"Pyruvate Kinase Deficiency Clinical management"

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University Medical Centre Utrecht, University of Utrecht, ERN-
EuroBloodNet subnetwork: Red cells.
Utrecht – the Netherlands
13 February 2020
Conflicts of interest and disclaimer

Advisory board: Agios

Research support: Novartis, Bayer, Agios, Mechatronics, ZonMW.

Content contains personal opinion of the presenter
Practical issues before starting

- 30-35min presentation (30 slides max) + 15 min Q&A session
- Microphones will be muted by host to avoid back noise
- Please, stop your video to improve internet connexion
- Send your questions during the presentation through the chat, they will be gathered and answered after the presentations.
1. PK Deficiency shares the clinical picture with many other hereditary hemolytic anemia’s

2. Many complications go unnoticed until irreversible damage has been done

3. Screening for possible complications should be considered

   *(there is often treatment available)*

4. This is also applicable for so-called “mild” transfusion independent PK Deficiency

**Learning objectives:**

1. Diagnosis of PK Deficiency

2. Specific Neonatological/Paediatric aspects of PK Deficiency
Outline

1. Disclosures/ Personal info
2. Introduction
3. Organ damage
4. When to transfuse
5. When to chelate
6. When to splenectomize
7. Stem cell transplantation
8. New treatment options:
   1. Mitapivat
   2. Gene therapy
9. Acknowledgements and Q&A
The Global Burden of Disease Study 2010
Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010

Years Lived with Disability (YLD)

- 6,916,000,000
- 772,000,000

- 21,000,000 - dm
- 29,000,000 - copd
- 68,000,000 - anemia

2010

Lancet, 15 dec 2012, 4 jan 2013
Icons: C Shannon, J Cabesaz, A Coscovelina, M Vanco.
## Global causes of anemia YLD:

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cause</th>
<th>Global</th>
<th>AP HI</th>
<th>Eurp Western</th>
<th>Australasia</th>
<th>NA HI</th>
<th>Eurp Central</th>
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<tbody>
<tr>
<td>Females</td>
<td>Iron-deficiency anemia</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>11</td>
<td>1</td>
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<td>Males</td>
<td>Iron-deficiency anemia</td>
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<td>2</td>
<td>4</td>
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<td>13</td>
<td>2</td>
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<tr>
<td>Males</td>
<td>Hookworm disease</td>
<td>3</td>
<td>30</td>
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<td>Hookworm disease</td>
<td>4</td>
<td>28</td>
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<td>Females</td>
<td>Sickle cell disorders</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Females</td>
<td>Thalassemias</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Males</td>
<td>Sickle cell disorders</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Females</td>
<td>Malaria</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>Thalassemias</td>
<td>9</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Males</td>
<td>Malaria</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Additional Causes

- Females: Iron-deficiency anemia, Hookworm disease, Sickle cell disorders, Thalassemias, Malaria, CKD (unspecified), Schistosomiasis, Uterine fibroids, Schistosomiasis, Other tropical diseases, Other infectious diseases, Other hemoglobinopathies, Other endocrine, Other endocrine, Other infectious diseases, Other hemoglobinopathies, CKD (due to diabetes), CKD (due to hypertension), Other gynecological diseases, CKD (due to hypertension), CKD (due to diabetes), Gastritis and duodenitis, G6PD deficiency, Maternal hemorrhage, Gastritis and duodenitis, G6PD deficiency, Peptic ulcer disease, Peptic ulcer disease.
Causes of hereditary anemia:
Causes of hereditary hemolytic anemia (HHA)

Hemoglobin disorder:
- Hemoglobinopathies
- Thalassemia’s

Red cell enzyme disorders (non-spherocytic HHA):
- G6PD- Deficiency
- Pyruvate Kinase Deficiency

Red cell membrane disorders:
- Spherocytosis
- Stomatocytosis

Other:
- Congenital dyserythropoietic anemia’s (CDA)
Enzymes of the red blood cell

6-Phosphogluconate dehydrogenase
6-Phosphogluconolactonase
Acetylcholinesterase
Adenine phosphoribosyl transferase
Adenosine deaminase
Adenylate kinase
Aldolase
AMP deaminase
Bisphosphoglycerate mutase
Carbonic anhydrase I
Carbonic anhydrase II
Catalase
Cytochrome b5 reductase
δ-ALA dehydrase
Enolase
Galactokinase
Galactose-1-P-uridyltransferase
γ-Glutamylcysteine synthetase
Glucose phosphate isomerase
Glucose-6-phosphate dehydrogenase
Gluthathione peroxidase
Gluthathione reductase
Glutathione synthetase
Glutathione-S-transferase
Glyceraldehyde 3-phosphate dehydrogenase
Glyoxalase I
Hexokinase
Hypoxanthine-guanine phosphoribosyl transferase
ITPase
Lactate dehydrogenase
NADPH diaphorase
Phosphofructokinase
Phosphoglucomutase
Phosphoglycerate kinase
Pyrimidine-5’-nucleotidase
Pyruvate kinase
Triosephosphate isomerase
Uroporphyrinogen 1 synthase

courtesy Richard van Wijk : R.vanWijk@umcutrecht.nl
Pyruvate kinase (PK)

- **Key enzyme of glycolysis:** sole source of energy for the red blood cell
- **Catalyses the irreversible phosphoryl group transfer from phosphoenolpyruvate to ADP**

\[ \text{pyruvate + ATP} \]

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**Blood** (2005) courtesy Richard van Wijk: R.vanWijk@umcutrecht.nl
IN TOTAL, RARE DISEASE IMPACT

30 MILLION

1 in 10 AMERICANS

Source: National Institutes of Health
The various forms of Hereditary hemolytic anemia are rare.

Hereditary hemolytic anemia is common.
How severe is mild?

Do I Diagnose Hereditary hemolytic anemia?
How Do I Diagnose Hereditary hemolytic anemia?

5th March Eurobloodnet webinar:

Dr Paola Bianchi: Recommendations on pyruvate kinase deficiency

https://www.eurobloodnet.eu/education/webinars/8/recommendations-on-pyruvate-kinase-deficiency-diagnosis

PK-deficiency: R.vanWijk@umcutrecht.nl (free service)
Organ damage
Patients with the same genotype have different phenotypes
Retinopathy?
Cardiac arrhythmia
Cardiomyopathy
Iron Overload
Asplenia
Gallstones
Liver cirrhosis
Osteonecrosis
Osteoporosis

Stroke?
Endocrinopathy
Hearing problems?
Pulmonary hypertension
Extra-medullary Hematopoiesis
Nephropathy
Leg ulcers
Do all patients with Hereditary anemia share the same problems?
Retinopathy?
Cardiac arrhythmia
Cardiomyopathy
Iron Overload
Asplenia
Gallstones
Liver cirrhosis
Osteonecrosis
Osteoporosis

Main Body

Superior Sagittal Sinus
Corpus Callosum
Pons
Cerebellum
Medulla
Pharynx
Spinal Cord
Tongue
Epiglottis
Trachea
Thyroid Gland
Superior Vena Cava
Pulmonary Artery
Pulmonary Artery
Heart
Spleen
Kidney
Kidney
Iliac Artery
Iliac Vein
Bladder

Stroke
Endocrinopathy
Hearing problems?
Pulmonary hypertension
Extra-medullary Hematopoiesis
Nefropathy
Leg ulcers
Adults with PK Deficiency Had Higher Rates of Splenectomy, Cholecystectomy and Gallstones Over the Previous 8 Years

Comparisons with the General Population

ERT: Ever Regularly Transfused; NRT: Never Regularly Transfused (but transfused at least once); NT: Never Transfused. All comparisons are based on 2-sided Fisher's exact test

*p<0.05 for PK Deficiency NHS population versus matched general population; †p<0.001 for PK Deficiency NHS population versus matched general population

EAT: Ever Regularly Transfused; NRT: Never Regularly Transfused (but transfused at least once); NT: Never Transfused. All comparisons are based on 2-sided Fisher's exact test

*b<0.05 for PK Deficiency NHS population versus matched general population; †p<0.001 for PK Deficiency NHS population versus matched general population

Adults with PK Deficiency had Higher Lifetime Rates of Pulmonary Hypertension, Osteoporosis and Liver Cirrhosis

ERT: Ever Regularly Transfused; NRT: Never Regularly Transfused (but transfused at least once); NT: Never Transfused. All comparisons are based on 2-sided Fisher’s exact test.

*p<0.05 for PK Deficiency NHS population versus matched general population; †p<0.001 for PK Deficiency NHS population versus matched general population.

<table>
<thead>
<tr>
<th>Condition</th>
<th>General population*</th>
<th>HNSHA# (screened for organ damage)</th>
<th>PK deficiency## (non-screened)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>n.a.</td>
<td>30</td>
<td>254</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>3%</td>
<td>17%</td>
<td>3%</td>
</tr>
<tr>
<td>Hypertension**</td>
<td>&lt;1%</td>
<td>10%</td>
<td>11%</td>
</tr>
<tr>
<td>Thrombotic event</td>
<td>&lt;1%</td>
<td>68%</td>
<td>48%</td>
</tr>
<tr>
<td>Iron overload (liver)</td>
<td>&lt;1%</td>
<td>39%</td>
<td>n.r.</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>7%</td>
<td>3%</td>
<td>n.r.</td>
</tr>
<tr>
<td>Renal failure</td>
<td>4%</td>
<td>3%</td>
<td>n.r.</td>
</tr>
<tr>
<td>Cholecystectomy</td>
<td>&lt;1%</td>
<td>73%</td>
<td>40%</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>3%</td>
<td>15%</td>
<td>n.r.</td>
</tr>
<tr>
<td>Fractures</td>
<td>7%</td>
<td>0%</td>
<td>17%</td>
</tr>
<tr>
<td>Leg ulceration</td>
<td>&lt;1%</td>
<td>7%</td>
<td>2%</td>
</tr>
<tr>
<td>Low testosterone</td>
<td>2%</td>
<td>14%</td>
<td>0%</td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
<td>49%</td>
<td>50%</td>
<td>n.r.</td>
</tr>
<tr>
<td>IGF-1 deficiency</td>
<td>2%</td>
<td>43%</td>
<td>3%</td>
</tr>
</tbody>
</table>

n.r. not reported; IGF: insulin-like growth factor deficiency was defined as >-2 SD from healthy controls

* disease prevalence in the general Dutch population

** defined as tricuspid regurgitant jet flow velocity >2.5m/s by cardiac ultrasound.

#HNSHA: Hereditary Nonspherocytic Hemolytic Anemia. (23 PK deficiency, 4 G6PD deficiency, 2 HK deficiency, 1 GCL deficiency)


## Data cited from: Grace et al. Blood 2018
Treatment of organ damage: examples and suggestions (use Eurobloodnet expertise):

- Microalbuminuria
- Osteoporosis
- Endocrine problems
- Iron overload
- Heart failure
- Leg Ulcers
- Vitamin and Zinc deficiency
- EMH

- ACE-inhibition
- Bisphosphonates
- Suppletion
- Chelation
- Blood transfusion, specific therapy
- Topical nitroglyceride, transfusion
- Suppletion
- Blood transfusion
Organ damage in Hemolytic anemia including PKD is underdiagnosed and prevalent.
How to recognise iron overload in your patients
Iron overload in Pyruvate kinase deficiency

A. Ferritin based

B. MRI based

van Beers et al. Haematologica 2019
Ferritin versus LIC in PK Deficiency

B. MRI based

van Beers et al. Haematologica 2019
**Ferritin versus LIC per disease category**

- Sickle cell disease
- B-thalassemia
- Other hemoglobin disorders
- Pyruvate kinase deficiency
- Other enzyme disorders
- Hereditary spherocytosis
- Other membrane disorders

---

Van Straaten et al. Am J Hematol 2019
Sensitivity of ferritin combined with transferrin saturation to predict iron overload.

Table 3: predictive value of ferritin, TSAT and LIC

<table>
<thead>
<tr>
<th></th>
<th>ferritin ≥1000</th>
<th>ferritin ≥500</th>
<th>ferritin ≥500 or TSAT≥45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N=112</td>
<td>LIC≥3 LIC≥7</td>
<td>LIC≥3 LIC≥7</td>
<td>LIC≥3 LIC≥7 N=51 LIC≥7</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>41% 58%</td>
<td>76% 92%</td>
<td>87% 100%</td>
</tr>
</tbody>
</table>

“At a ferritin cut off of 500 ng/mL, the sensitivity for LIC >3 mg/g DW was 90% and the specificity was 67%”

PKD –NHS study:
“In patients with a transferrin saturation >45% or a ferritin >500 ng/ml, the sensitivity to predict LIC >3 mg/g DW was 92%”

Van Straaten et al. Am J Hematol 2019
Van Beers et al. Haematologica 2019
Consider to diagnose iron overload by MRI in all with TSAT > 45% or Ferritin > 500
When to

Transfuse
Considerations when to transfuse in PK deficiency

- Controversial topic
- Traditionally: very tolerant to anemia...
- But 2-3 dpg levels are comparable to SCD e.g.
- Aging patient with complications differs from younger patient without complications
- Depends on “needs” or “activity” of patient
- Good chelation is available; iron overload is not a major decision driver
- Antibody formation no major issue when extended matching is performed
- No specific target hemoglobin. *(e.g. do not use Thalassemia targets)*
Organ damage and treatment in thalassemia intermedia

Taher et al. Blood 2010
Considering to
transfuse
is a
personalized medicine
shared decision
When to Splenectomize
Consider splenectomy if patient is transfusion-dependent or severely anemic

Iolascon et al. Haematologica 2017
Stem cell transplantation in pyruvate kinase deficiency
### Results of stem cell transplantation in PK-deficiency

<table>
<thead>
<tr>
<th></th>
<th>Survivor</th>
<th>Non-survivor</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age in years</strong></td>
<td>7.5 – 3.0 (0.8-41)</td>
<td>17.4 – 15.2 (6-39)</td>
<td>0.036*</td>
</tr>
<tr>
<td><strong>Asian hospital</strong></td>
<td>8/11 (73%)</td>
<td>0/5</td>
<td>0.026*</td>
</tr>
<tr>
<td><strong>Splenectomy performed</strong></td>
<td>3/11 (27%)</td>
<td>4/5 (80%)</td>
<td>0.106</td>
</tr>
<tr>
<td><strong>Mena Hb (g/dL) (N=13)</strong></td>
<td>6.0 – 5.5 (4.5-7.9)</td>
<td>7.1 – 6.9 (6.0-8.1)</td>
<td>0.112</td>
</tr>
<tr>
<td><strong>Pre-transplant ferritin (ng/ml) (n=12)</strong></td>
<td>804 – 771 (206-1650)</td>
<td>2167 – 675 (596-7026)</td>
<td>0.432</td>
</tr>
<tr>
<td><strong>Myeloablation</strong></td>
<td>6/11 (55%)</td>
<td>4/5 (80%)</td>
<td>0.588</td>
</tr>
<tr>
<td><strong>Graft type</strong></td>
<td></td>
<td></td>
<td>0.507</td>
</tr>
<tr>
<td>MSD</td>
<td>2/11 (18%)</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>MUD</td>
<td>6/11 (55%)</td>
<td>3/5 (60%)</td>
<td></td>
</tr>
<tr>
<td>CORD</td>
<td>2/11 (18%)</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>MFD</td>
<td>1/11 (9%)</td>
<td>2/5 (40%)</td>
<td></td>
</tr>
<tr>
<td><strong>Transplant source</strong></td>
<td></td>
<td></td>
<td>0.333</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>4/11 (36%)</td>
<td>4/5 (80%)</td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>5/11 (45%)</td>
<td>1/5 (20%)</td>
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</tr>
<tr>
<td>Cord blood</td>
<td>2/11 (18%)</td>
<td>0/5</td>
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</tr>
<tr>
<td><strong>GvHD</strong></td>
<td></td>
<td></td>
<td>0.015*</td>
</tr>
<tr>
<td>None</td>
<td>7/11 (64%)</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>1/11 (9%)</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>1/11 (9%)</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>0/11</td>
<td>1/5 (20%)</td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>2/11 (18%)</td>
<td>4/5 (80%)</td>
<td></td>
</tr>
</tbody>
</table>
Results of stem cell transplantation in PK-deficiency

Survival

Time at endpoint (years)

Survival (%)

Overall survival
Age <10 years
Age ≥10 years
Stem cell transplantation can be curative treatment, in pyruvate kinase deficiency, but...
New treatment options?
Safety and Efficacy of Mitapivat in Pyruvate Kinase Deficiency

Rachael F. Grace, M.D., Christian Rose, M.D.,* D. Mark Layton, M.B., B.S., Frédéric Galactéros, M.D., Wilma Barcellini, M.D., D. Holmes Morton, M.D., Eduard J. van Beers, M.D., Hassan Yaish, M.D., Yaddanapudi Ravindranath, M.D., Kevin H.M. Kuo, M.D., Sujit Sheth, M.D., Janet L. Kwiatkowski, M.D., M.S.C.E., Ann J. Barbier, M.D., Ph.D., Susan Bodie, Pharm.D., Bruce Silver, M.D., Lei Hua, Ph.D., Charles Kung, Ph.D., Peter Hawkins, Ph.D., Marie-Hélène Jouvin, M.D., Chris Bowden, M.D., and Bertil Glader, M.D., Ph.D.

ABSTRACT

N Engl J Med 381;10  NEJM.org  SEPTEMBER 5, 2019
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mitapivat, 50 mg Twice Daily (N=27)</th>
<th>Mitapivat, 300 mg Twice Daily (N=25)</th>
<th>All Patients (N=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex — no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>9 (33)</td>
<td>11 (44)</td>
<td>20 (38)</td>
</tr>
<tr>
<td>Male</td>
<td>18 (67)</td>
<td>14 (56)</td>
<td>32 (62)</td>
</tr>
<tr>
<td>Median age (range) — yr</td>
<td>28 (18–58)</td>
<td>40 (20–61)</td>
<td>34 (18–61)</td>
</tr>
<tr>
<td>Race — no. (%)†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>22 (81)</td>
<td>21 (84)</td>
<td>43 (83)</td>
</tr>
<tr>
<td>Asian</td>
<td>2 (7)</td>
<td>1 (4)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Not reported</td>
<td>2 (7)</td>
<td>1 (4)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (4)</td>
<td>2 (8)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>PKLR mutation type — no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missense/missense</td>
<td>15 (56)</td>
<td>17 (68)</td>
<td>32 (62)</td>
</tr>
<tr>
<td>Missense/non-missense</td>
<td>6 (22)</td>
<td>4 (16)</td>
<td>10 (19)</td>
</tr>
<tr>
<td>Non-missense/non-missense</td>
<td>6 (22)</td>
<td>4 (16)</td>
<td>10 (19)</td>
</tr>
<tr>
<td>Median hemoglobin (range) — g/dl</td>
<td>9.6 (6.9–12.3)</td>
<td>8.6 (6.5–12.0)</td>
<td>8.9 (6.5–12.3)</td>
</tr>
<tr>
<td>Splenectomy — no. (%)‡</td>
<td>23 (85)</td>
<td>20 (80)</td>
<td>43 (83)</td>
</tr>
<tr>
<td>Cholecystectomy — no. (%)</td>
<td>19 (70)</td>
<td>19 (76)</td>
<td>38 (73)</td>
</tr>
<tr>
<td>Chelation therapy before enrollment — no. (%)</td>
<td>14 (52)</td>
<td>11 (44)</td>
<td>25 (48)</td>
</tr>
<tr>
<td>Median ferritin (range) — ng/ml</td>
<td>723 (41–3254)</td>
<td>775 (346–2518)</td>
<td>764 (41–3254)</td>
</tr>
<tr>
<td>Osteoporosis — no. (%)</td>
<td>5 (19)</td>
<td>3 (12)</td>
<td>8 (15)</td>
</tr>
<tr>
<td>Completion of 24-wk core period — no. (%)§</td>
<td>21 (78)</td>
<td>22 (88)</td>
<td>43 (83)</td>
</tr>
</tbody>
</table>
50% (26/52) of patients had an increase from baseline of more than 1.0 g/dL in Hemoglobin (Hb) level

- Mean maximum increase in the Hb was 3.4 g/dL (range 1.1 – 5.8 g/dL)
- Median time until first observed increase of >1.0 g/dL in Hb was 10 days (range 7 to 187 days)
- All patients who had an average hemoglobin increase from baseline of >1.0 g/dL had at least one missense PKLR mutation

*Patients homozygous for R479H.

Average Change in Hemoglobin by PK-R Protein Level

*Patients homozygous for R479H.

Gene therapy
in
pyruvate kinase deficiency

Courtesy: dr. J.C. Segovia
jc.segovia@ciemat.es
Efficacy of PK deficiency gene therapy in mouse model

- Improved hematological parameters:
  - Reb blood cell counts
  - Hemoglobin
  - Hematocrit
  - Reticulocytosis
  - Erythrocyte half-life
  - Erythropoietin levels
  - Erythroid differentiation

- Organs
  - Spleen
  - Liver
    - Iron deposits
    - Extramedullary hematopoiesis

Meza et al. Mol Ther 2009 Courtesy: dr. J.C. Segovia
jc.segovia@ciemat.es
Who are the patients eligible for being enrolled?

Group 1:  
Never regularly transfused  
+/- acute transfusion

Group 2:  
Regularly transfused before splenectomy  
Less anemic  
Hg>8.7 gr/dl  
25%

Group 3:  
Regularly transfused before splenectomy  
Less anemic  
Hg≤8.7 gr/dl  
29%

Group 4:  
Regularly transfused after splenectomy  
9%

Group 5:  
37%

Courtesy: dr. J.C. Segovia  
jc.segovia@ciemat.es
Overview of the clinical protocol

Severe PKD patient (Still transfusion dependent after splenectomy)

Peripheral blood harvest

Hematopoietic stem cell mobilization

G-CSF + plerixafor

Purification

Hematopoietic Stem cells CD34+

In vitro transduction

Viral vector
cryopreservation

Corrected stem cells

Testing/release while frozen

Intravenous infusion of corrected cells

Hematopoietic conditioning (myeloablation)

Courtesy: dr. J.C. Segovia
jc.segovia@ciemat.es
Acknowledgements

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dr. J.C. Segovia Ciemat-Ciberer Madrid Spain
(Q&A gene therapy: jc.segovia@ciemat.es)

Questions: e.j.vanbeers-3@umcutrecht.nl
1. PK Deficiency shares the clinical picture with many other hereditary hemolytic anemia’s

2. Many complications go unnoticed until irreversible damage has been done

3. Screening for possible complications should be considered

   *(there is often treatment available)*

4. This is also applicable for so-called “mild” transfusion independent PK Deficiency
Supplemental slides
### Anemia

<table>
<thead>
<tr>
<th>Erythrocyte parameters</th>
<th>Mouse strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy mouse</td>
</tr>
<tr>
<td>RBC</td>
<td>$10.5 \times 10^{12}/L$</td>
</tr>
<tr>
<td>HGB</td>
<td>$13.8 \text{ g/dL}$</td>
</tr>
<tr>
<td>HCT</td>
<td>$46.2%$</td>
</tr>
</tbody>
</table>

### Reticulocytosis

Towards a gene therapy clinical trial for PKD

**Orphan Drug Designation**

EU/3/14/1330

DRU-2016-5168
DRIVE PK: Phase 2, Open Label, Randomized Study of Safety and Efficacy in Patients with PK Deficiency

Study design

Open-label, global, phase 2 study: 14 centers in the US, Canada, and EU

PK-deficient adults who are not regularly transfused
(ClinicalTrials.gov NCT02476916)

Randomization
Stratified by PKLR genotype (none excluded)

Arm 1
300 mg BID

Arm 2
50 mg BID

6-month core dosing period

1 2 3 6 9 12 16 20 24

Assessment points (weeks)

Primary endpoints:
- Safety and tolerability

Secondary endpoints:
- Pharmacokinetics of AG-348
- PD response: ATP, 2,3-DPG
- Indicators of clinical activity: hemoglobin, reticulocyte count, and other hematologic parameters

Extension arm

Not regularly transfused = no more than 3 units of red blood cells transfused in the 12 months prior to the first day of study dosing and no transfusions within 4 months of the first day of study dosing

All patients provided written informed consent

2,3-DPG = 2,3-diphosphoglycerate; BID = twice daily; PD = pharmacodynamic

Patient Disposition by Randomized Dose

65 Assessed for eligibility
13 Excluded (did not meet eligibility criteria)

52 Randomized

- 52 Entered Core Period
  - 50 mg BID
    - N=27
    - 6 Discontinued treatment
      - 2 Adverse event
        - Pharyngitis / nausea
        - Hemolysis
      - 1 Investigator decision
      - 3 Withdraw consent
  - 300 mg BID
    - N=25
    - 3 Discontinued treatment
      - 2 Adverse event
        - Hyperglycemia
        - Pleural effusion
      - 1 Investigator decision

- 43 Completed Core
  - Completed core period
    - N=21 (77.8%)

- 36 Entered Extension
  - Entered extension phase
    - N=18

- 19 Ongoing
  - Ongoing in extension phase
    - N=8
  - Ongoing in extension phase
    - N=11

Treatment ongoing, N = 19

Safety Findings

• The vast majority of AEs were:
  – CTCAE Grade 1 or 2
  – Non-serious events
  – Transient
  – Self-limiting

• No clinically meaningful trends in BMD (total hip, total lumbar spine, and femoral neck) were evident over median of 17 months

• Changes from baseline in sex hormone levels, the result of off-target aromatase inhibition, were observed in males, with most values of testosterone and estradiol remaining within the normal range

• Interpretation of sex hormone data in females was confounded by variability in menopausal status and hormonal contraception use, and is the subject of further investigation

Most Common Adverse Events

- The most common adverse events were transient and generally resolved within 7 days for patients with headache (92%), insomnia (47%), and nausea (78%).

### Incidence of Treatment-Emergent Adverse Events by Randomized Dose

<table>
<thead>
<tr>
<th>Most common adverse events occurring in ≥15% of the overall population — no. of patients (%)</th>
<th>Core Period</th>
<th>Core Period + Extension Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mitapivat 50 mg Twice Daily N=27</td>
<td>Mitapivat 300 mg Twice Daily N=25</td>
</tr>
<tr>
<td>Headache</td>
<td>9 (33)</td>
<td>14 (56)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>5 (19)</td>
<td>16 (64)</td>
</tr>
<tr>
<td>Nausea</td>
<td>10 (37)</td>
<td>10 (40)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>7 (26)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Hot flush</td>
<td>2 (7)</td>
<td>7 (28)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>5 (19)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4 (15)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2 (7)</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3 (11)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Influenza</td>
<td>6 (22)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Cough</td>
<td>4 (15)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>5 (19)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Oropharyngeal pain</td>
<td>3 (11)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>1 (4)</td>
<td>5 (20)</td>
</tr>
</tbody>
</table>

*Headache was transient and generally resolved within several days. †Insomnia typically occurred within 14 days of initiating mitapivat, was self-resolving (generally <7 days) and was not unexpected on the basis of off-target antagonistic or inverse agonist activity against the histamine H3 receptor. ‡Hot flush events were transient and generally reported within the first 7 days of treatment and resolved without treatment within 3 days. Events did not correspond to changes in hormone levels or correlate with age or sex.

### Table 4. Multivariate analysis for determinants of complication rate

<table>
<thead>
<tr>
<th>Complication/parameter</th>
<th>RR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EMH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &gt; 35 y</td>
<td>0.85</td>
<td>0.46-1.58</td>
<td>.610</td>
</tr>
<tr>
<td>Ferritin ≥ 1000 µg/L</td>
<td>0.85</td>
<td>0.51-1.44</td>
<td>.548</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>0.44</td>
<td>0.26-0.73</td>
<td>.001*</td>
</tr>
<tr>
<td>Transfusion</td>
<td>0.06</td>
<td>0.03-0.09</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>0.52</td>
<td>0.30-0.91</td>
<td>.022*</td>
</tr>
<tr>
<td><strong>PHT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &gt; 35 y</td>
<td>2.59</td>
<td>1.08-6.19</td>
<td>.032*</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>4.11</td>
<td>1.99-8.47</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>Transfusion</td>
<td>0.33</td>
<td>0.18-0.58</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>0.42</td>
<td>0.20-0.90</td>
<td>.025*</td>
</tr>
<tr>
<td>Iron chelation</td>
<td>0.53</td>
<td>0.29-0.95</td>
<td>.032*</td>
</tr>
<tr>
<td><strong>HF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splenectomy</td>
<td>2.88</td>
<td>0.99-8.32</td>
<td>.051</td>
</tr>
<tr>
<td>Transfusion</td>
<td>0.06</td>
<td>0.02-0.17</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>1.84</td>
<td>0.98-3.47</td>
<td>.057</td>
</tr>
<tr>
<td>Iron chelation</td>
<td>0.45</td>
<td>0.18-1.12</td>
<td>.086</td>
</tr>
<tr>
<td><strong>Thrombosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &gt; 35 y</td>
<td>2.60</td>
<td>1.39-4.87</td>
<td>.003*</td>
</tr>
<tr>
<td>Female</td>
<td>1.27</td>
<td>0.74-2.19</td>
<td>.387</td>
</tr>
<tr>
<td>Hb ≥ 90 g/L</td>
<td>0.41</td>
<td>0.23-0.71</td>
<td>.001*</td>
</tr>
<tr>
<td>Ferritin ≥ 1000 µg/L</td>
<td>1.86</td>
<td>1.09-3.16</td>
<td>.023*</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>6.59</td>
<td>3.09-14.05</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>Transfusion</td>
<td>0.28</td>
<td>0.16-0.48</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>0.56</td>
<td>0.28-1.10</td>
<td>.090</td>
</tr>
<tr>
<td>Iron chelation</td>
<td>0.97</td>
<td>0.56-1.68</td>
<td>.912</td>
</tr>
<tr>
<td><strong>Cholelithiasis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &gt; 35 y</td>
<td>2.76</td>
<td>1.56-4.87</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>Female</td>
<td>1.96</td>
<td>1.18-3.25</td>
<td>.010*</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>5.19</td>
<td>2.72-9.90</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>Transfusion</td>
<td>0.36</td>
<td>0.21-0.62</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>0.55</td>
<td>0.29-1.02</td>
<td>.058</td>
</tr>
<tr>
<td>Iron chelation</td>
<td>0.30</td>
<td>0.18-0.51</td>
<td>&lt; .001*</td>
</tr>
</tbody>
</table>