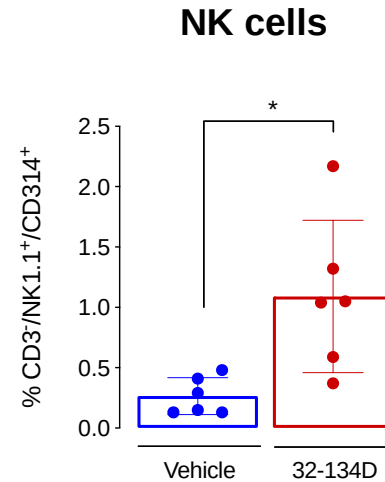
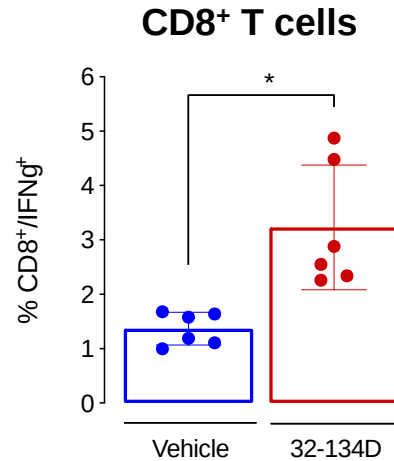


Co-Administration of 32-134D Significantly Improves the Response to Anti-PD1 Immunotherapy

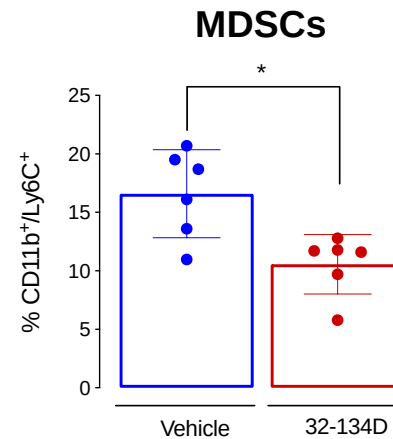
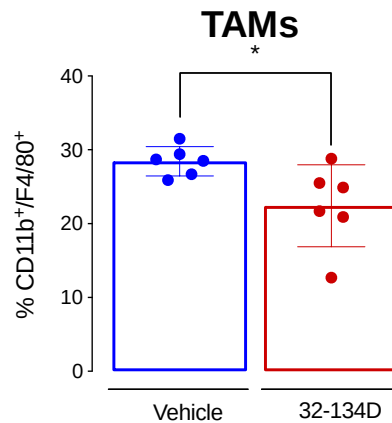


Treatment with HIF Inhibitor 32-134D Alters the Tumor Immune Cell Microenvironment

These cells mediate anti-tumor immunity and are the cells targeted by anti-PD1 therapy

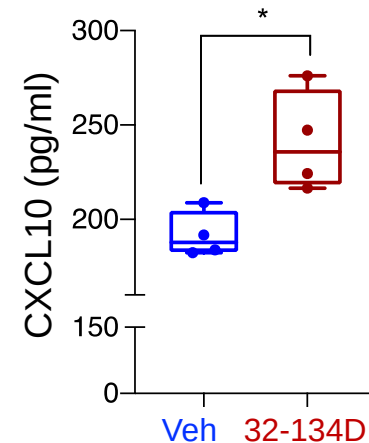
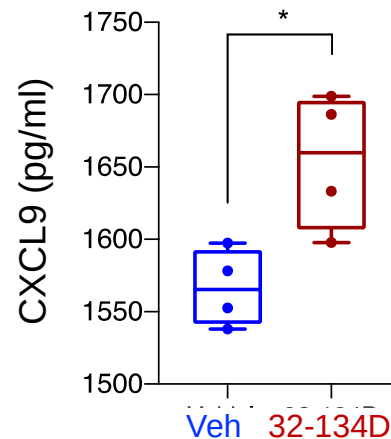


These cells mediate immunosuppression and resistance to anti-PD1 therapy

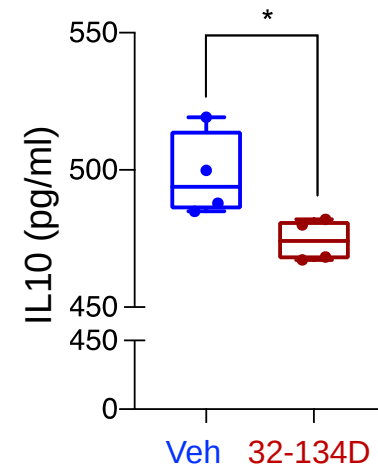
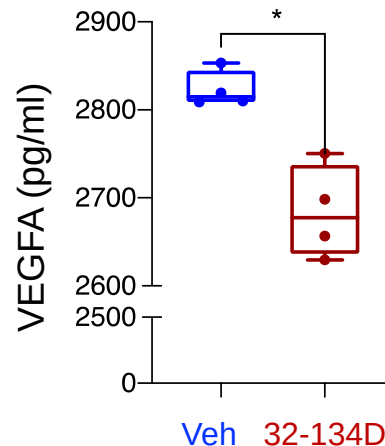


32-134D Alters Cytokine/Chemokine Expression in Tumor To Restore Anti-Tumor Immunity and Response to Anti-PD1

These chemokines recruit CD8⁺ T cells and NK cells, which mediate anti-tumor immunity.



These cytokines recruit TAMs, MDSCs, and Tregs, which mediate immunosuppression.



Effects of 32-134D in Hepatocellular Carcinoma

Induces degradation of HIF-1 α and HIF-2 α

Blocks expression of multiple angiogenic growth factors
and inhibits tumor vascularization

Converts immunosuppressive tumor microenvironment
to one favoring anti-tumor immunity

Sensitizes HCC to anti-PD1 therapy

2024 Update: Two New HIF Inhibitors With Broad Anti-Cancer Activity

Induce degradation of HIF-1 α and HIF-2 α

Block expression of multiple angiogenic growth factors
and inhibit tumor vascularization

Convert immunosuppressive tumor microenvironment
to one favoring anti-tumor immunity

Sensitize breast, colorectal, lung, melanoma,
pancreatic and prostate cancer to immunotherapy

Novel HIF Inhibitors Increase Complete Responses To Immunotherapy by >20-Fold in All Common Cancers

<u>Cancer</u>	<u>Response Rate</u>	
	<u>ICB</u>	<u>ICB+HIFi</u>
Breast, HER2 ⁺	0/5	5/5
Breast, TNBC	1/5	4/10
Colorectal	0/5	3/5
Lung	0/5	5/5
Melanoma	0/5	5/10
Pancreatic	0/10	5/10
Prostate	<u>0/5</u>	<u>5/10</u>
	1/40	32/55
	2.5%	58%

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Final Thoughts

Basic science discoveries lead to unpredicted, transformative advances in medicine

Taking advantage of serendipity requires an open mind and broad preparation - students should not specialize too quickly

Venture capital must do more venturing and less "me too" investing

AI will become a powerful tool for scientific discovery

There will continue to be important scientific discoveries made without using AI

The high cost of preclinical and clinical studies in the U.S. makes Europe - ITALY! - an attractive environment for drug development

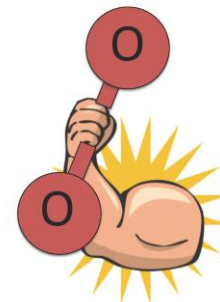
Acknowledgments



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