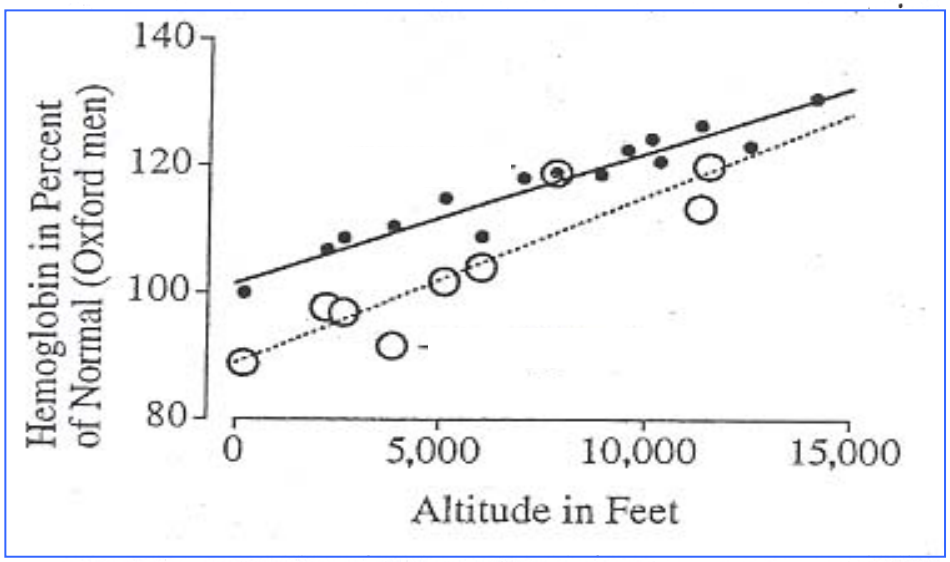
Oxford-Yale expedition to Pike's Peak, Colorado

J. S. Haldane and colleagues - July 1911



Signalling hypoxia in cells

O_2

↓

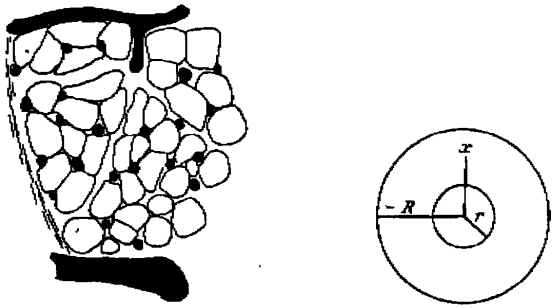
Sensor

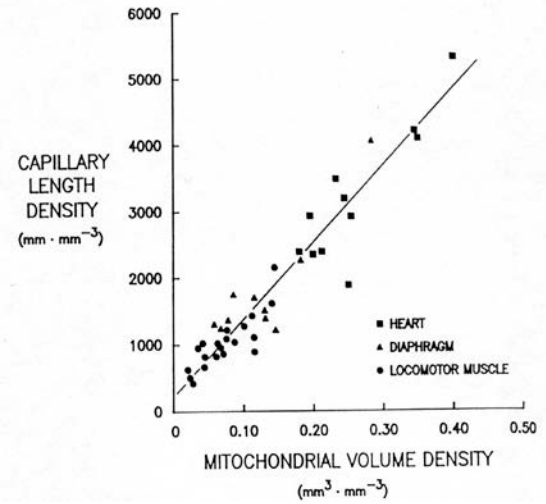
↓

Epo

Krogh

J. Physiol 1919 52 409-415





Signalling hypoxia in cells

O_2

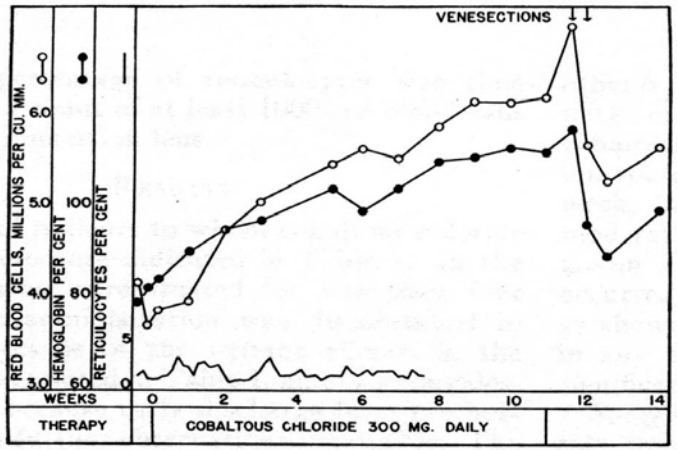
↓

Sensor

↓

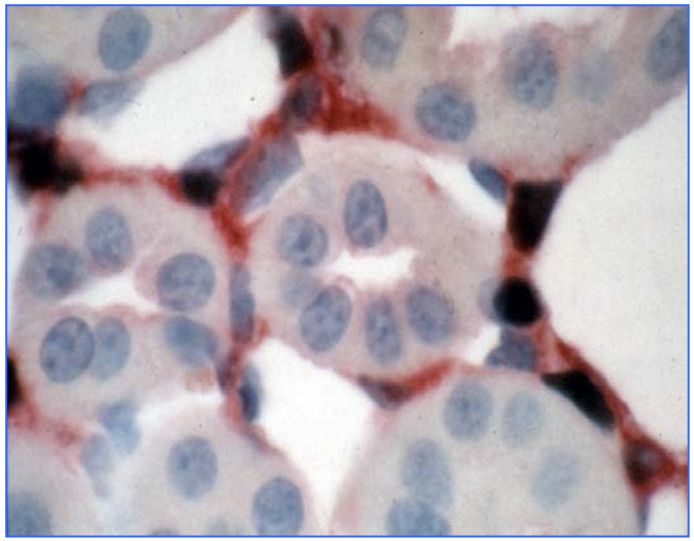
Epo

Cobalt poisoning causes erythrocytosis without obvious metabolic compromise



Transgenic marker gene strategy indicates that erythropoietin producing cells are

Interstitial fibroblasts



Dissection of hypoxia signalling pathways

Transcription factors

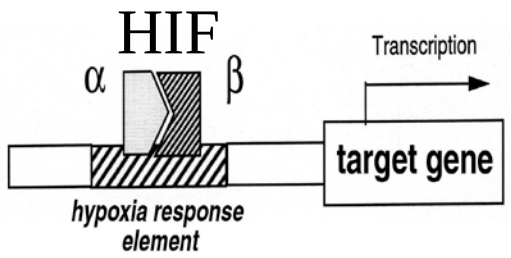
ERYTHROPOIETIN

Angiogenesis

Vasomotor regulators

Matrix metabolism

Transporters



Iron metabolism

Ion Channels

Redox control

Glucose metabolism

Growth factors

PHOSPHOGLYCERATE KINASE

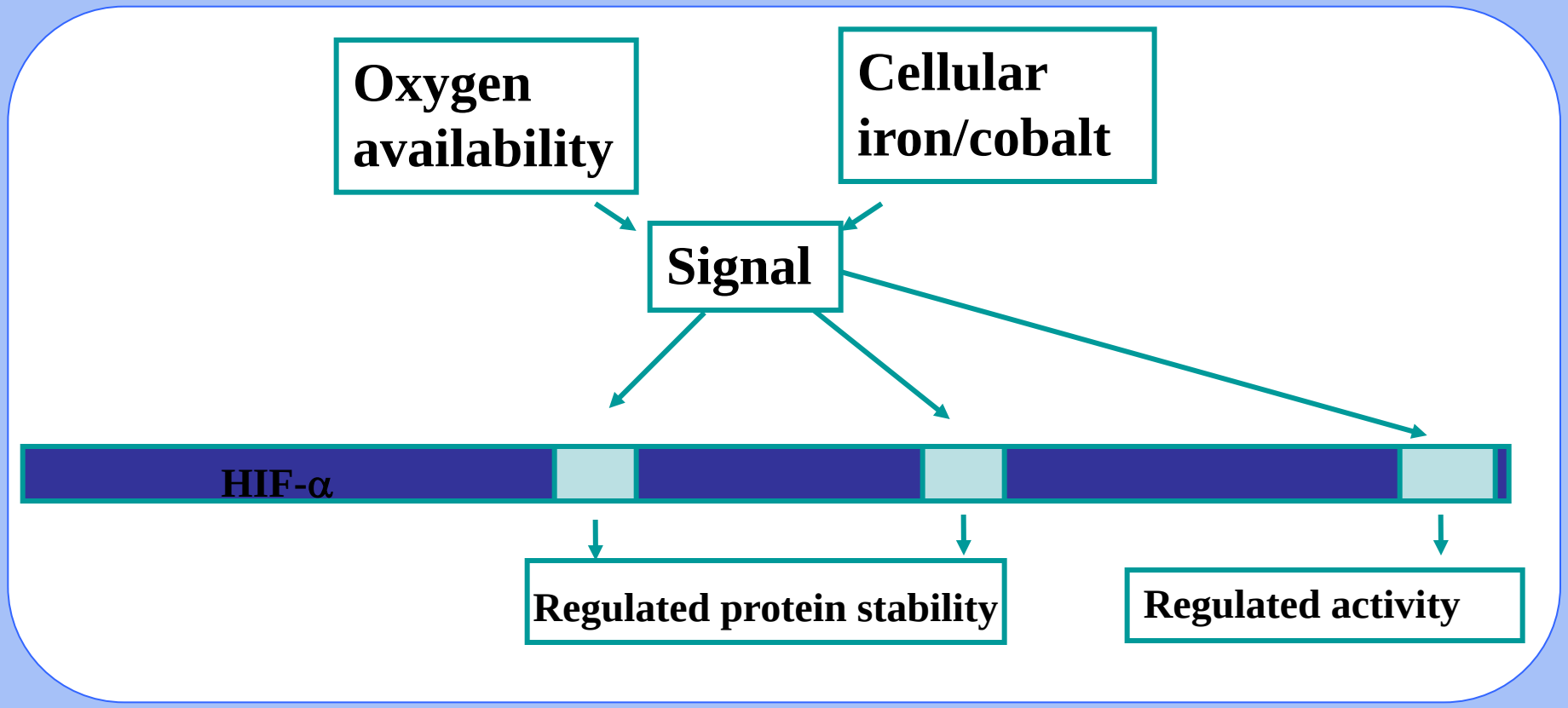
Mitochondrial control

Oncogenes

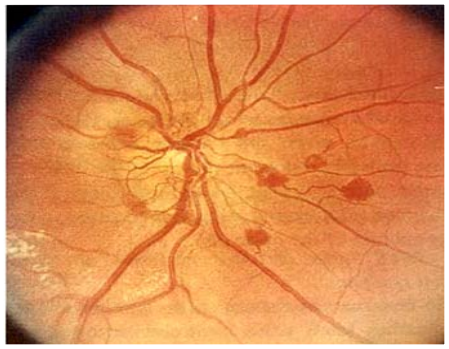
Fat metabolism

Apoptotic regulators

Regulation of HIF by oxygen



The von Hippel-lindau tumour supprerssor is directly linked to hypoxia pathways

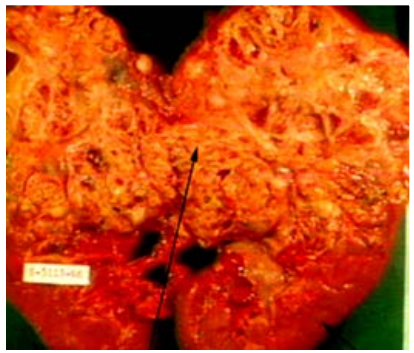


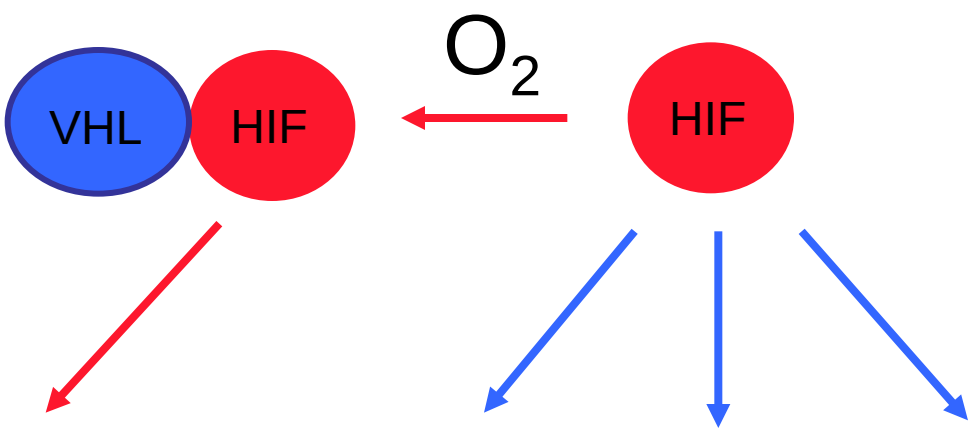
Certain types of kidney cancer are associated with

Excessive red cell production (erythropoietin)

Excessive blood vessel growth (angiogenesis)

These cancer are defective for the VHL tumour supprssor **(VHL)**





Signalling modification is prolyl hydroxylation

Biochemical analysis

Heat labile extract

~~Non-enzymatic oxidation~~

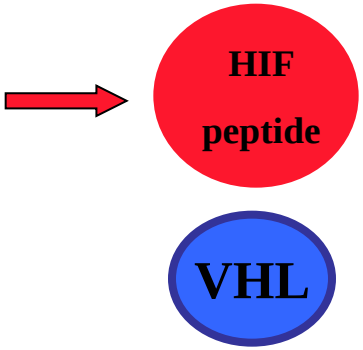
~~NADH/NADHoxidase~~

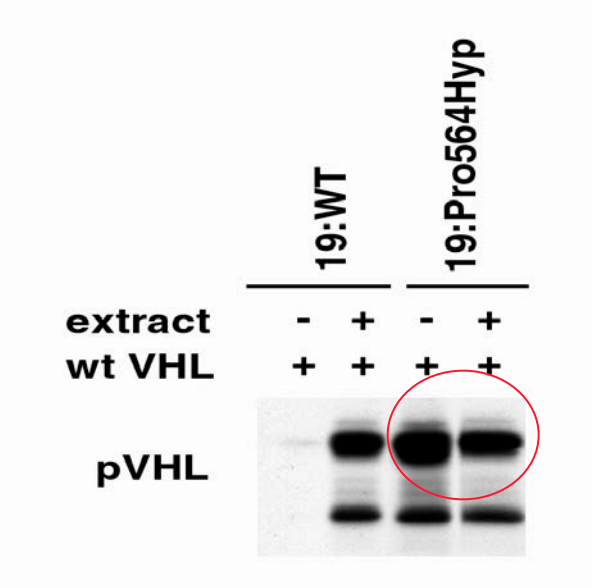
Oxygen

Iron

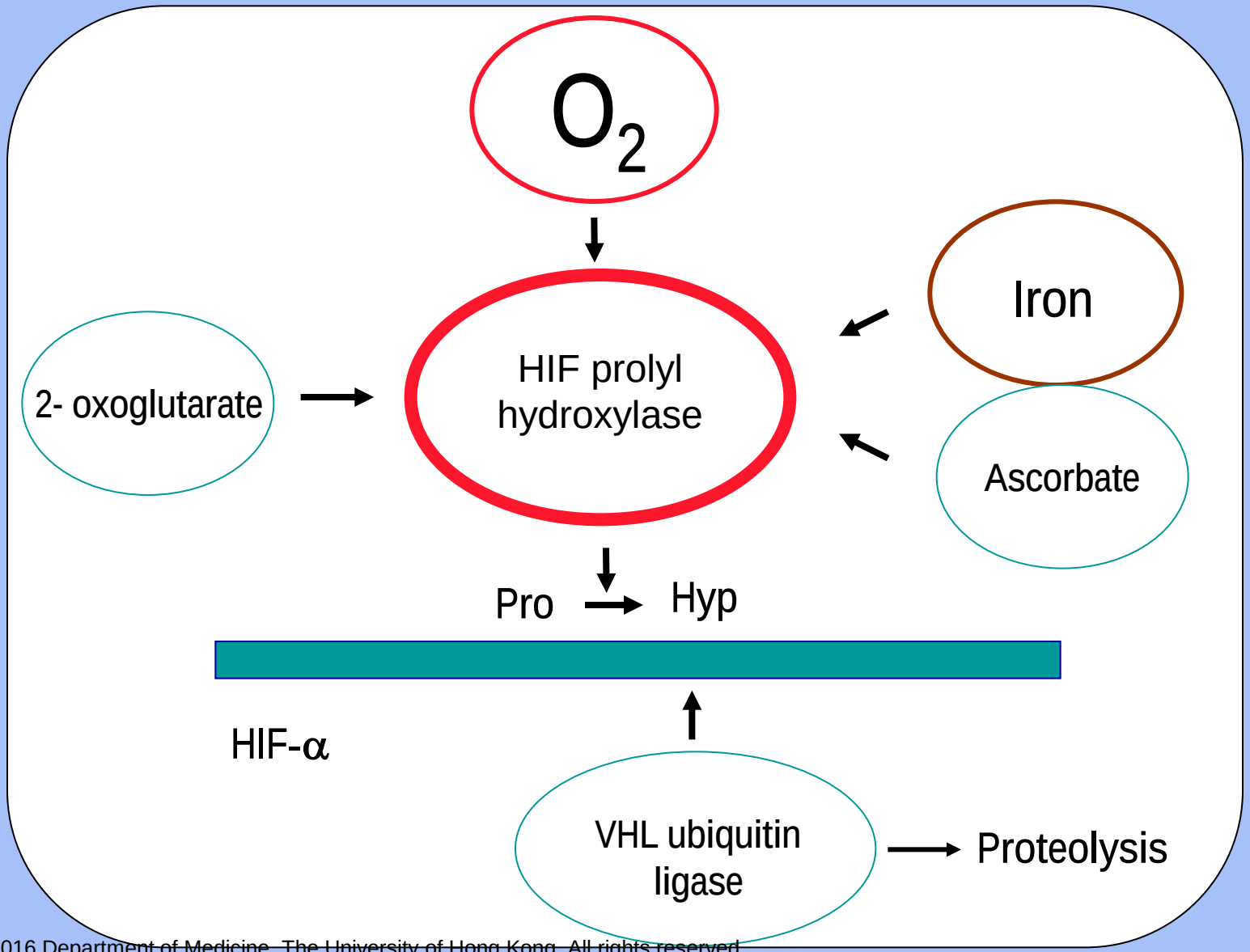
~~ATP~~

Treatment with cell extract

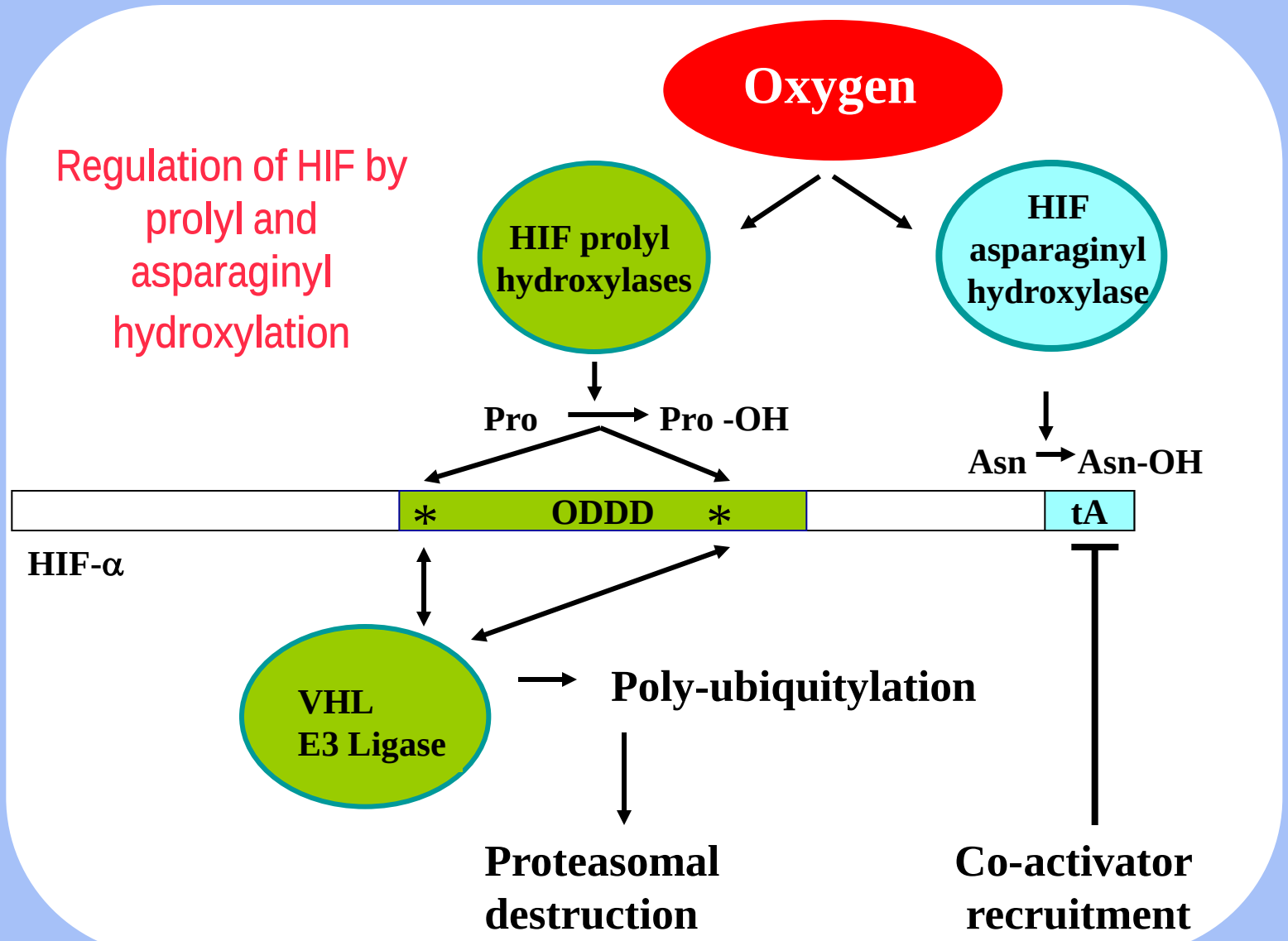




HIF proyl hydroxylation implies a mechanism of oxygen sensing

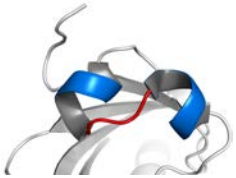


HIF prolyl hydroxylases - a set of Fe(II) and 2-oxoglutarate dioxygenases that are conserved throughout the animal kingdom



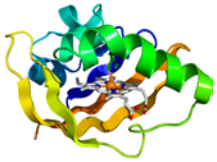
Implications of Darwinian evolution for understanding (and translating) biology

Basic helix-loop-helix proteins



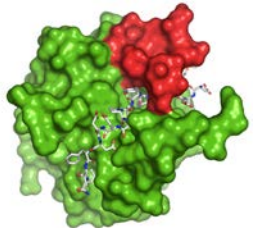
(eukaryotes; yeast, plants, animals)

PAS domain proteins



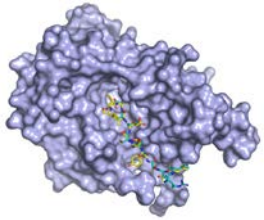
(sensing functions, many phyla; incl. prokaryotes, eukaryotes)

PHD – like enzymes



(range of non-metazoan as well as non-metazoan species)

FIH – like enzymes



(range of species incl. prokaryotes and eukaryotes)

bHLH – PAS – ODD - CAD

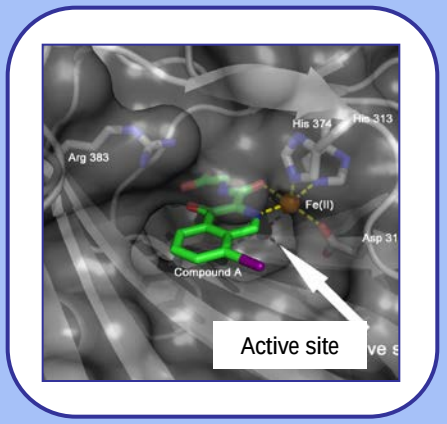
Hypoxia inducible factor (HIF)
(basic-helix-loop-helix PAS proteins)

Therapeutic potential of activating HIF pathways by inhibition of HIF hydroxylases

Erythropoiesis -Epo, Iron metabolism

Angiogenesis –Growth factors, Receptors, Matrix

Cytoprotection –Energy metabolism, Anti-oxidants



Wound healing – Inflammation, oxygen delivery

Stem cell behaviour – Transcription factors

Adiposity –Lipid metabolism, adipose differentiation

Glucose homestasis – Energy metabolism

Respiratory stimulant – Channels glomus cells

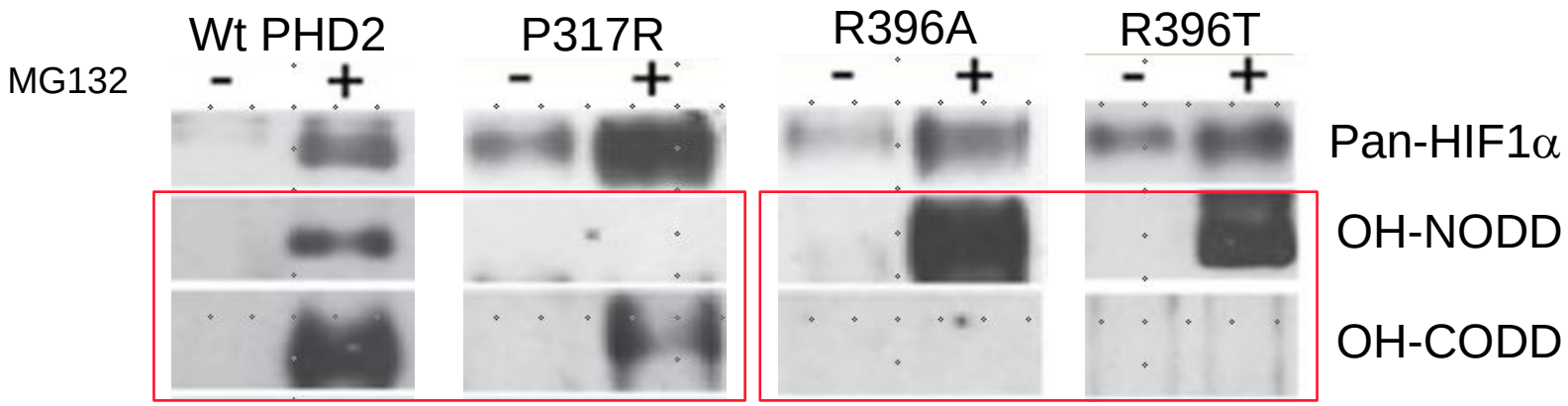
Co-evolution of HIF hydroxylase pathways and animals

Would it be possible to design more selective small molecule interventions on the PHD/HIF system?

Mammalian HIF has two hydroxylation sites NODD (N-terminal) and CODD (C-terminal)
<u>evidence for selective hydroxylase activity from human genetics</u>

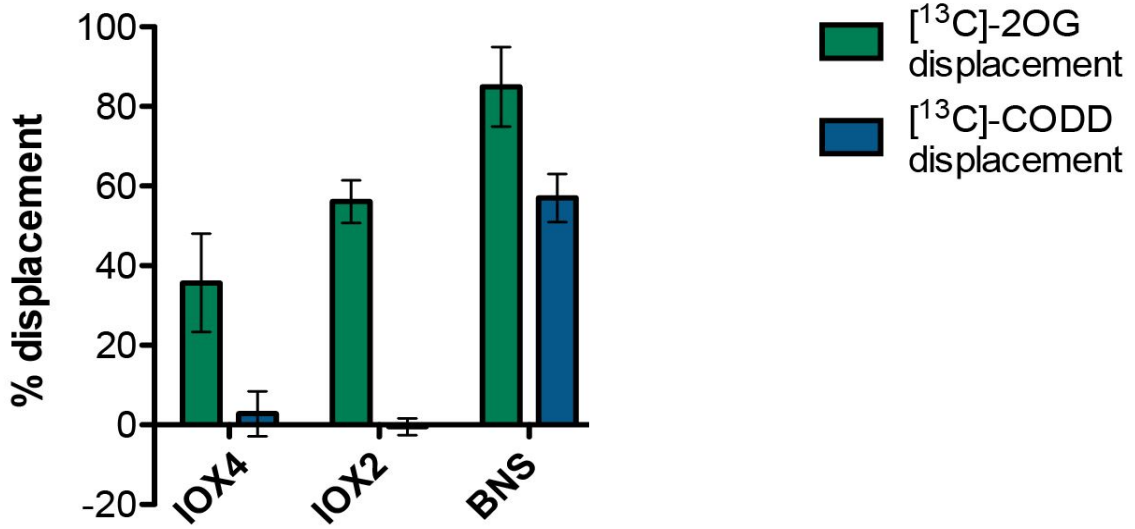
A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis

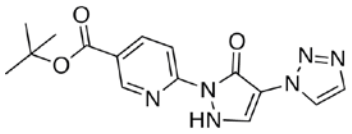
Melanie J. Percy*, Quan Zhao†, Adrian Flores‡, Claire Harrison‡, Terence R. J. Lappin§, Patrick H. Maxwell¶, Mary Frances McMullin§||, and Frank S. Lee†**



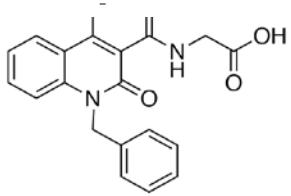
- Re-introduction of wild type and mutant human PHD2 into MEFs that are null for all 3PHDs
- Assay HIF status by hydroxy-amino acid specific antibodies – block of degradation with MG132

Would it be possible to design more selective small molecule interventions on the PHD/HIF system?

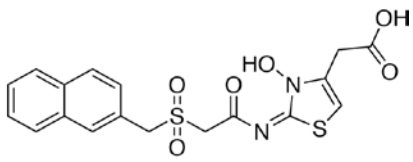




IOX4



IOX2



BNS

Multiple HIF/PHD isoforms co-evolved with specialist oxygen delivery systems in animal evolution

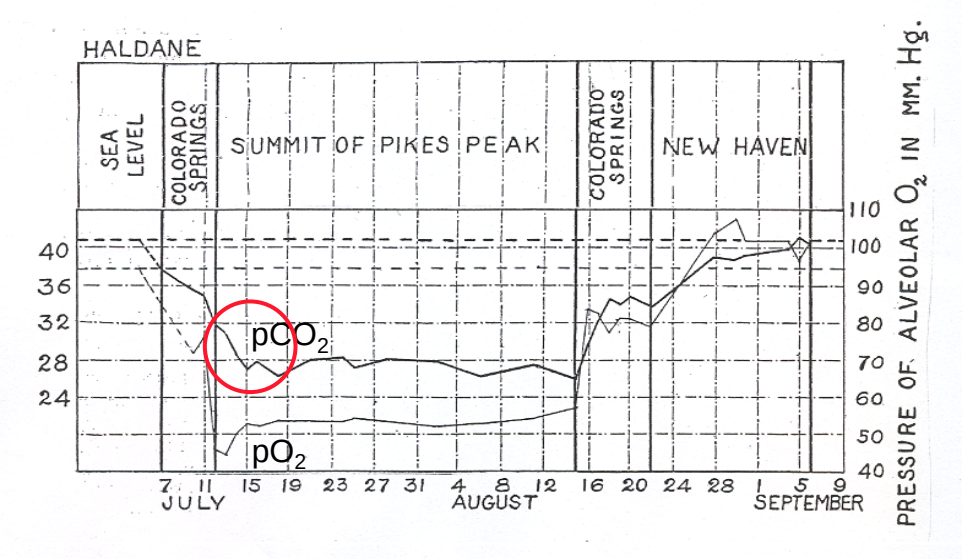
Relative isoform specificity of small molecule inhibitors of PHD/HIF hydroxylation should be possible

Would this be useful?

Altitude physiology and the concept of oxygen sensing

J.S. Haldane and colleagues , Pike's Peak 1911

Haldane, Fitzgerald, Schneider, Henderson and Douglas at top of Pike's peak, 1911



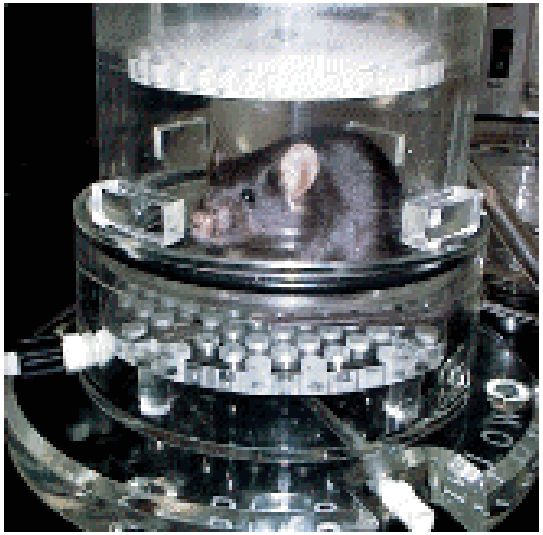
Acute acclimatization

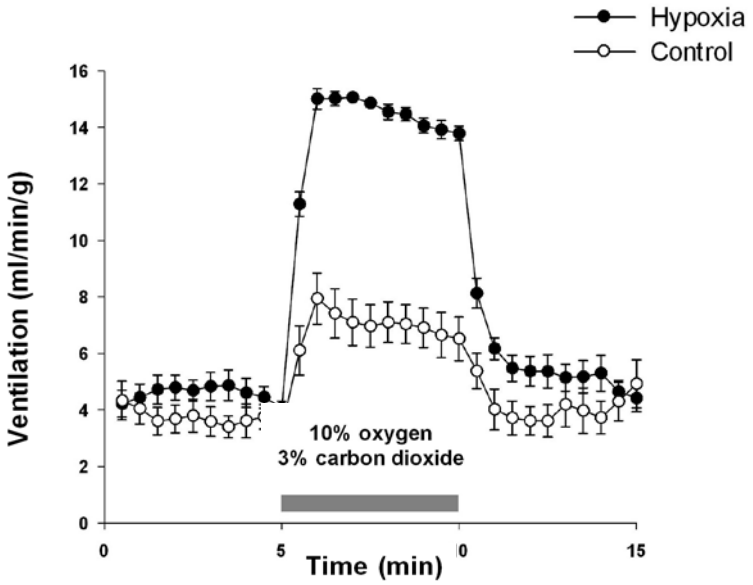
pre-exposure to hypoxia (days)

pre-increased sensitivity to acute hypoxia (minutes)

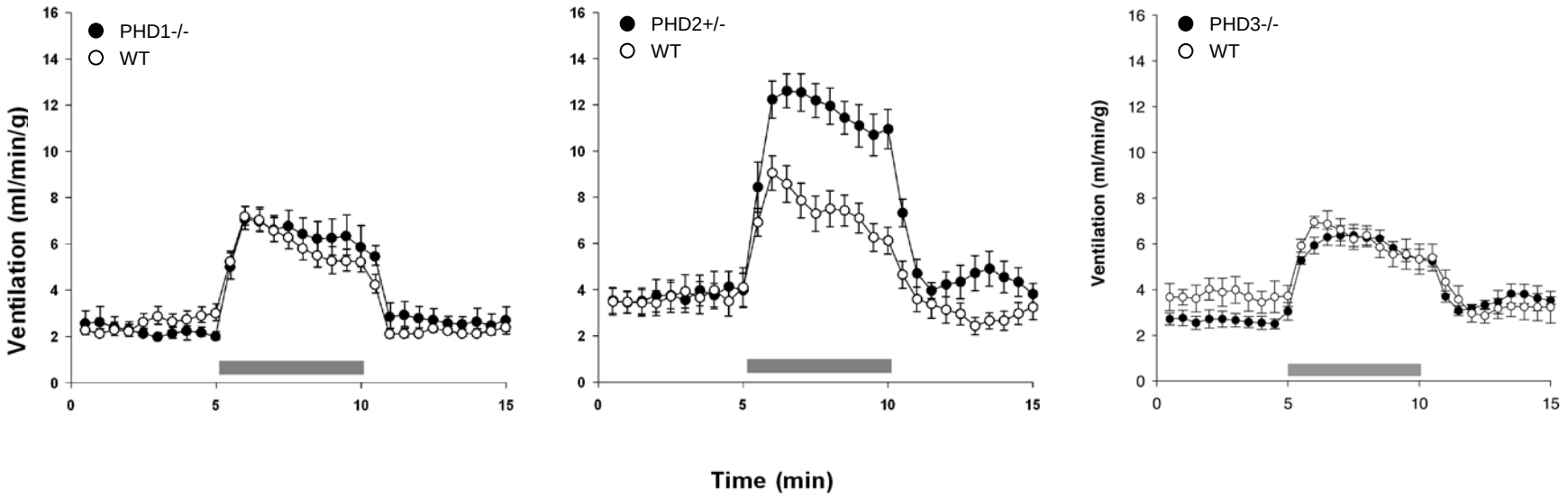
Are specific components of the hypoxia response linked to specific components of the HIF/PHD system?

Ventilatory sensitivity to acute hypoxia pre- and post- 7 days at 10% oxygen (ca. 5000m)

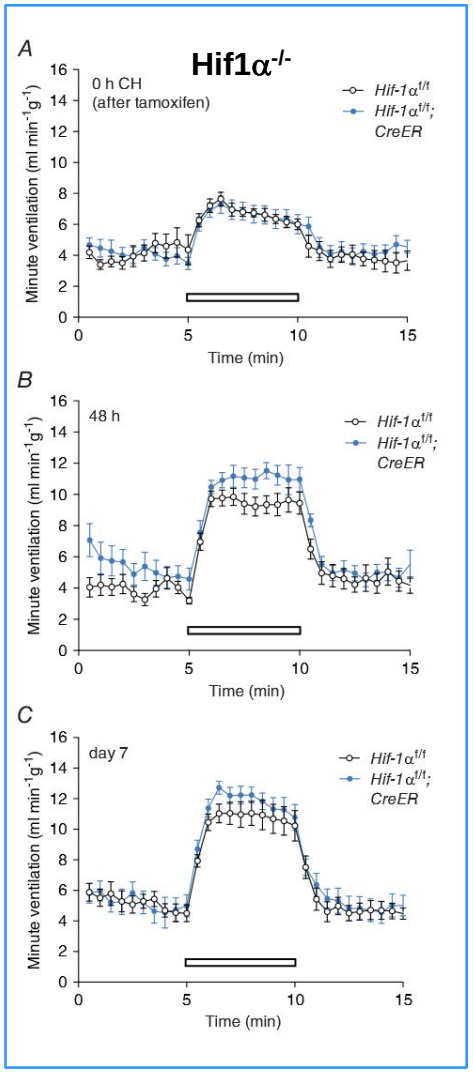




Enhanced hypoxic ventilatory response in Phd2$^{+/-}$ mice (10% O2 + 3%CO2)



Ventilation acclimatization and erythropoietic responses to simulated altitude are largely drive by the HIF-2α isoform

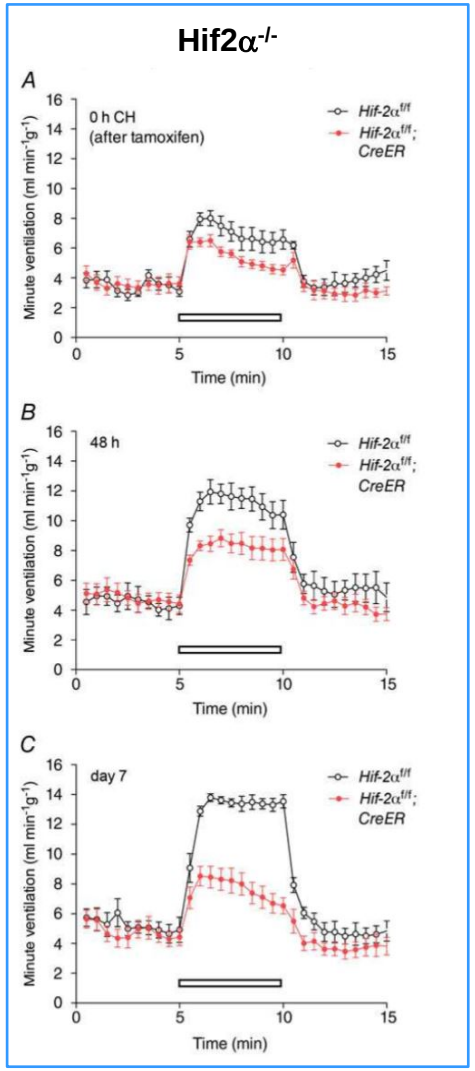


Conditional inactivation of HIF-α by Rosa 26 driven CreER

Animals studied 10days after first dose of tamoxifen

Sensitizing exposure 10% O_2 (up to 7 days)

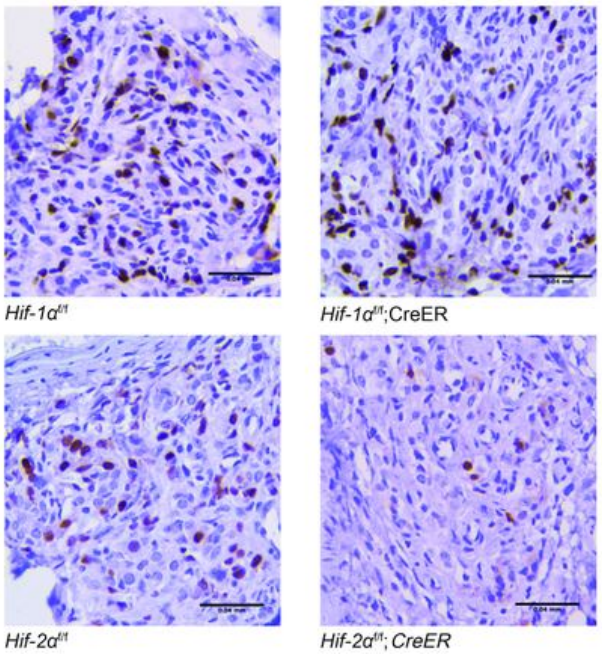
Acute exposure 10%O_2 + 3 CO_2 (5 min)

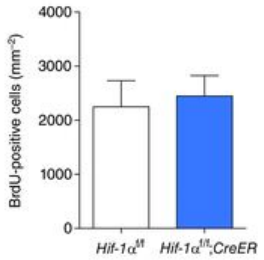


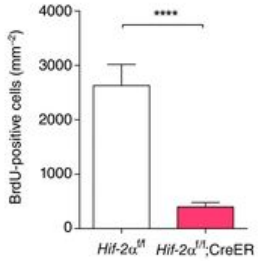
Ventilation acclimatization to simulated altitude is largely driven by HIF-2α

Inducible inactivation of HIF-1α or HIF-2α in adult life

Proliferation of cells in the carotid body after 7 days exposure to 10% oxygen – BrDU labelling

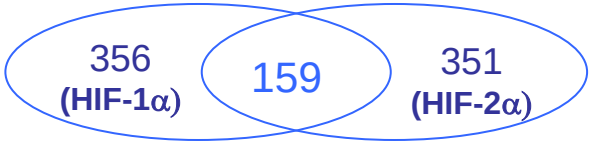


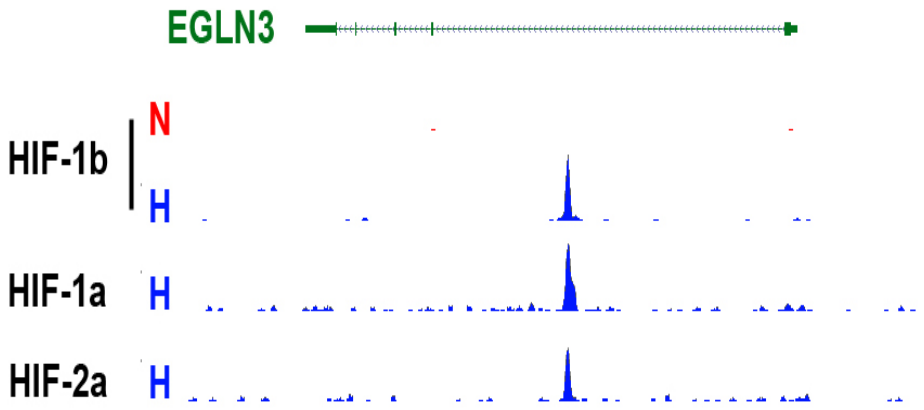




Pan-genomic analysis of binding of HIF binding

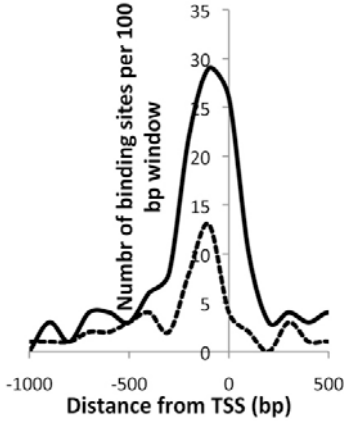
Comparison of HIF-1α and HIF-2α binding by ChIP-seq (MCF7 breast cancer cells)

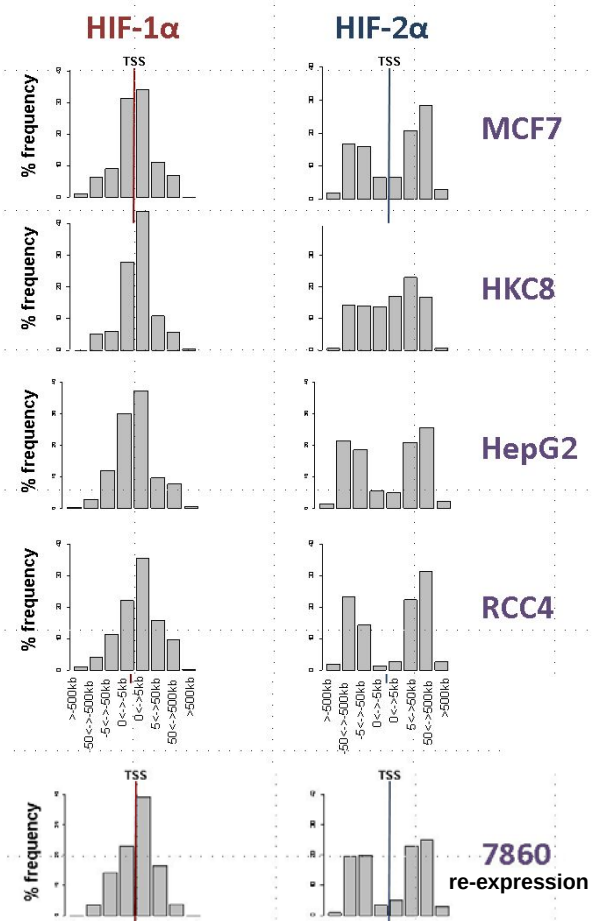




Pan-genomic analyses reveal differences in the transcriptional architecture of HIF-1α binding versus HIF-2α binding

HIF-1α binding sites cluster much more strongly at promoters than HIF-2α binding sites



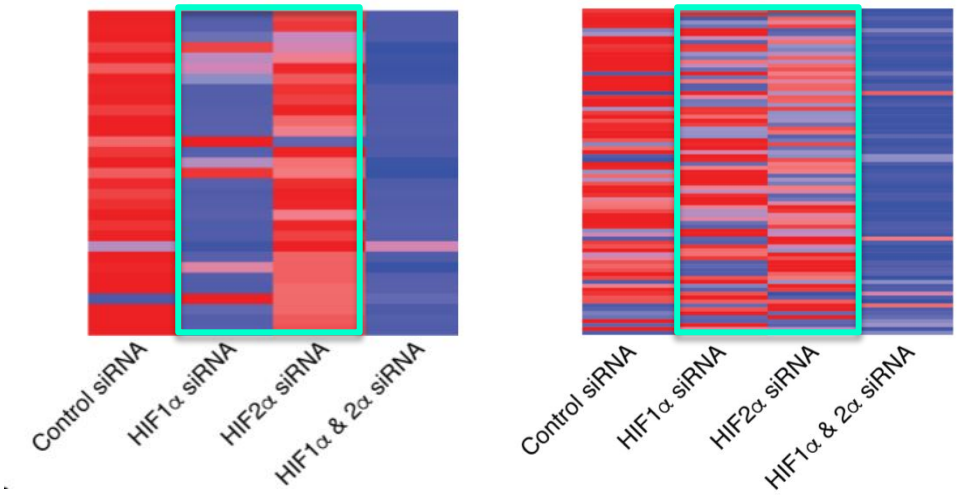


Marked isoform selective activity of chromatin bound HIF-α is strongly distant dependent

Near complete preferential action of HIF-2 on genes lying at a distance from DNA binding site

Genes lying close (<2Kb) to their HIF binding site

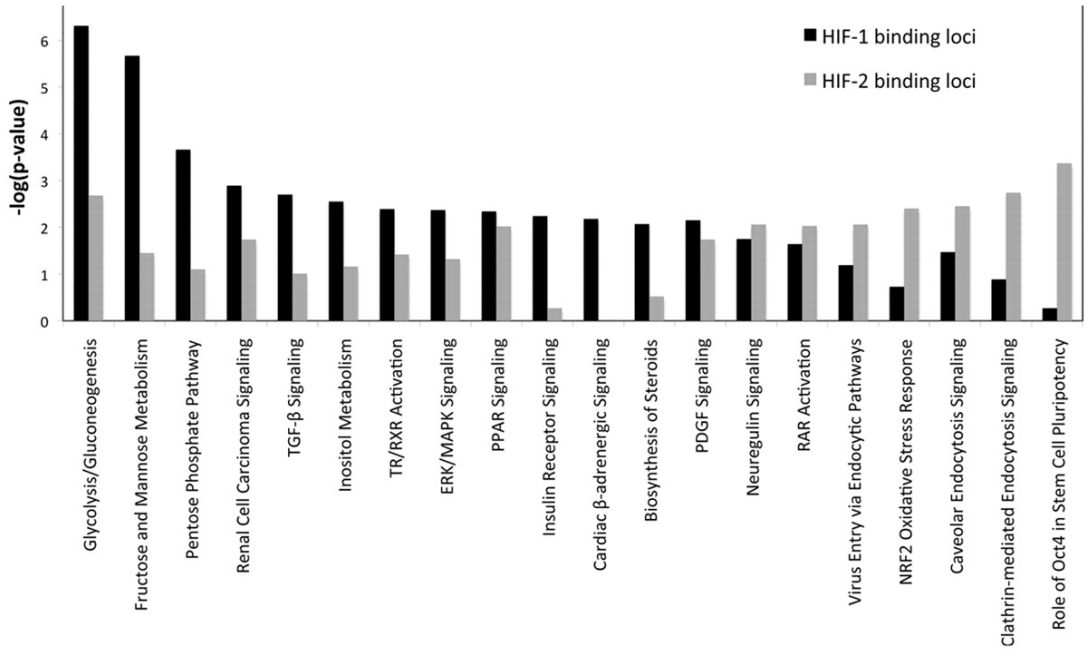
Genes lying distant (>10Kb) from their HIF binding site



Very incomplete separation of HIF-1 vs HIF-2 function by gene ontogeny programs

HIF-1

Metabolic, cytostatic responses (less oxygen demand) ?



HIF-2

Reparative responses (more oxygen supply)

Evolution of distinct characteristics of the HIF response

HIF-1 (early invertebrate form) versus HIF-2 (more modern vertebrate form)

Differences in transcriptional biochemistry - produced by agnostic variation

- HIF-1 dominant action at promoters – widely expressed genes)
- HIF-2 enhancer action; dominant action on RNA regulatory networks – more cell-type specific genes

A fundamental design flaw for rationale drug development

Differences in physiological function - produced by Darwinian selection

- HIF-1 generally expressed, core role in development, general cellular functions, metabolism
- HIF-2 cell-specific expression, major roles in higher animal physiological adaption

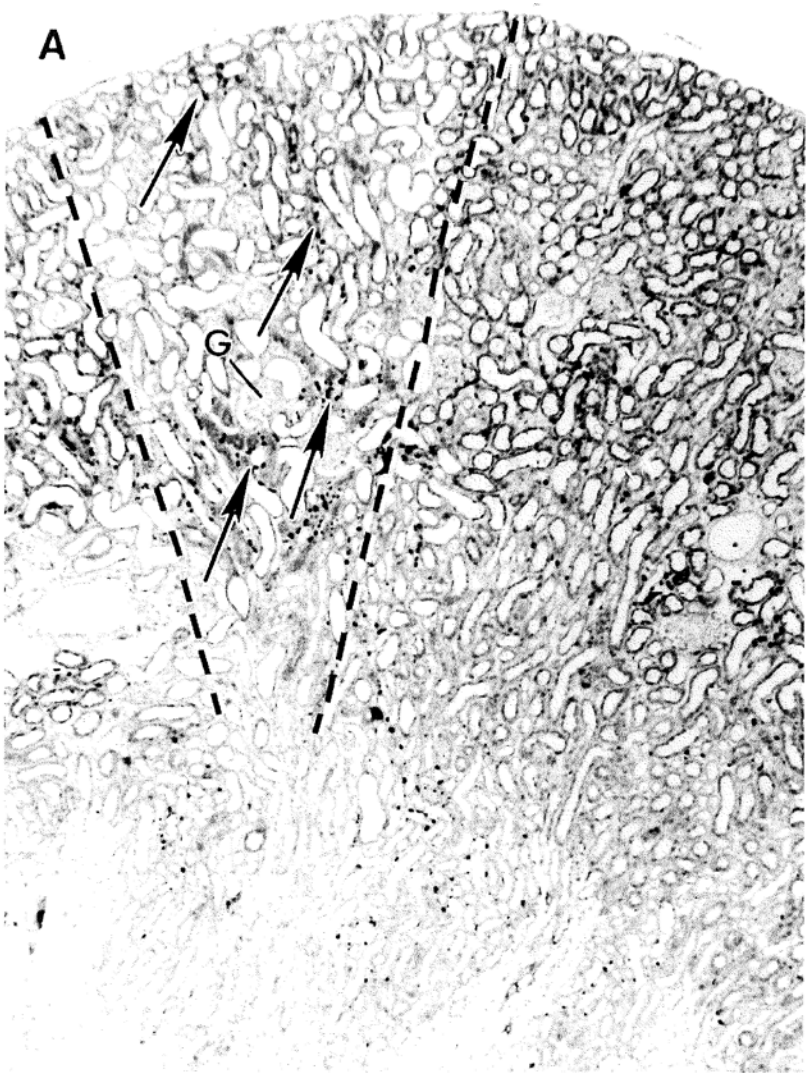
Darwinian biology is at odds with rational reductionist drug design

But might there be drug anyway?

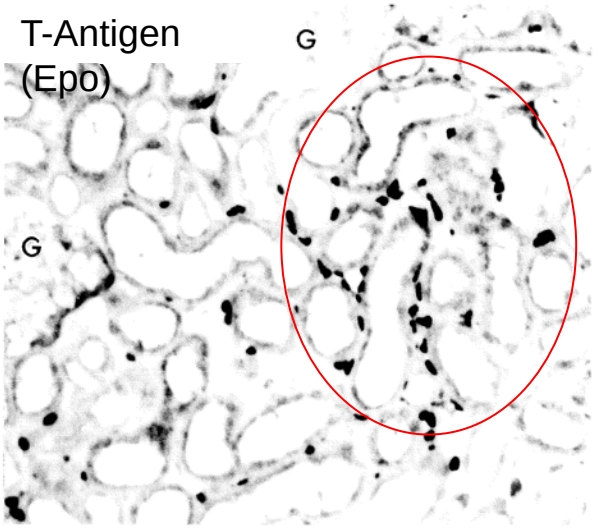
So what about renal medicine?

Why does Epo production fail in diseased kidneys?

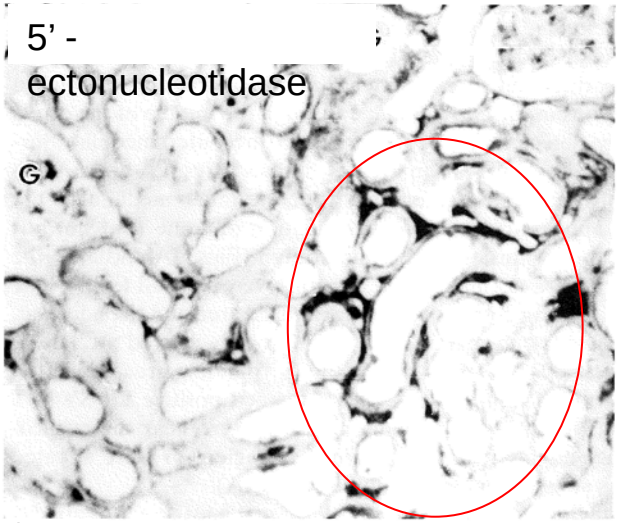
Loss of Epo production in locally injured kidney – changes in morphology and markers in interstitial fibroblasts



T-Antigen (Epo)



5’ - ectonucleotidase



Erythropoietin-producing potential in diseased kidneys?

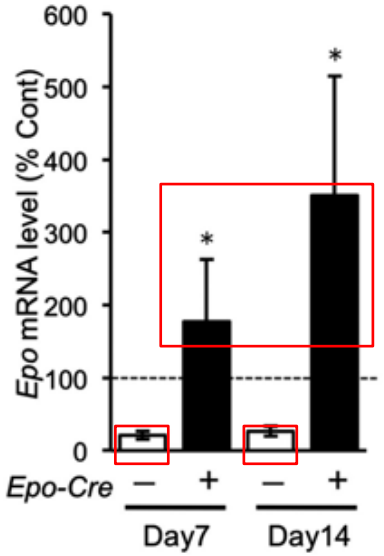
Total inactivation of HIF prolyl hydroxylases reveals enhanced Epo-producing potential of diseased kidneys

Souma *et al.* JASN 08/06/2015

PHD1, 2, 3 triple k/o (Epo-Cre)

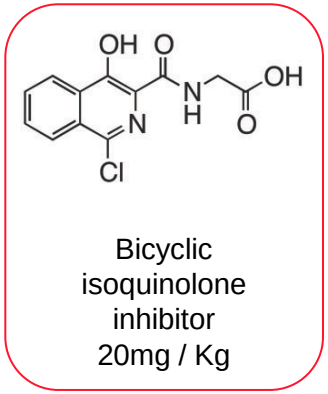
↓

Unilateral ureteric obstruction model



Action of HIF prolyl hydroxylase inhibitor to increase plasma-EPO levels in healthy controls and in HD patients with and without remaining renal tissue.

Bernhardt W M *et al.* JASN 2010;21:2151-2156

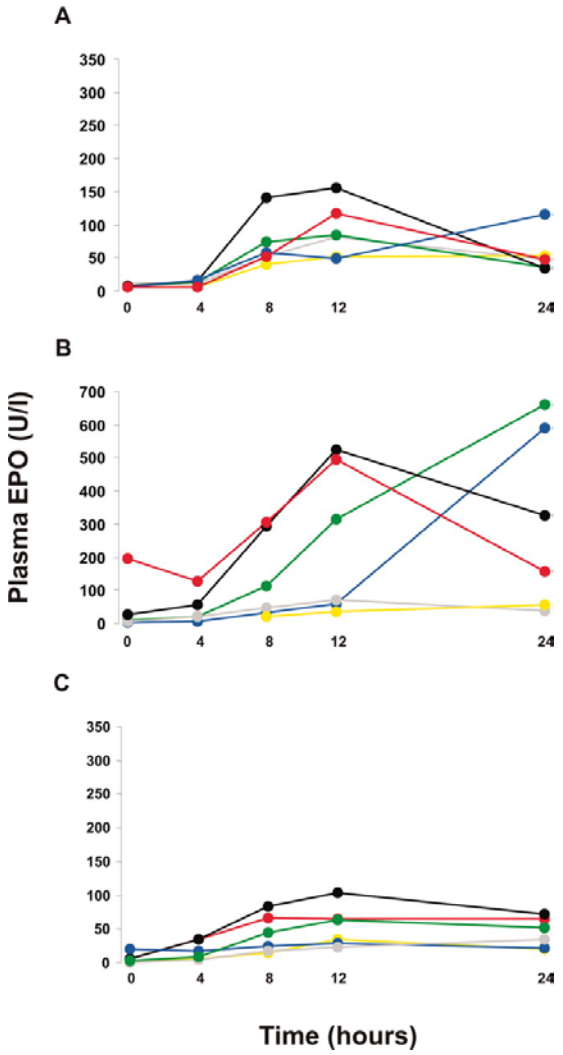


Bicyclic isoquinolone inhibitor 20mg / Kg

Control (normal subjects)

Nephric HD patients

Anephric HD patients



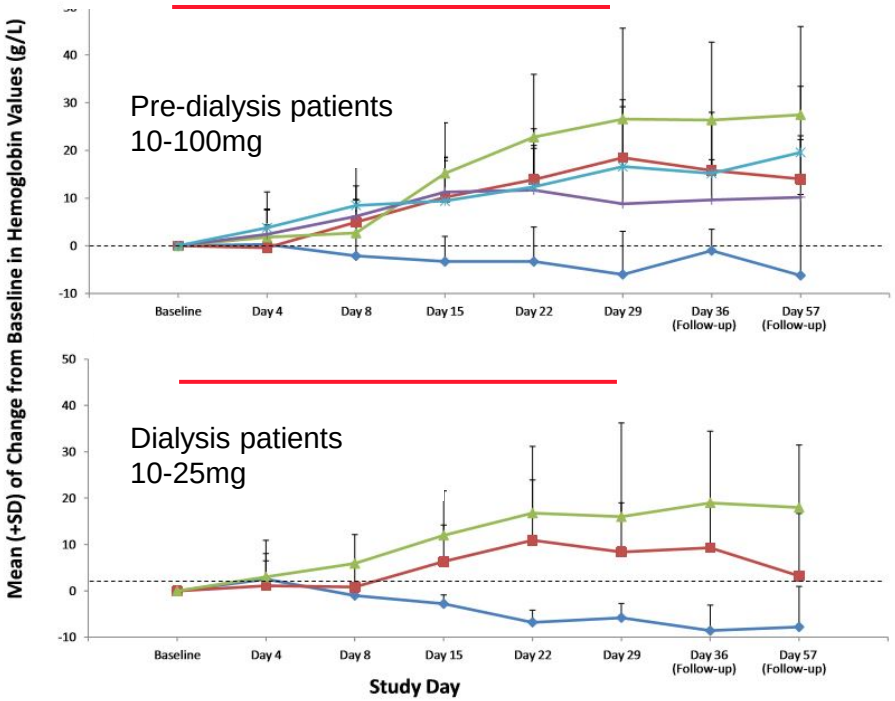
Strategy for PHI in renal anaemia?

↑ Aim to stimulate the general hypoxia response?
+ Benefits?
+ Risks?

↓ Aim to stimulate Epo production alone?

HIF prolyl hydroxylase inhibitors appear safe and effective in anaemia correction in medium term (months) studies

Randomized placebo controlled dose ranging study of GSK1278863 over 28 days ***AJKD 67 861-871 (2016)***



Lower plasma Epo levels (effective dose 10-25mg od)
Better iron balance?
Effective in the setting of inflammation?

Define molecular mechanism at the molecular level

Define integrated physiology at the physiological level

Define clinical effects at the clinical level

Nothing (much) else will do

With grateful thanks to:
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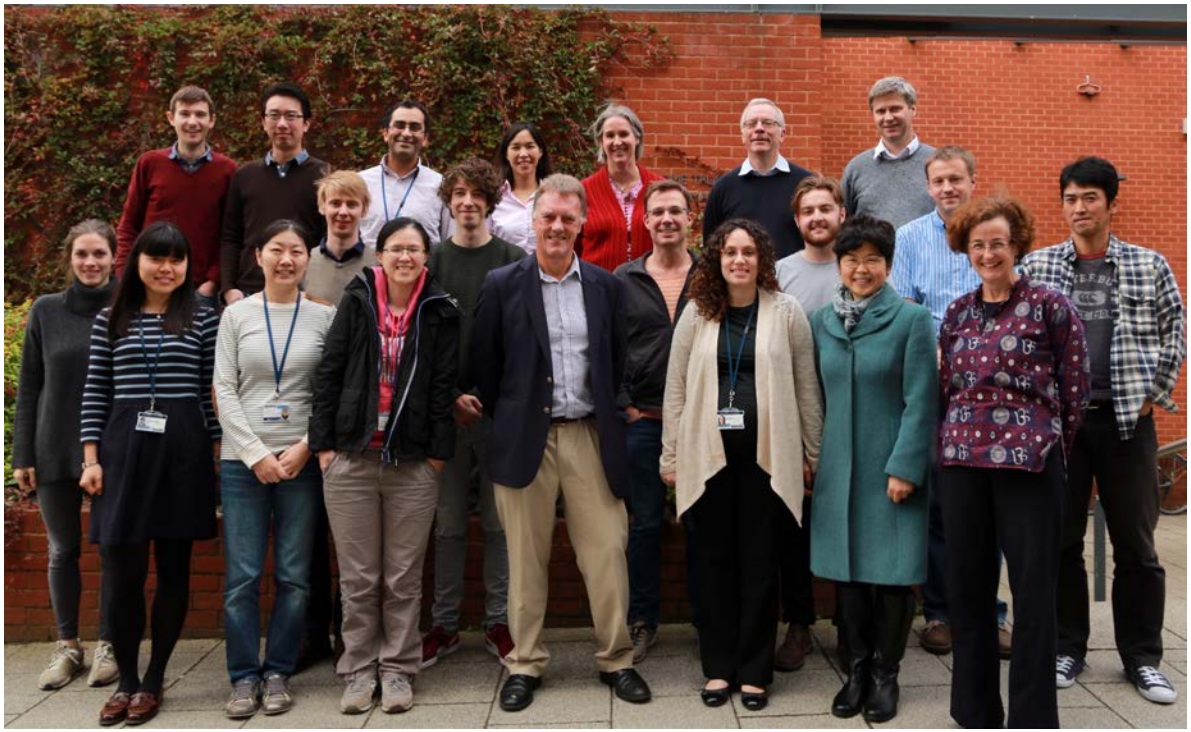
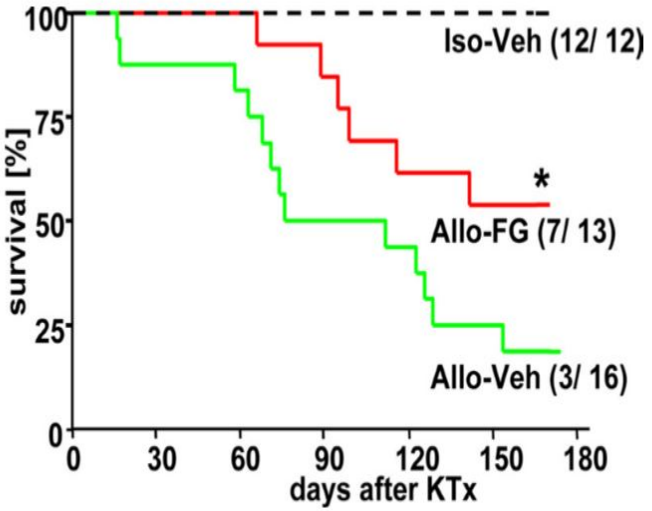


Immune cell phenotypes following modulation of HIF prolyl hydroxylases

Single dose of prolyl hydroxylase inhibitor (FG-4497) to donor results in sustained improvement in rat kidney allograft survival

Bernhardt et al (Kai Eckardt and colleagues, Erlangen) PNAS 106 21276-21281 (2009)



Severe lymphoid dysregulation following sustained (8 week) general shPHD2 k/d

Astu Yamamoto and Chris Pugh – unpublished data