Prolyl Hydroxylase Inhibitors

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- University Clinic Erlangen
- Community Hospital Nuremberg
Prolyl Hydroxylase Inhibitors

- HIF regulation and the role of PHDs
- Stimulation of EPO production
  - Rational: overcoming limitations of rhEPO
  - Prerequisite: preserved production capacity
  - Challenges: (un) specificity
- Tissue protection
  - Medical Need
  - Experimental evidence
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Oxygen sensing and the HIF system

a “simple” hydroxylation reaction

O$_2$ oxoglutarate

CO$_2$ succinate

PHD-I

PHD-1 PHD-2 PHD-3 FIH

VHL

HIF 1α /2α

HIF 1α /2α

degradation

HIF-dependent genes

regulators of cell-specific expression

Oxoglutarate analogues can be used as competitive inhibitors of PHDs (PHD-I), i.e. HIF stabilizers

Examples:

More than 100 different compounds developed

Rose et al., Chem Soc Rev 2011
Rabinowitz, J Med Chem 2013
Oxygen sensing and the HIF system

Oxoglutarate analogues can be used as competitive inhibitors of PHDs (PHD-I), i.e. HIF stabilizers

Rose et al., Chem Soc Rev 2011
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"simple" hydroxylation reaction

Oxoglutarate analogues can be used as competitive inhibitors of PHDs (PHD-I), i.e. HIF stabilizers

Chem Soc Rev 2011
Hypoxia Inducible Factor (HIF)

**Cell Survival**
- ADM
- EPO
- IGFBP1-3
- TGFα

**Transcriptional regulation**
- ETS1
- DEC1-2

**Erythropoiesis**
- Epo

**Iron metabolism**
- Ceruloplasmin
- TRF
- TRFR

**Proliferation**
- CyclinD1
- IGF2
- IGFBP1-3

**Angiogenesis**
- VEGF
- VEGFR1
- LEP
- EGF

**Mitochondrial function**
- PDK
- COX4-1
- LON

**Vascular tone**
- ADM
- iNOS
- ET1
- BNP

**Cell motility**
- CXCR4
- c-Met

**Glucose metabolism**
- GLUT1
- HK
- LDH1
- PGK
- ENO1

**pH regulation**
- CA9
- MCT4
- NHE1

**Apoptosis**
- BNIP3
- NIX

**Extracellular matrix metabolism**
- PAI1
- MMP2
- FN
- UPAR

**Adapted from Schofield & Ratcliffe, Nat Rev Mol Cell Biol 2004**
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Inadequately low EPO as cause of renal anemia

Rh EPO
- effective in almost all patients
- overall safe
- with relatively few limitations:
  - biological: high costs, limited stability
  - parenteral dosing required
  - occasionally immunogenic \( \rightarrow \) PRCA
  - efficacy limited by iron availability
  - risks when targeting normal Hb levels

- Rational for new therapies
- Interest in market participation
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Hct and EPO dose in HD patients depending on altitude

Effect of a PHD-I on EPO production in rats

Model of gentamycin-induced renal anemia

Plasma EPO

EPO mRNA

Liver

Kidney

Flamme et al. PLoS 2014
Single dose study with a PHD-I in humans (FG 2216)

Bernhardt et al.
J Am Soc Nephrol  2010

preserved EPO production capacity
Fate of renal EPO producing cells (REPs)

PHD-I can stimulate renal EPO in ESRD

- Capacity preserved
- Perhaps cells are simply not hypoxic enough

Souma T et al., *J Am Soc Nephrol* 2013
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Specificity of EPO regulation

- HIF 1α/2α
- HIF 1α/2α
- HIF β
- HIF-dependent genes
- EPO

O₂ oxoglutarate

CO₂ succinate

PHD-1
PHD-2
PHD-3
FIH

regulators of cell-specific expression

degradation
Oxygen sensing and the HIF system

Specificity of EPO regulation

1. HIF-2 is the important transcription factor
Oxygen sensing and the HIF system

Specificity of EPO regulation

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2. PHD-2 is of particular relevance
Oxygen sensing and the HIF system

Which of these aspects helps to confine the PHD-I effect to EPO?

Specificity of EPO regulation

1. HIF-2 is the important transcription factor
2. PHD-2 is of particular relevance
3. Cell-specific regulators restrict EPO gene expression (renal fibroblasts, hepatocytes, Ito-cells, astrocytes, ….)
4. EPO the most sensitive HIF-target gene
Oxygen sensing and the HIF system

**Rare mutations causing HIF activation lead to polycythemia**

- **PHD-2**  
  Percy et al., *PNAS* 2006  
  Ladroue et al., *NEJM* 2008

- **HIF-2**  
  Percy et al., *NEJM* 2008  
  Percy et al., *Blood* 2008  
  Gale et al., *Blood* 2008  
  Furlow et al., *JBC* 2009

- **VHL**  
  - VHL-syndrome (cancer)  
  - Chuvash polycythemia
Chuvashia – a Russian Republic

Chuvash polycythemia
- endemic in Chuvash population in Russia
- majority likely originated from a single founder event
- 598 C > T mutation in VHL; \( \rightarrow R200W (\text{Arg} \rightarrow \text{Trp}) \)

Mild inhibition of HIF-2α Degradation
- Polycythemia
- No enhanced tumor incidence
- Reduced life expectancy (due to polycythemia ?)
- Mild organomegaly
- Pulmonary hypertension
Chuvash mutation and pulmonary hypertension

observed in patients …
and reproduced in mice

$VHL^{R200W}$ (Arg → Trp)

HIF-1α → HIF-2α

→ MYC

+ p21Cip1

- cell proliferation +

Hickey et al.,
*J Clin Invest* 2010
Oxygen sensing and the HIF system

O_{2}\text{ oxoglutarate} \quad \text{CO}_2\text{ succinate} \quad \text{VHL} \quad \text{degredation}

1. HIF 1\alpha / 2\alpha
2. PHD-1, PHD-2, PHD-3, FIH
3. HIF \beta
4. HIF-dependent genes
5. EPO

Rare mutations causing HIF activation lead to polycytemia

- PHD-2  
  Percy et al., PNAS 2006  
  Ladroue et al., NEJM 2008

- HIF-2  
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  - Chuvash polycythemia
Oxygen sensing and the HIF system

Limitations of selectivity

1. Although EPO is the most sensitive HIF target, inducing the HIF pathway does **not** selectively induce HIF

2. PHDs and FIH modulate other pathways

3. Other oxoglutarate dependent di-oxygenases may be inhibited by oxoglutarate analogues
Potential spectrum of PHD-I effects

1. *On*-target effects via HIF-induction of other genes
2. *On*-target effects of inhibiting PHDs that are independent of HIF
3. *Off*-target effects, e.g. via inhibition of other dioxygenases

<table>
<thead>
<tr>
<th>Neutral</th>
<th>Beneficial</th>
<th>Harmful</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficult to detect</td>
<td>- Improved iron utilisation *</td>
<td>- Increase in pulmonary artery pressure</td>
</tr>
<tr>
<td></td>
<td>- Blood pressure reduction *</td>
<td>- Destabilisation of atherosclerotic plaques</td>
</tr>
<tr>
<td></td>
<td>- Lipid lowering effect *</td>
<td>- Enhanced growth of renal cysts</td>
</tr>
<tr>
<td></td>
<td>- Reduced progression of kidney disease</td>
<td>- Tumor progression</td>
</tr>
<tr>
<td></td>
<td>- …</td>
<td>- …</td>
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</table>

* Evidence in humans
Some ongoing trials with PHD-I in renal anemia

<table>
<thead>
<tr>
<th>Company</th>
<th>Molecule</th>
<th>Ph</th>
<th>Condition</th>
<th>N</th>
<th>Comparator</th>
<th>Endpoints</th>
<th>NCT</th>
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</thead>
<tbody>
<tr>
<td>Fibrogen / Astellas</td>
<td>FG-2216</td>
<td>II</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Fibrogen / Astellas</td>
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<td>III</td>
<td>HD/PD</td>
<td>1425</td>
<td>epoetin alfa</td>
<td>MACE</td>
<td>02174731</td>
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<tr>
<td>Astra Zeneca</td>
<td>FG-4592</td>
<td>III</td>
<td>ND CKD</td>
<td>2600</td>
<td>Placebo</td>
<td>MACE</td>
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<td>III</td>
<td>ND CKD</td>
<td>570</td>
<td>darbepoetin alfa</td>
<td>Hb response</td>
<td>02021318</td>
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<td>Bayer</td>
<td>BAY 85-3934</td>
<td>II</td>
<td>ND CKD</td>
<td>228</td>
<td>epoetin</td>
<td>Hb response</td>
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<td></td>
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<td>HD</td>
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<td>Hb response</td>
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<td>GSK</td>
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<td>ND CKD</td>
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<td>Epoetin</td>
<td>Hb response</td>
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<tr>
<td></td>
<td></td>
<td>II</td>
<td>HD</td>
<td>20 *</td>
<td>---</td>
<td>Hb response</td>
<td>02075463</td>
</tr>
<tr>
<td>Akebia</td>
<td>AKB6548</td>
<td>II b</td>
<td>ND CKD</td>
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<td>Placebo</td>
<td>Hb response</td>
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<td>Japan Tobacco Inc</td>
<td>JTZ-951</td>
<td>I</td>
<td></td>
<td></td>
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* hyporesponsive patients
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Tissue protection - against (hypoxic) injury

- **Brain**
- **Heart**
- **Kidney**
- **Liver**
- **Gut**
- **Eye**
- **Limbs**
- **Systemic**

<table>
<thead>
<tr>
<th><strong>Tissue</strong></th>
<th><strong>Events</strong></th>
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<tbody>
<tr>
<td>Brain</td>
<td>Stroke, intracerebral hemorrhage, chronic vascular disease, degenerative diseases, …</td>
</tr>
<tr>
<td>Heart</td>
<td>Acute myocardial infarction, post-MI remodeling, chronic ischemic heart disease, …</td>
</tr>
<tr>
<td>Kidney</td>
<td>Acute kidney injury, kidney transplantation, chronic kidney disease, …</td>
</tr>
<tr>
<td>Liver</td>
<td>Acute ischemic injury, chronic hepatitis, hepatic congestion, …</td>
</tr>
<tr>
<td>Gut</td>
<td>Acute intestinal ischemia, chronic inflammatory bowel disease, …</td>
</tr>
<tr>
<td>Eye</td>
<td>Acute retinal infarction, glaucoma, …</td>
</tr>
<tr>
<td>Limbs</td>
<td>Acute vascular occlusion, peripheral artery disease, wounds …</td>
</tr>
<tr>
<td>Systemic</td>
<td>Sepsis, multiorgan failure, general anesthesia, cardiothoracic surgery, …</td>
</tr>
</tbody>
</table>

**Huge medical need!**

**Short term therapy may be sufficient!**
Effect of HIF induction in organ donors

Fisher → Lewis allogenic rat kidney transplant model

treatment of the donor with a single dose of FG 4497 (6 hours before nephrectomy)

Bernhardt et al., PNAS 2009
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Summary

• PHD-Is represent a fascinating translation of recent knowledge on hypoxia sensing into clinical application.

• PHD-Is can stimulate endogenous EPO production in healthy individuals and patients with CKD.

• Short term use is not associated with obvious toxicity, but long-term benefits and risks need to be carefully evaluated.

• Use of PHD-I for tissue and organ protection has a huge potential that warrants clinical studies.