Elucidation of oxygen sensing mechanisms in human and animal cells

Peter J. Ratcliffe

Nobel Lecture - December 2019

Oxford-Yale expedition to Pike's Peak, Colorado
J. S. Haldane and colleagues - July 1911

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

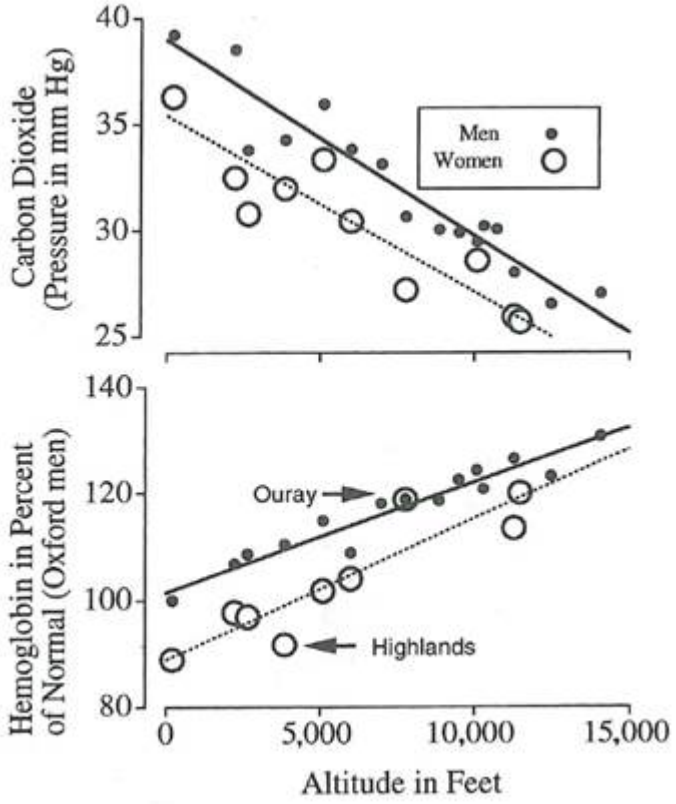
VIII. *The Changes in the Breathing and the Blood at Various High Altitudes.*

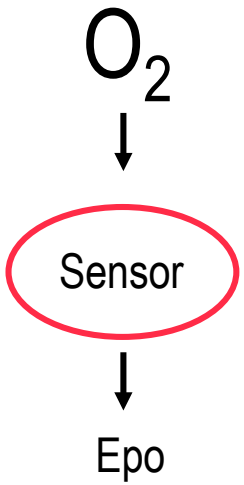
By Mabel Purefoy FitzGerald.

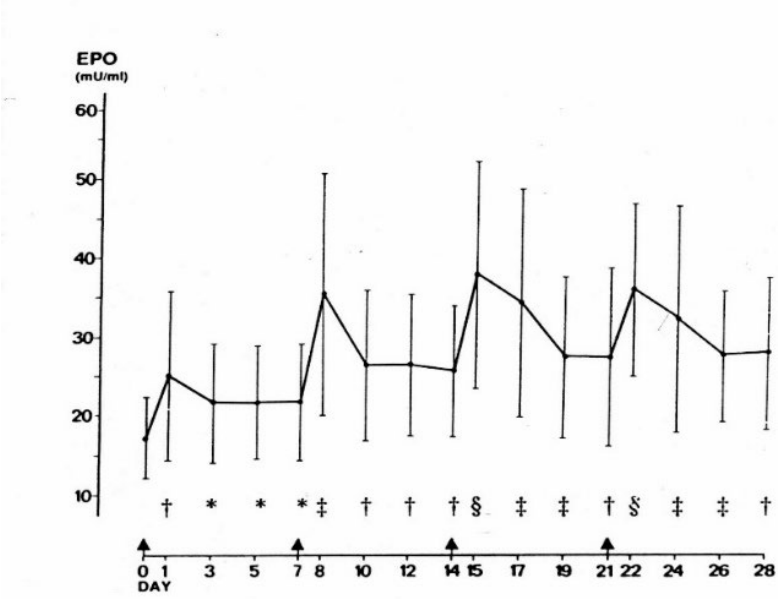
Communicated by Dr. J. S. Haldane, *F.R.S.*

(Received January 16,—Read February 20, 1913.)

Fig. 3.3. Mabel FitzGerald, measuring the hemoglobin in the blood by diluting a sample of blood in one of two tubes until it matches the color of the standard in the other tube. Reprinted from Colorado Springs Herald Telegraph, *July 8, 1911.*







Extra-ordinary sensitivity of the erythropoietin response to changes in blood oxygen availability – responses to donation of blood

Lorentz et al. Transfusion 31 650-654 (1991)

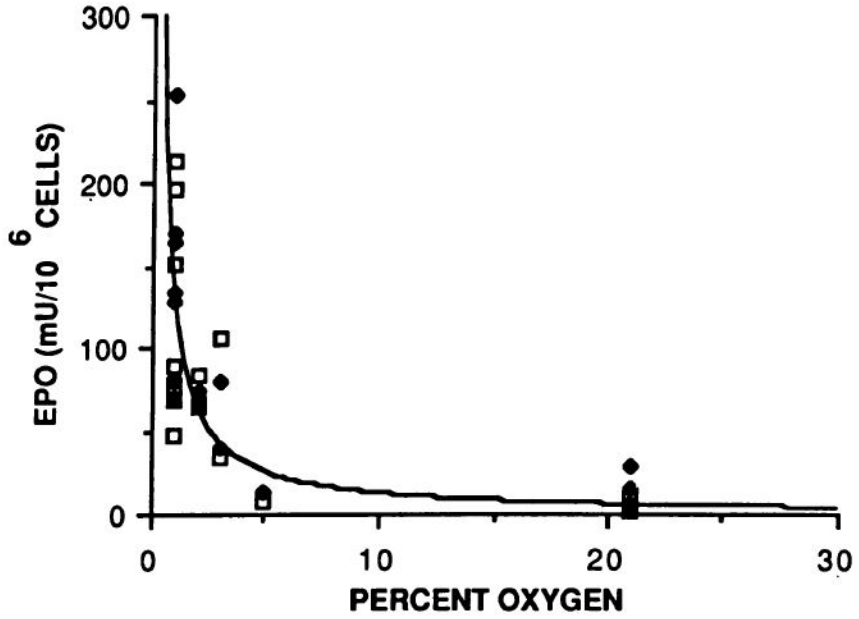
Development of a cellular model for study of Epo regulation by Oxygen

Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 7972–7976, November 1987
Cell Biology

The regulated expression of erythropoietin by two human hepatoma cell lines

MARK A. GOLDBERG, G. ALLISON GLASS, JAMES M. CUNNINGHAM, AND H. FRANKLIN BUNN

Howard Hughes Medical Institute, Division of Hematology, Brigham and Women's Hospital, and the Department of Medicine, Harvard Medical School, Boston, MA 02115



Widespread operation of hypoxia signalling pathways

6-92 WED 10:42

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5 August 1992

Dr P J Ratcliffe
Room 420
Institute of Molecular Medicine
John Radcliffe Hospital
Oxford
OX3 9DU

Dear Dr Ratcliffe,

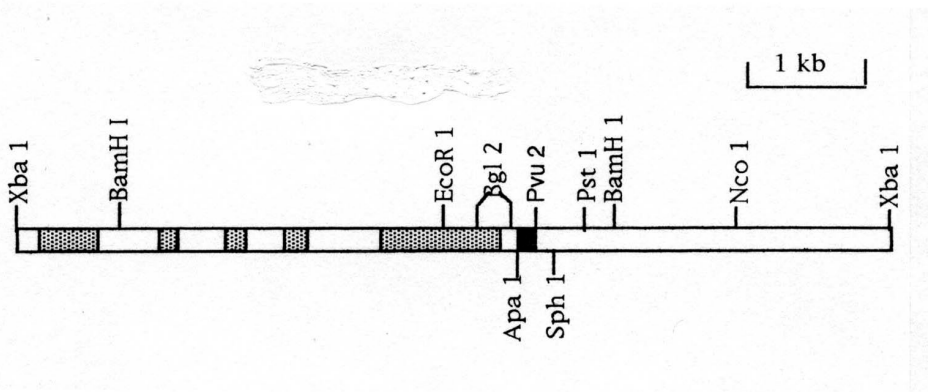
I am very sorry about the long delay in reaching a decision on your manuscript, particularly as in the event we are unable to offer to publish it. As I explained on the telephone, we had great difficulty obtaining referees reports; your manuscript has, however, now been seen by two reviewers whose comments are attached.

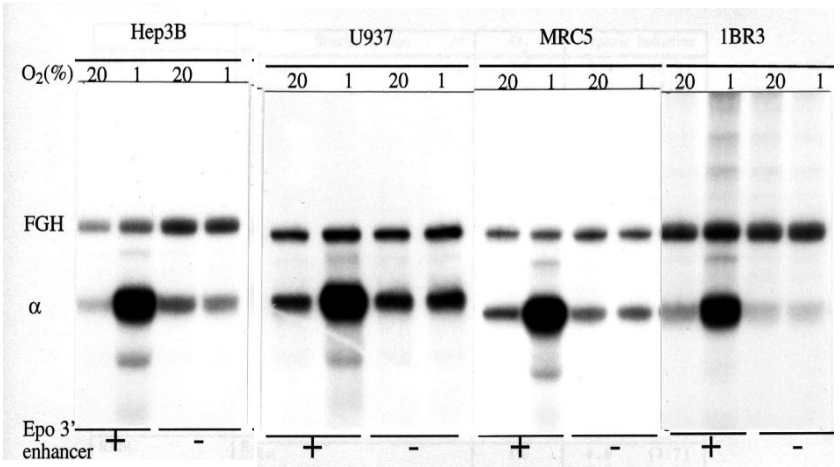
While it is clear from the essentially favourable comments of reviewer 1 that your findings will be of interest to others in the field, reviewer 2 is not persuaded that they represent a sufficient advance in our understanding of the mechanisms of genetic response to hypoxia to justify publication in Nature. Given also the discrepancy mentioned by reviewer 1, we have sadly concluded, on balance, that your paper would be better placed in a more specialized journal, particularly given the competition for space.

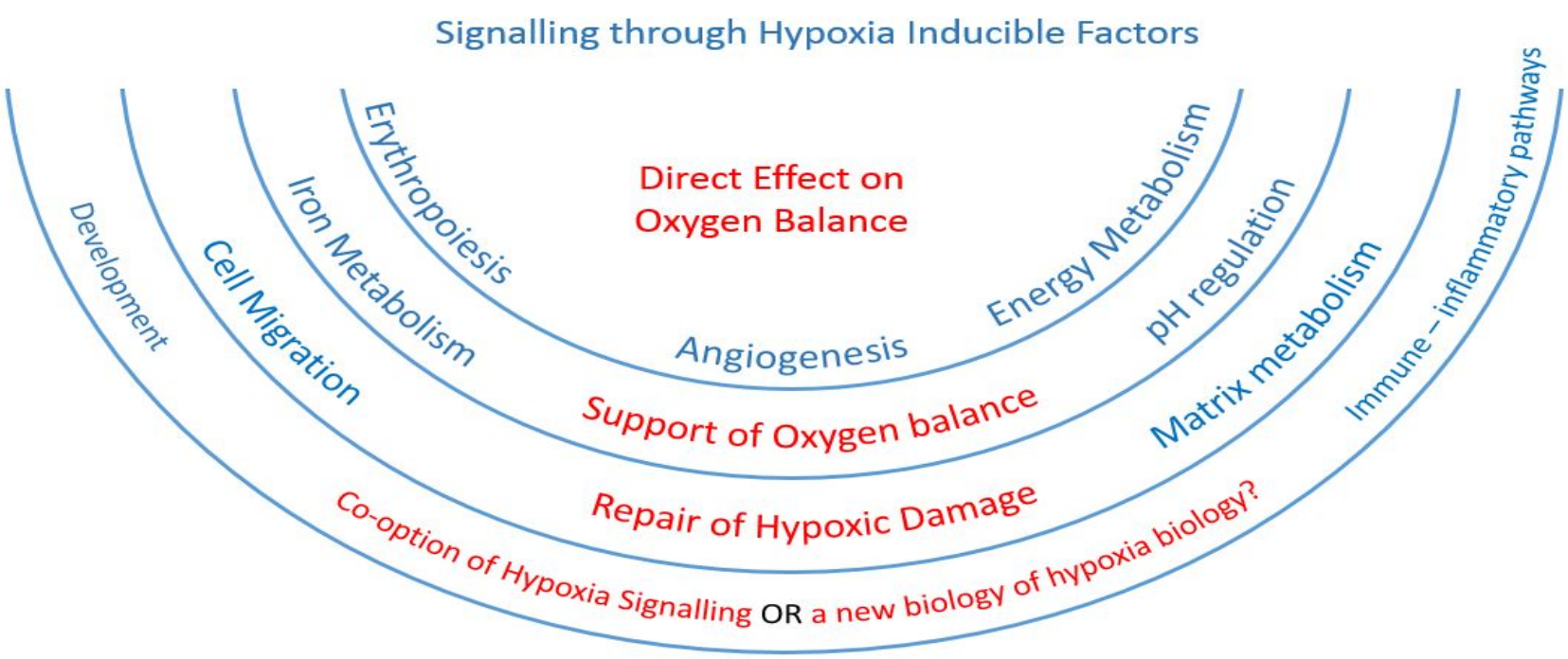
I realize that this must be from your point of view a very disappointing outcome, particularly after such an unfortunate delay, but we do hope that you will understand our reasons for the decision, and that you will find our reviewers' comments helpful when a new version of your manuscript for resubmission elsewhere.

Yours sincerely,

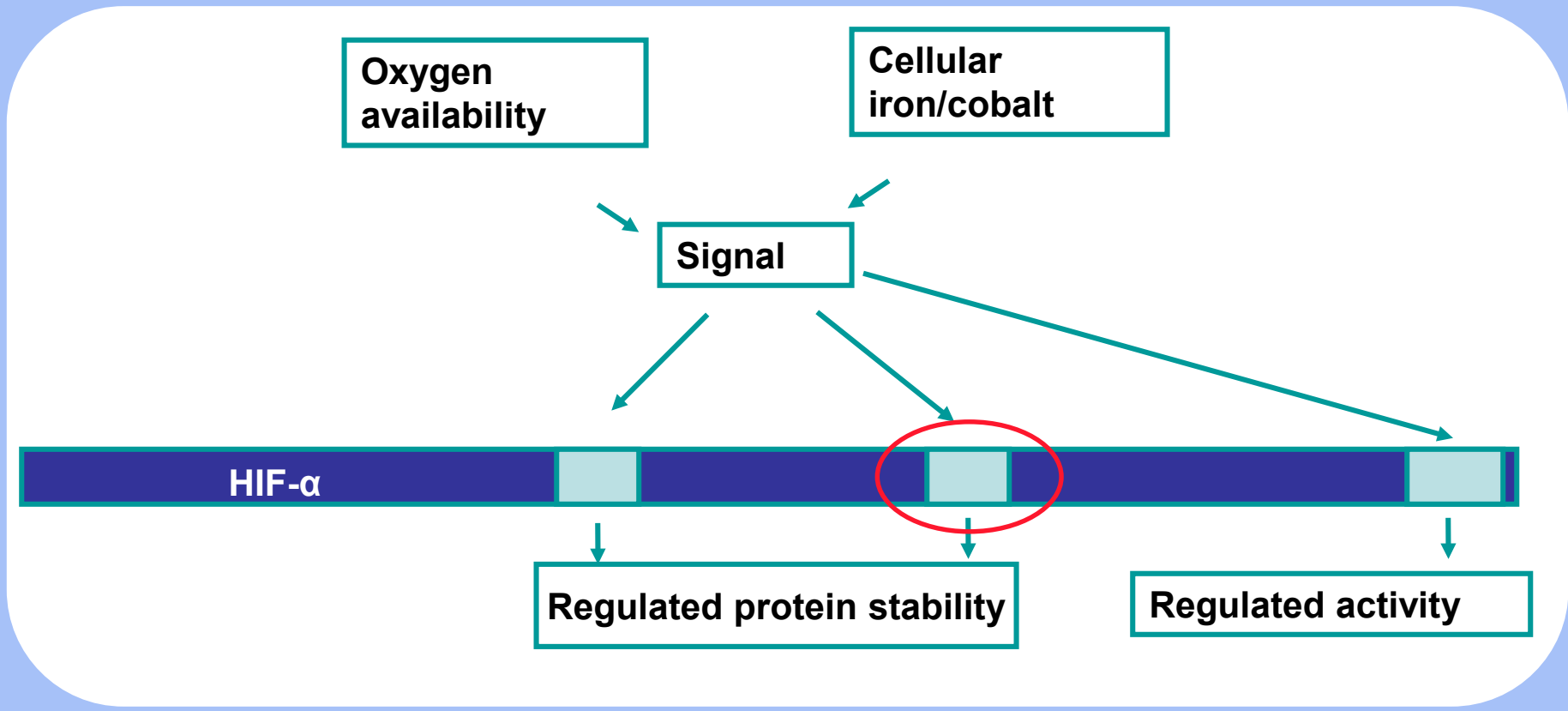
Associate Editor






Signalling through Hypoxia Inducible Factors
Direct Effect on
Oxygen Balance
Erythropoiesis
Energy Metabolism
Iron Metabolism
pH regulation
Angiogenesis
Matrix metabolism
Development
Immune – inflammatory pathways
Cell Migration
Support of Oxygen balance
Repair of Hypoxic Damage
Co-option of Hypoxia Signalling OR a new biology of hypoxia biology?

Regulation of HIF by oxygen

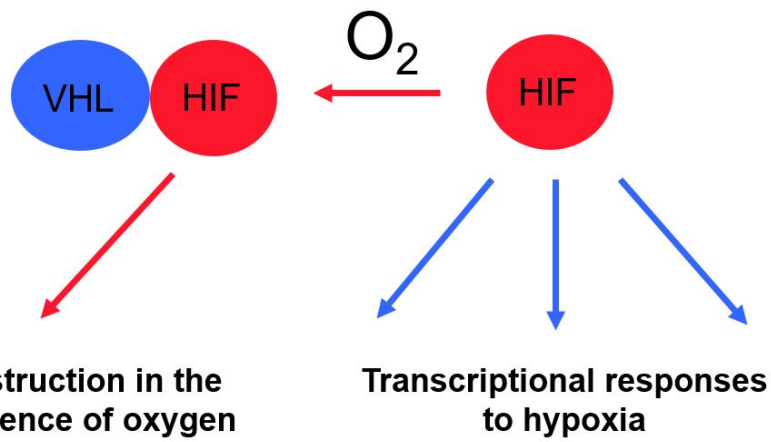


Sequencing the human genome suggests existence of HIF paralogues

Multiple hypoxia inducible transcripts identified

Making anti-EPAS antibodies (PM9) proves regulation by oxygen

Transcripts constitutively upregulated in VHL defective cells



Distortion from HIF-1 to HIF-2 during RCC development

Oxygen sensing pathway

Role in Cancer Treatment by HIF-2 antagonists

Signalling modification is prolyl hydroxylation

Biochemical analysis

Heat labile extract

~~Non-enzymatic oxidation~~

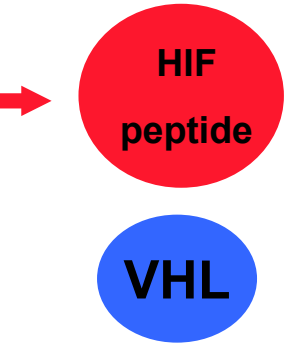
~~NADH/NADHoxidase~~

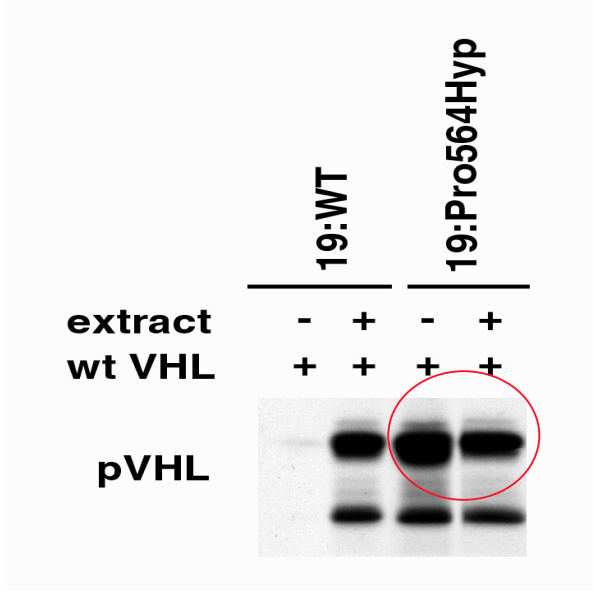
Oxygen

Iron

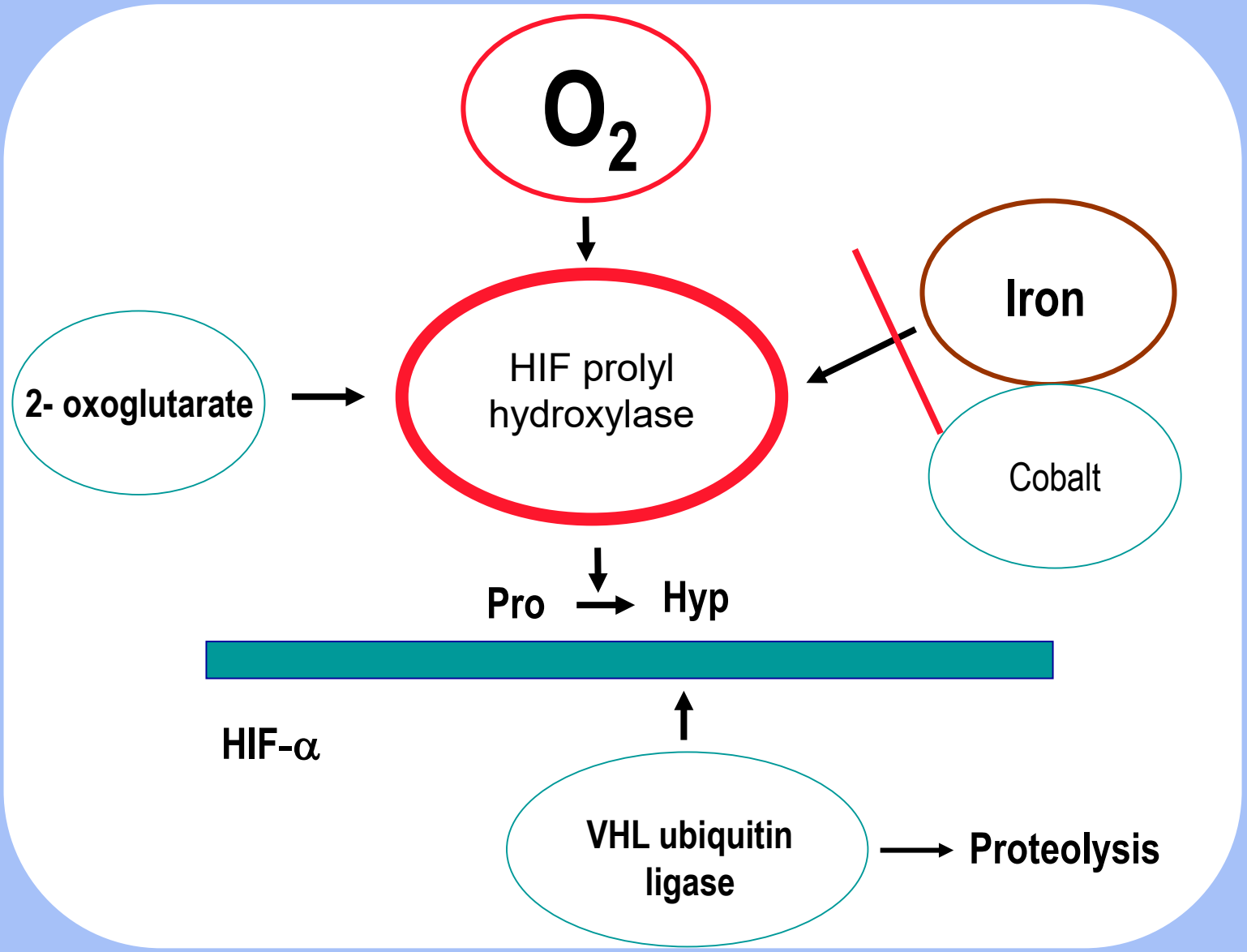
~~ATP~~

Treatment with cell extract

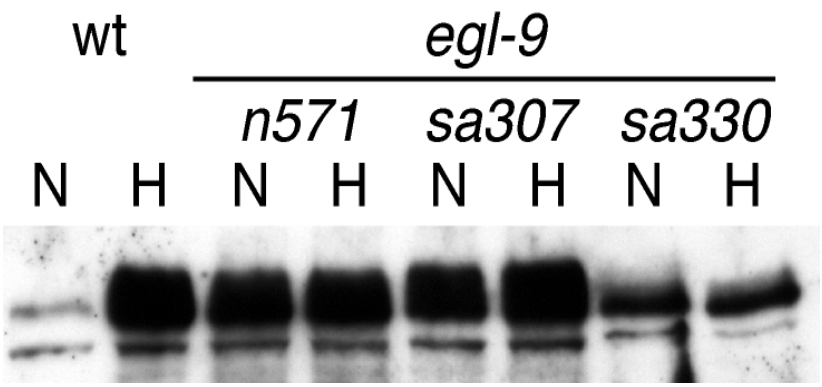




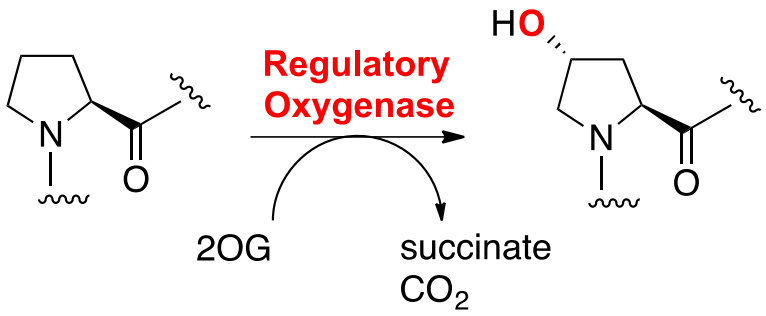
HIF prolyl hydroxylation implies a mechanism of oxygen sensing



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



O_2


HO
Regulatory
Oxygenase
N
O
N
O
2OG
succinate
CO_2

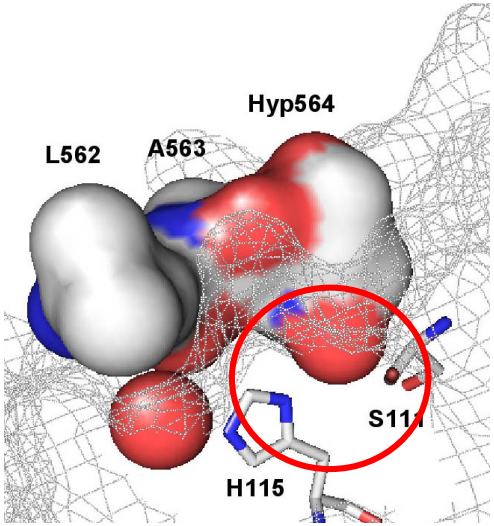
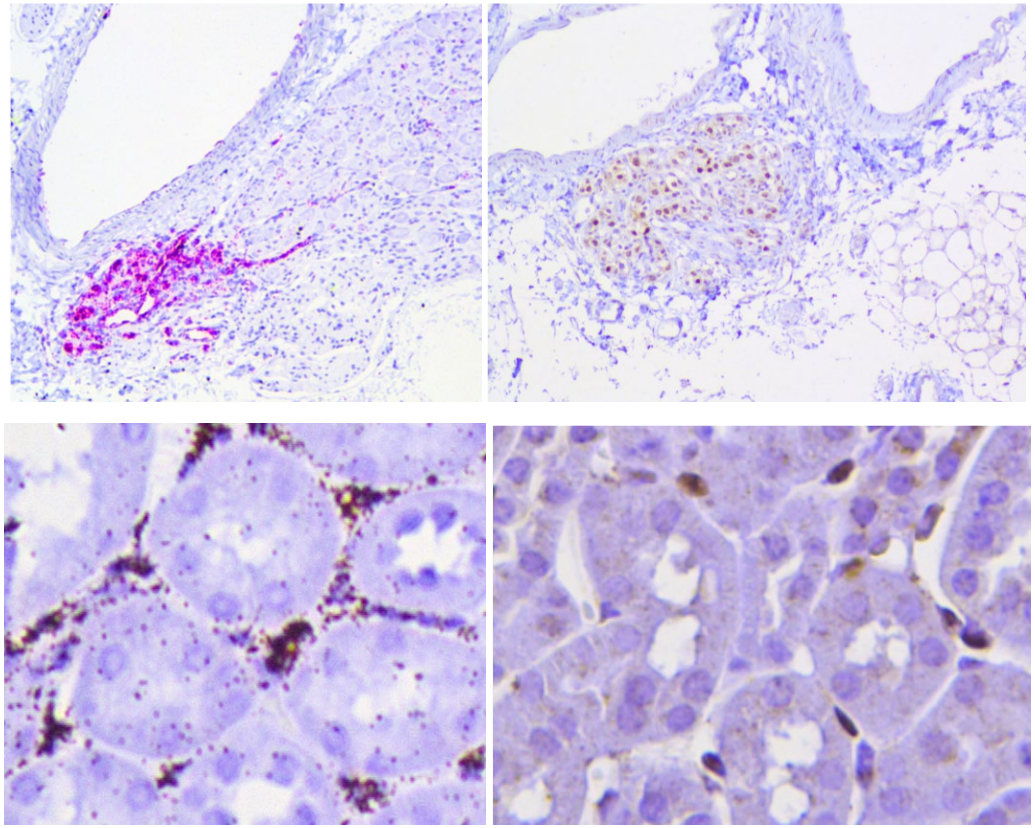
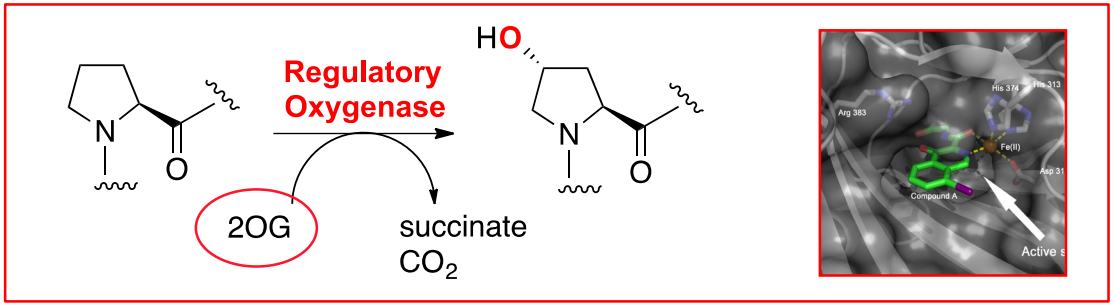

Hyp564
L562
A563
S111
H115

Fig. 3.3. Mabel FitzGerald, measuring the hemoglobin in the blood by diluting a sample of blood in one of two tubes until it matches the color of the standard in the other tube. Reprinted from Colorado Springs Herald Telegraph, *July 8, 1911.*

HIF-2 mRNA

HIF-2 IHC





Signalling through Hypoxia Inducible Factors

Direct Effect on
Oxygen Balance

Erythropoiesis

Iron Metabolism

Angiogenesis

Energy Metabolism

pH regulation

Matrix metabolism

Immune – inflammatory pathways

Development

Cell Migration

Support of Oxygen balance

Repair of Hypoxic Damage

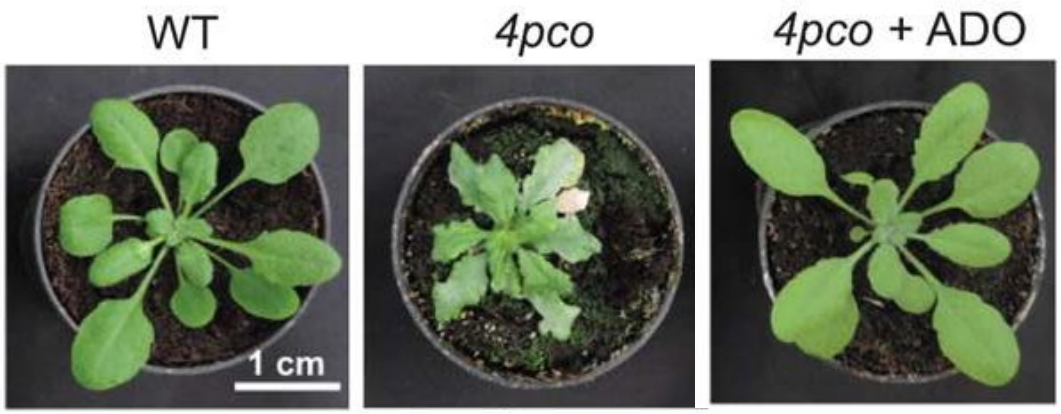
Co-option of Hypoxia Signalling OR a new biology of hypoxia biology?

Protein Oxidation in Signalling hypoxia
Evolutionary Origins?

All eukaryotic kingdoms use protein oxidation and proteolysis to signal oxygen levels

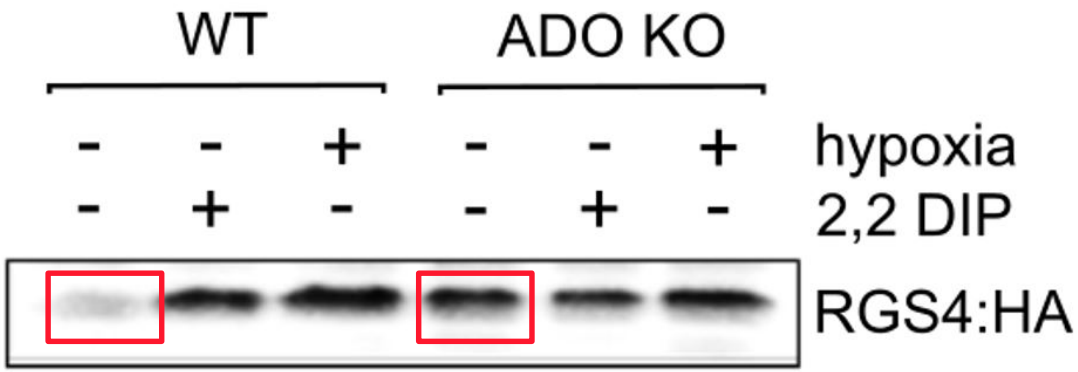
Funghi *Schizosaccharomyces pombe*	Animals *Homo sapiens*	Protists *Dictyostelium discoideum*	Plants *Arabidopsis thaliana*
Ofd1 - Prolyl 3 hydroxylase	Prolyl 4 hydroxylase	Prolyl 4 hydroxylase	Cysteine oxidases
SRE proteolysis	Hypoxia inducible factors	Skp1 ubiquitin ligase	MCxxx N-end rule
Sterol response	VHL ubiquitin ligase	Culmination factors	Ethylene response factors

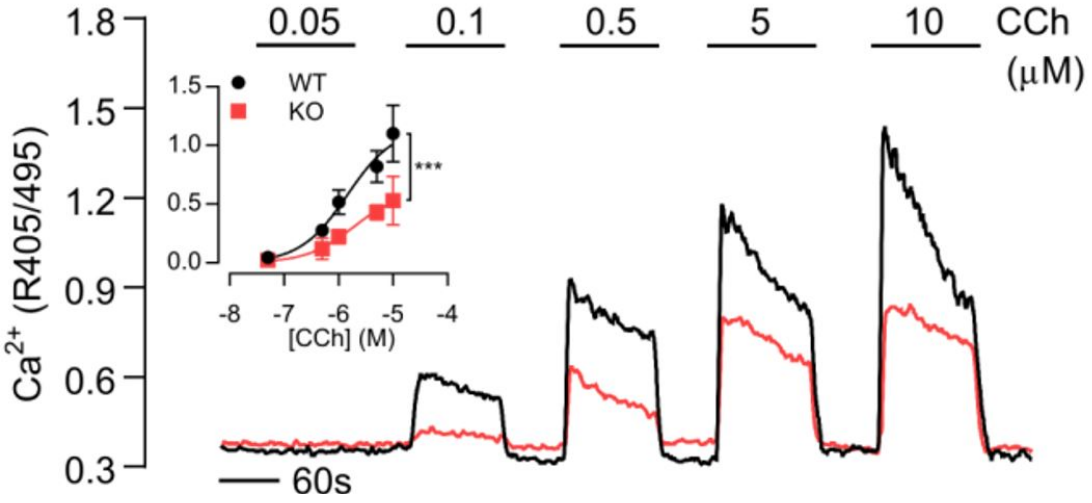
A conserved oxygen sensing mechanism in plants and animals?



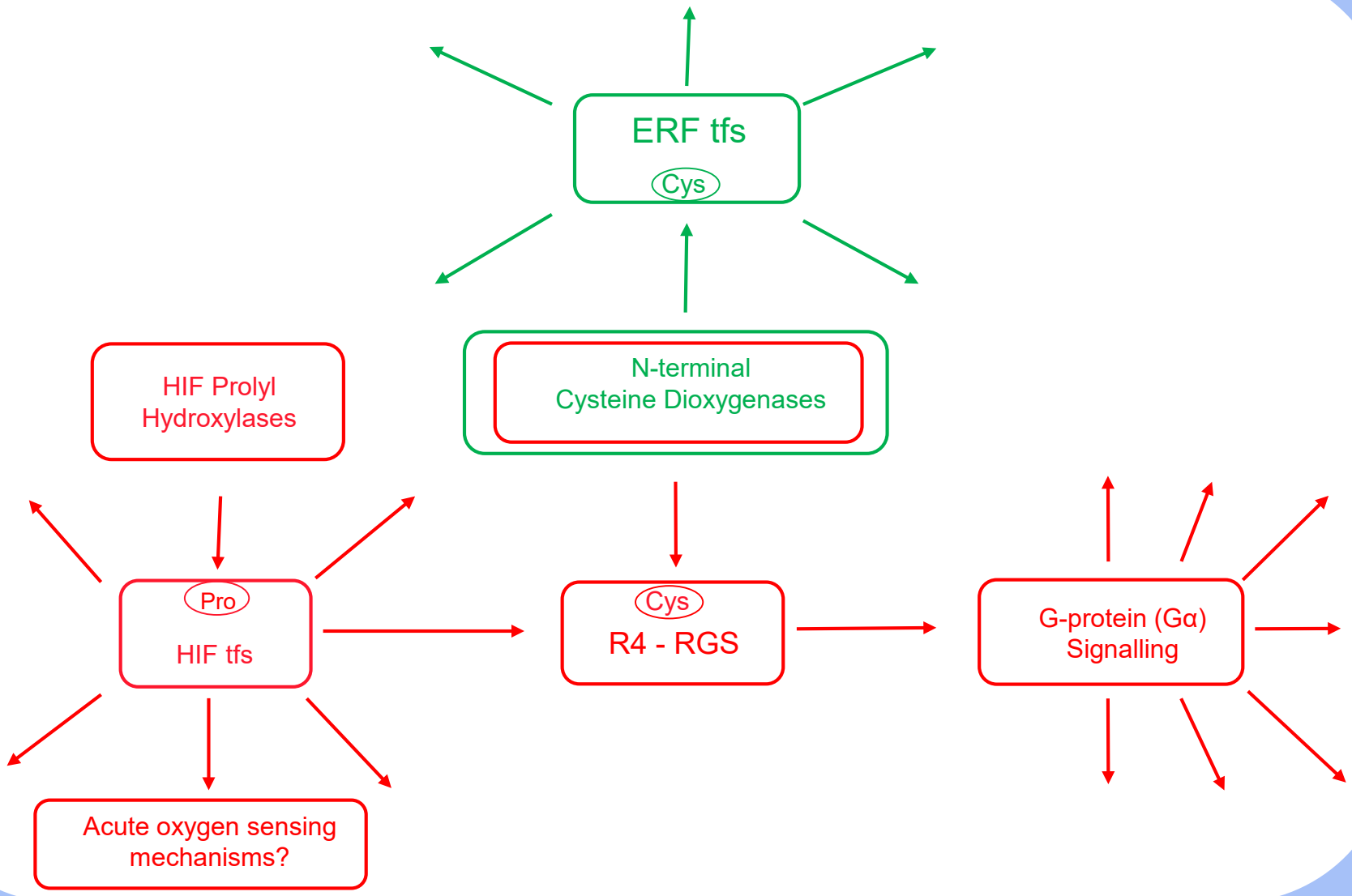
Oxygen sensing by enzymatic protein oxidation

A conserved N-terminal cysteine dioxygenase regulates G-protein signalling in human cells



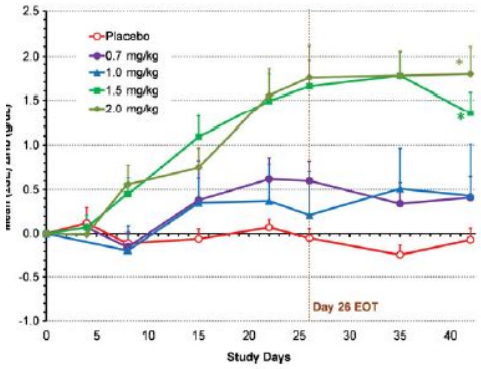


Modulation and integration of oxygen sensing systems employing enzymatic protein oxidation linked to degradation

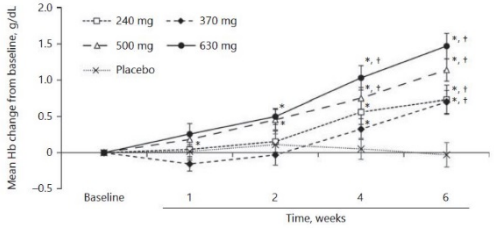


Clinical trials show efficacy of prolyl hydroxylase inhibitors in raising haemoglobin levels in pre-dialysis and dialysis patients

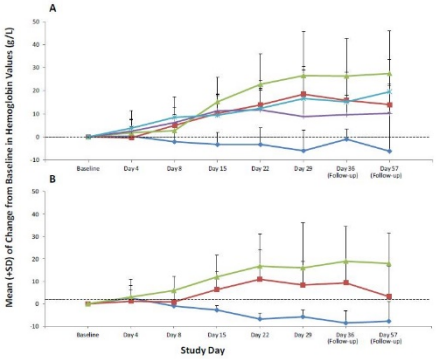
Roxadustat, Fibrogen



Vadadustat, Akebia



Daprodustat, GSK



'You can't always get what you want
But if you try sometime you find
You get what you need'

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