Update on Renal Anaemia

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Pathogenesis of anaemia in patients with CKD
Uremic toxicity and inflammation both exacerbate anemia of CKD

General characteristics of 239 ESA-naive CKD Stages 1–5 male patients stratified according to the presence of anaemia (according to WHO)

Table 1. General characteristics of 239 ESA-naive CKD Stages 1–5 male patients stratified according to the presence of anaemia in males (according to WHO)\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>All patients, N = 239</th>
<th>Haemoglobin ≤ 13.0 g/dL, N = 56</th>
<th>Haemoglobin &gt; 13.0 g/dL, N = 183</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>53 (46–63)</td>
<td>53 (45–63)</td>
<td>55 (47–64)</td>
<td>0.4</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m(^2)</td>
<td>48 (5–106)</td>
<td>44 (14–80)</td>
<td>69 (28–95)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>22</td>
<td>48</td>
<td>14</td>
<td>0.002</td>
</tr>
<tr>
<td>Previous CVD, %</td>
<td>33</td>
<td>34</td>
<td>32</td>
<td>0.6</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>26.3 (24.1–28.3)</td>
<td>26.1 (23.8–28.1)</td>
<td>26.4 (25.2–28.2)</td>
<td>0.6</td>
</tr>
<tr>
<td>s-Albumin, g/dL</td>
<td>4.0 (3.7–4.3)</td>
<td>4.0 (3.7–4.3)</td>
<td>4.0 (3.7–4.4)</td>
<td>0.4</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>13.1 (9.0–21.2)</td>
<td>16.0 (9.9–23.0)</td>
<td>11.0 (6.2–16.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PTH, pg/mL</td>
<td>127 (55–168)</td>
<td>131 (59–178)</td>
<td>84 (51–151)</td>
<td>0.05</td>
</tr>
<tr>
<td>Total testosterone, nmol/L</td>
<td>12.4 (7.4–14.9)</td>
<td>8.4 (4.8–13.2)</td>
<td>12.8 (9.7–15.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^a\)Data are expressed as medians (interquartile ranges) or percentages. CVD, cardiovascular disease; BMI, body mass index.

General characteristics of 126 ESA-treated prevalent male HD patients stratified according to median weekly ESA dose normalized per kg of body weight (IU/kg/week)


<table>
<thead>
<tr>
<th></th>
<th>All patients, N = 126</th>
<th>ESA ≤ 121 IU/kg/week, N = 63</th>
<th>ESA &gt; 121 IU/kg/week, N = 63</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>63 (49–73)</td>
<td>64 (47–76)</td>
<td>62 (49–70)</td>
<td>0.4</td>
</tr>
<tr>
<td>Vintage, months</td>
<td>25 (14–55)</td>
<td>28 (10–58)</td>
<td>24 (17–54)</td>
<td>0.8</td>
</tr>
<tr>
<td>Davies comorbidity score, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>19</td>
<td>20</td>
<td>17</td>
<td>0.1</td>
</tr>
<tr>
<td>Middle</td>
<td>54</td>
<td>60</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>26</td>
<td>19</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.6 (21.3–27.5)</td>
<td>25.4 (22.5–28.2)</td>
<td>22.3 (20.0–25.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>s-Albumin, g/dL</td>
<td>3.5 (3.2–3.8)</td>
<td>3.5 (3.3–3.8)</td>
<td>3.5 (3.1–3.8)</td>
<td>0.4</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>6.8 (2.9–21.0)</td>
<td>6.4 (2.5–17.0)</td>
<td>8.7 (3.4–27.7)</td>
<td>0.2</td>
</tr>
<tr>
<td>Haemoglobin, g/dL</td>
<td>12.2 (11.0–13.4)</td>
<td>12.1 (11.1–13.3)</td>
<td>12.3 (11.2–13.3)</td>
<td>0.2</td>
</tr>
<tr>
<td>Hypochromic RBC, %</td>
<td>1.1 (0.5–4.1)</td>
<td>0.8 (0.4–1.5)</td>
<td>2.6 (0.6–5.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Intravenous iron medication, %</td>
<td>69</td>
<td>76</td>
<td>63</td>
<td>0.2</td>
</tr>
<tr>
<td>SHBG, nmol/L</td>
<td>27 (20–39)</td>
<td>27 (19–42)</td>
<td>26 (20–38)</td>
<td>0.9</td>
</tr>
<tr>
<td>Total testosterone, nmol/L</td>
<td>10.0 (7.2–12.0)</td>
<td>11.0 (8.4–13.0)</td>
<td>8.0 (6.6–12.0)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

aData are expressed as medians (interquartile ranges) or percentages. BMI, body mass index.
Erythropoiesis in chronic kidney disease

Lankhorst C.E., Wish J.B., Blood Rev., 2010, 24, 39-47
The development of ESAs and IV iron preparations over the last 20 years

Macdougall IC and Ashenden M. Adv Chronic Kidney Dis 2009;16:117−130
The development of ESAs and IV iron preparations over the last 20 years

ESAs

- Darbepoetin alfa (Aranesp) licensed in US and Europe (2001). Epoetin alfa (Eprex; Erypo) and eopoetin beta (NeoRecormon) licensed in Europe (1990)

IV iron preparations

- Increasing use of IV iron in conjunction with EPO
- Imferon (iron dextran) withdrawn from market by Fisons (1992)
- Use of HMW iron dextran (Dexferrum) decreasing
- Main IV iron preparations used: LMW iron dextran (INFed, Cosmofer), iron sucrose (Venofer), and iron gluconate (Ferrlecit)

Peginesatide (OMONTYS) lincend for HD in the US, 2012

Epoetin delta (Dynepo) launched in Germany and UK (2007)
- (Binocrit/Hemax/Abseamed) and Retacrit/Silapo licensed in Europe (2007).

Macdougall IC and Ashenden M. Adv Chronic Kidney Dis 2009;16:117-130
Currently available Erythropoiesis – Stimulating Agents

**Table 1. Currently Available Erythropoiesis-Stimulating Agents**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Active Compound</th>
<th>Manufacturing Process</th>
<th>Year Licensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epoetin alfa/beta (Epogen, Eprex, Erypo, NeoRecormon)</td>
<td>Recombinant human EPO</td>
<td>Recombinant DNA technology; EPO cDNA/gene–transfected CHO cells</td>
<td>1989 (Epogen, in US); 1990 (Eprex/Erypo/NeoRecormon, in Europe)</td>
</tr>
<tr>
<td>Epoetin delta (Dynepo)</td>
<td>Recombinant human EPO</td>
<td>Recombinant DNA technology; EPO cDNA/gene–transfected human cells</td>
<td>2006 (outside of US); product withdrawn by Shire in 2009</td>
</tr>
<tr>
<td>“Biosimilar” epoetins (Binocrit, Hexal, Retacrit, Silapo, Eporatio)</td>
<td>Recombinant human EPO</td>
<td>Recombinant DNA technology; EPO cDNA/gene–transfected CHO cells</td>
<td>2009 onward</td>
</tr>
<tr>
<td>Nonapproved or locally approved “copy” epoetins</td>
<td>Recombinant human EPO</td>
<td>Recombinant DNA technology; EPO cDNA/gene–transfected human cells</td>
<td>Available in many countries outside of US and Europe, eg, India, China, Thailand, Argentina, Brazil</td>
</tr>
<tr>
<td>Darbepoetin alfa (Aranesp)</td>
<td>Hyperglycosylated recombinant human EPO analogue</td>
<td>Recombinant DNA technology; mutated EPO cDNA–transfected CHO cells</td>
<td>2001 (both US and Europe)</td>
</tr>
<tr>
<td>C.E.R.A. (Mircera)</td>
<td>Pegylated recombinant human EPO analogue</td>
<td></td>
<td>2009 (outside of US only)</td>
</tr>
</tbody>
</table>

*Abbreviations: EPO, erythropoietin; cDNA, complementary DNA; C.E.R.A., continuous erythropoietin receptor activator; CHO, Chinese hamster ovary; US, United States.*
Anaemia treatment in CKD patients
What we know about anaemia treatment in CKD in 2012?

- Generally well tolerated
- Treatment increases Hb and reduces need for blood transfusions
- Higher Hb concentration obtained with higher ESAs may lead to increased the risk of adverse events (predominantly CV events)
- Probably improves patients’ quality of life
  - Relationships for most subscales particularly vitality, exercise capacity is sigmoid
    - major increases occur between 7 to 9-10 g/dl Hb
    - Effects of Hb levels is off at 9-11 g/dL Hb
    - In most studies no added benefit at higher Hb except for “vitality” or “fatigue” that are measurable up to 13 g/dl
Results from high-quality trials assessing effect of hemoglobin target on HRQOL

<table>
<thead>
<tr>
<th>Trial</th>
<th>Population, patient characteristics</th>
<th>Number(^a)</th>
<th>High vs. low hemoglobin target (g/dl)</th>
<th>Positive HRQOL differences between arms</th>
<th>Support 2006 hemoglobin target of 11 g/dl or higher?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Besarab et al.(^1)</td>
<td>Hemodialysis, all had heart failure or CAD</td>
<td>1233</td>
<td>9–11 vs. 13–15</td>
<td>None (improved physical function according to 1998 publication)</td>
<td>No</td>
</tr>
<tr>
<td>CanEPO(^2)(^b)</td>
<td>Hemodialysis, all had LVD or LVH</td>
<td>78</td>
<td>9.5–11 vs. 11.5–13</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Foley et al.(^2)(^3)</td>
<td>Hemodialysis, free of marked comorbidity</td>
<td>94</td>
<td>9.5–10.5 vs. 13–14</td>
<td>Fatigue, depression, relationships</td>
<td>Possibly in healthier subjects</td>
</tr>
<tr>
<td>Parfrey et al.(^1)(^9)</td>
<td>Hemodialysis, no CAD or LVD</td>
<td>324</td>
<td>9.5–11.5 vs. 13.5–14.5</td>
<td>Vitality</td>
<td>Possibly; significantly increased risk of stroke</td>
</tr>
<tr>
<td>Roger et al.(^2)(^2)</td>
<td>CKD stage 3 and 4, excluded uncontrolled angina, class III or IV HF, severe chronic respiratory disease, symptomatic peripheral vascular disease, or fistula placement</td>
<td>155</td>
<td>9–10 vs. 12–13</td>
<td>None</td>
<td>No</td>
</tr>
</tbody>
</table>

\(^a\)Number of patients included in the study.

\(^b\)CanEPO: CanHemoEPO, European, American, and Canadian collaborative study

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Kidney Int. Suppl., 2012, 2 (4)
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Ashish Upadhyay, MD (Assistant Project-Director)
## Grading of recommendations

<table>
<thead>
<tr>
<th>Quality of evidence</th>
<th>High</th>
<th>A</th>
<th>Strength of recommendation</th>
<th>Level 1: Strong</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate</td>
<td>B</td>
<td>“We recommend... should”</td>
<td>Level 2: Weak</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>C</td>
<td></td>
<td>“We suggest... might”</td>
</tr>
<tr>
<td></td>
<td>Very low</td>
<td>D</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Guideline Outline

Chapter 1:
Diagnosis and Evaluation of Anemia in CKD

Chapter 2:
Use of Iron to Treat Anemia in CKD

Chapter 3:
Use of ESAs and Other Agents to Treat Anemia in CKD

Chapter 4:
Red Cell Transfusion to Treat Anemia in CKD
Guideline Outline

Chapter 1:
Diagnosis and Evaluation of Anemia in CKD

Chapter 2:
Use of Iron to Treat Anemia in CKD

Chapter 3:
Use of ESAs and Other Agents to Treat Anemia in CKD

Chapter 4:
Red Cell Transfusion to Treat Anemia in CKD
3.1 We recommend that prior to initiation of ESA therapy correctable causes of anemia be addressed (including iron deficiency and inflammatory states, if possible)  
(Grade 1A)

3.2 In initiating and maintaining ESA therapy, we recommend balancing the potential benefits of reducing blood transfusions and anemia-related symptoms against the risks of harm in individual patients (stroke, vascular access loss, hypertension)  
(Grade 1B)

3.3 We recommend using ESA therapy with great caution in CKD patients with active malignancy (in particular when cure is the anticipated outcome), or a history of malignancy or stroke  
(Grade 1C)

Balance benefit vs. risk; caution in patients with malignancies or stroke!
3.4.1 For CKD patients not on HD with Hb $\geq 10.0$ g/dL, we suggest that ESA therapy not be initiated (Grade 2D).

3.4.2 For CKD patients not on HD with Hb $< 10.0$ g/dL, we suggest that the decision whether to initiate ESA therapy be individualized based on the rate of fall of Hb, the risk of needing a transfusion, the risks related to ESA therapy and the presence of symptoms attributable to anemia (Grade 2C).

In non-HD patients no ESA until Hb below 10,0 g/dL!
For CKD patients on HD, we suggest that ESA therapy be used to avoid having the Hb concentration fall below 9.0 g/dL by starting ESA therapy when the Hb is between 9.0-10.0 g/dL (Grade 2B).

Individualization of therapy will be necessary as some patients may have improvements in quality of life at higher Hb and ESA therapy may be started above 10.0 g/dL. (Not Graded)
Rationale I

- Correction of severe anemia (Hb <9 g/dL): strong evidence for substantial benefit (QOL and transfusion requirements), but safety not sufficiently evaluated
- Only one trial in CKD patients on HD tested different target Hb values in patients with severe anemia (9.5-11 g/dL and >11 g/dL) vs placebo and found no difference in the degree of improvement between both targets
- Correction of moderate anemia (Hb >10 g/dL): moderate benefits offset by harm (US NHT trial, CREATE, CHOIR, TREAT)
- TREAT: 1 more stroke for 5 transfusion events prevented; absolute risk of stroke associated with ESA 8% in prior stroke vs 2%
What is the evidence for starting ESAs therapy in CKD patients on HD when Hb level between 9.0 - 10.0 g/dL?
What is the evidence for starting ESAs therapy in CKD patients on HD when Hb level between 9.0 - 10.0 g/dL?
Treatment with ESA

Rationale II

- Based on cancer trials oncology guideline recommends using ESA with great caution in patients with active malignancy, in particular when cure is the anticipated outcome
- Adverse results in cancer trials are consistent with post-hoc analysis in TREAT
- Some patients will be prepared to take risks, given that they experience an improvement in QOL when ESA therapy is initiated at Hb levels above 10 g/dL (→ individualized decision)
# TREAT Study – Malignancy

<table>
<thead>
<tr>
<th></th>
<th>Darbepoetin</th>
<th>Placebo</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer-related AE</td>
<td>139/2012 6.9%</td>
<td>130/2026 6.4%</td>
<td>0.53</td>
</tr>
<tr>
<td>Deaths attributed to cancer</td>
<td>39/2012 1.9%</td>
<td>25/2026 1.2%</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Subgroup: Baseline ✓ History of malignancy (n = 348)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All cause mortality</td>
<td>60/188 31.9%</td>
<td>37/160 23.1%</td>
<td>0.13</td>
</tr>
<tr>
<td>Deaths attributed to cancer</td>
<td>14/188 7.4%</td>
<td>1/160 0.6%</td>
<td>0.002</td>
</tr>
</tbody>
</table>

3.5 In general, we suggest that ESAs **not** be used to maintain Hb above 11.5 g/dL. 
*Grade 2C*

3.6 In all patients, we recommend that ESAs **not** be used to intentionally increase the Hb above 13 g/dL. 
*Grade 1A*

**Hb not above 11,5 g/dL; never above 13,0 g/dL!**

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Treatment with ESA

Rationale

- Lack of evidence for favorable risk benefit relationship when Hb is maintained above 11.5 g/dL
- Some patients will be prepared to take risks, given that they experience an improvement in QOL when Hb levels are maintained above 11.5 g/dL (→ individualized decision)
- Combined results of large RCTs indicate consistently that risks outweigh benefits if Hb is targeted above 13 g/dL

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We know we must individualize

One size does not fit all!!!!!

Practice Guidelines with patient specific recommendations needed
Treatment with ESA

3.8.1 We recommend determining the initial ESA dose by the patient's Hb, body weight and clinical circumstances (Grade 1D)

3.8.2 We recommend that ESA dose adjustments be made based on the patient's Hb, rate of change in Hb concentration, ESA dose and clinical circumstances (Grade 1B)

3.8.3 We suggest decreasing ESA dose in preference to withholding if a downward adjustment of Hb is needed (Grade 2C)

3.8.4 Re-evaluate ESA dose if the patient suffers an ESA-related adverse event, or the patient has an acute or progressive illness that may cause loss of ESA response (Not graded)

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Treatment with ESA

3.13.1 Classify patients as having ESA hyporesponsiveness if they have no increase in Hb from baseline after the first month of ESA treatment on appropriate weight-based dosage. *(Not Graded)*

3.13.2 In patients with ESA hyporesponsiveness, we suggest avoiding repeated escalations in ESA dose beyond double the initial weight-based dose. *(Grade 2D)*

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3.14.1 Classify patients as having loss of ESA response if after treatment with stable doses of ESA, they require 2 increases in ESA doses up to 50% beyond the dose at which they had been stable in an effort to maintain a stable Hb.

(Not Graded)

3.14.2 In patients with loss of ESA responsiveness, we suggest avoiding repeated escalations beyond double the dose at which they had been stable.

(Grade 2D)

Loss of ESA responsiveness:
2x dose increase to more than 50% of dose with stable Hb

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Rationale

- Relative resistance to ESAs is common
- Hyporesponsiveness is a powerful predictor of CV risk and mortality
- Hyporesponsive patients usually receive higher doses of ESAs
- Although the poor prognosis of hyporesponsive patients may be related to their co-morbidity, a toxic effect of high ESA doses cannot be excluded
- Given the uncertainty about toxic effects of ESAs and limited efficacy, it makes sense to limit / avoid dose escalation

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Treatment with ESA

3.16.1 We recommend not using androgens as an adjuvant to ESA treatment (Grade 1B)

3.16.2 We suggest not using adjuvants to ESA treatment including vitamin C, vitamin D, vitamin E, folic acid, L-carnitine, and pentoxifylline (Grade 2D)
Safety of anaemia treatment in 2012

- **Studies of higher Hb targets (NHCT, CCT, CREATE, CHOIR, TREAT)**
  - Increased cardiovascular risk
  - Increased thrombotic risk
  - Possible effect on cancer
  - Possible increased death risk
Normal versus low haematocrit
Probability of death or first non-fatal myocardial infarction

CHOIR: Increased CV risk with Hb correction to 13.5 g/dl in non-dialysis CKD patients

1,432 non-dialysis CKD patients from 130 US centres; comparing impact of Hb correction to 13.5 g/dL with correction to 11.3 g/dL on composite endpoint events (mortality, stroke, heart attack, hospitalisation) in patients on epoetin alfa therapy

Potential mechanisms of increased cardiovascular risk with targeting of higher Hb levels with ESAs

- Increased blood pressure
- Increased blood viscosity
- Direct effects of erythropoiesis stimulating agents
- Post-dialysis hemoconcentration in hemodialysis patients with large weight gains
- Oxidative stress from intravenous iron

Fishbane S, Nissenson A.R. Kidney Int. 2007; 49: 806-813
Activation of EPO Receptor
Non-erythropoietic actions of EPO that can potentially contribute to the development of hypertension, diabetic proliferative retinopathy, vascular remodeling, a-v fistula thrombosis, tumor growth and progression to ESRD

## Composite and Component End Points - TREAT study

<table>
<thead>
<tr>
<th>End points</th>
<th>Darbepoetin alfa (n = 2,012)</th>
<th>Placebo (n = 2,026)</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary composite cardiovascular endpoint</td>
<td>632 (31.4)</td>
<td>602 (29.7)</td>
<td>1.05 (0.94–1.17)</td>
<td>0.41</td>
</tr>
<tr>
<td>Death from any cause</td>
<td>412 (20.5)</td>
<td>395 (19.5)</td>
<td>1.05 (0.92–1.21)</td>
<td>0.48</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>124 (6.2)</td>
<td>129 (6.4)</td>
<td>0.96 (0.75–1.22)</td>
<td>0.73</td>
</tr>
<tr>
<td>Stroke</td>
<td>101 (5)</td>
<td>53 (2.8)</td>
<td>1.92 (1.38–2.68)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Heart failure</td>
<td>205 (10.2)</td>
<td>229 (11.3)</td>
<td>0.89 (0.74–1.08)</td>
<td>0.24</td>
</tr>
<tr>
<td>Myocardial ischemia</td>
<td>41 (2)</td>
<td>49 (2.4)</td>
<td>0.84 (0.55–1.27)</td>
<td>0.40</td>
</tr>
<tr>
<td>Primary composite renal endpoint (ESRD or death)</td>
<td>652 (32.4)</td>
<td>618 (30.5)</td>
<td>1.06 (0.95–1.19)</td>
<td>0.29</td>
</tr>
<tr>
<td>ESRD</td>
<td>338 (16.8)</td>
<td>330 (16.3)</td>
<td>1.02 (0.87–1.18)</td>
<td>0.83</td>
</tr>
</tbody>
</table>

### Additional adjudicated endpoints

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>Darbepoetin alfa (n = 2,012)</th>
<th>Placebo (n = 2,026)</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death from cardiovascular causes</td>
<td>259 (12.9)</td>
<td>250 (12.3)</td>
<td>1.05 (0.88–1.25)</td>
<td>0.61</td>
</tr>
<tr>
<td>Cardiac revascularization</td>
<td>84 (4.2)</td>
<td>117 (5.8)</td>
<td>0.71 (0.54–0.94)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

ESA hyporesponsiveness associated with poor survival

FDA analysis
Dose and responsiveness are inversely rated

ESA hyporesponsiveness associated with poor survival

FDA analysis
Dose and responsiveness are inversely rated

Higher ESA dose
Less ESA-responsive
Poor survival

Erythopoietic response and outcomes in kidney disease and type 2 diabetes – secondary analysis of TREAT study

Table 2. Rate of End Points and Adjusted Hazard Ratios. *

<table>
<thead>
<tr>
<th></th>
<th>Placebo (N = 1889)</th>
<th>Poor Response (N = 471)</th>
<th>Better Response (N = 1401)</th>
<th>Adjusted Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular composite</td>
<td>12.3 (11.3–13.4)</td>
<td>16.3 (14.0–18.9)</td>
<td>12.4 (11.2–13.6)</td>
<td>1.31 (1.09–1.59)</td>
</tr>
<tr>
<td>Death from any cause</td>
<td>7.5 (6.8–8.3)</td>
<td>9.9 (8.3–11.9)</td>
<td>7.5 (6.7–8.5)</td>
<td>1.41 (1.12–1.78)</td>
</tr>
<tr>
<td>Stroke</td>
<td>1.0 (0.8–1.4)</td>
<td>2.3 (1.6–3.4)</td>
<td>2.0 (1.6–2.6)</td>
<td>1.26 (0.78–2.02)</td>
</tr>
</tbody>
</table>

† Hazard ratios are for patients with a poor initial response as compared with those with a better initial response. Hazard ratios have been adjusted for 12 baseline covariates, including age, sex, race, history of cardiovascular disease, urinary protein-to-creatinine ratio, baseline estimated glomerular filtration rate, baseline albumin level, history of cardiac arrhythmia, glycated hemoglobin level, baseline hemoglobin level, history of diabetic neuropathy, and baseline C-reactive protein level.

Erythropoietic response and outcomes in kidney disease and type 2 diabetes – secondary analysis of TREAT study

Death, myocardial infarction, stroke, heart failure, or hospitalization for myocardial infarction

Death from any cause

Fatal or nonfatal stroke

Greater ESA doses associated with higher mortality

All Cause Mortality Hazard Ratio

Cardiovascular Mortality Hazard Ratio

n=58,058 dialysis patients

Higher dosages of ESA – higher rate of all-cause mortality in haemodialysis patients

Zhang Yi et al. Kidney Int. 2011; 80: 663-669
ESA responsiveness and mortality in haemodialysis patients

<table>
<thead>
<tr>
<th>Major (Frequent)</th>
<th>Minor (Less Common)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency</td>
<td>Poor compliance, poor adherence to ESA therapy</td>
</tr>
<tr>
<td>Infection, inflammation, Underdialysis</td>
<td>Blood loss</td>
</tr>
<tr>
<td></td>
<td>Hyperparathyroidism</td>
</tr>
<tr>
<td></td>
<td>Aluminum toxicity (rare nowadays)</td>
</tr>
<tr>
<td></td>
<td>Vitamin $\text{B}_{12}$ or folate deficiency</td>
</tr>
<tr>
<td></td>
<td>Hemolysis</td>
</tr>
<tr>
<td></td>
<td>Primary bone marrow disorders</td>
</tr>
<tr>
<td></td>
<td>(e.g., myelodysplastic syndrome)</td>
</tr>
<tr>
<td></td>
<td>Hemoglobinopathies (e.g., sickle cell disease)</td>
</tr>
<tr>
<td></td>
<td>ACE inhibitors, angiotensin receptor blockers</td>
</tr>
<tr>
<td></td>
<td>Carnitine deficiency</td>
</tr>
<tr>
<td></td>
<td>Anti-EPO antibodies causing PRCA</td>
</tr>
</tbody>
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# Future Erythropoiesis – Stimulating Agents

## Table 2. Future Erythropoiesis-Stimulating Agents

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<td>Various</td>
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<td>Phase 2</td>
</tr>
</tbody>
</table>

Abbreviations: *EPO*, erythropoietin; *GATA*-2, GATA-binding protein 2; *HIF*, hypoxia inducible factor.
Peptide Drug: Advantages Over Proteins

**PROTEINS**
- Require Cold Storage
- Expensive to Manufacture
- Immunogenic

**AFFYMAX PEPTIDES**
- Improved Stability
- Less Expensive to Manufacture
- Low Immunogenicity Potential
- Flexible Formulations and Delivery
- Equivalent Potency/Efficacy
- Improved PK/PD Profile
Background – Peginesatide (formerly known as Hematide™)

- Synthetic, PEGylated investigational, peptide-based erythropoiesis-stimulating agent (ESA)
- Binds to and stimulates the EPO receptor
- No structural homology with EPO
- Approved by the FDA for the treatment of anemia due to CKD in patients on dialysis (2012)
Dimeric EPO Receptor:
both EPO and Hematide activate signal transduction cascade

Erythropoiesis

Adapted from: Eur Arch Psychiatry Clin Neuro 2001 251: 179-184

Hematide signaling: Qing Fan et al., Exp. Hematol. 2006, 34, 1303
## Half-Life and EPOR Residence Time for Peginesatide and Other ESAs

In Vitro, Peginesatide Shows a Prolonged Residence Time (17X) on the Erythropoietin Receptor Compared With Other ESAs*

<table>
<thead>
<tr>
<th>ESA</th>
<th>Mean $t_{1/2}$ (hr)</th>
<th>EPOR Residence Time $t_{1/2}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epoetin</td>
<td>6.3</td>
<td>77</td>
</tr>
<tr>
<td>Darbepoetin</td>
<td>17.8</td>
<td>70</td>
</tr>
<tr>
<td>Peginesatide</td>
<td>47.9</td>
<td>1313</td>
</tr>
</tbody>
</table>

*The clinical significance of prolonged residence time is unknown.

### Table 3. Peginesatide Phase 3 Clinical Trials Overview

<table>
<thead>
<tr>
<th>Study</th>
<th>Description</th>
<th>Sample Size (region)</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEARL 1</td>
<td>Correction study: peginesatide vs darbepoetin alfa in nondialysis patients (SC)</td>
<td>~330 vs 165 (US)</td>
<td>Efficacy of peginesatide noninferior to darbepoetin; increased HR for composite safety end point at 1.32 for peginesatide vs darbepoetin alfa</td>
</tr>
<tr>
<td>PEARL 2</td>
<td>Correction study: peginesatide vs darbepoetin alfa in nondialysis patients (SC)</td>
<td>~330 vs 165 (US and Europe)</td>
<td></td>
</tr>
<tr>
<td>EMERALD 1</td>
<td>Maintenance study: peginesatide vs epoetin alfa in dialysis patients (IV)</td>
<td>~540 vs 270 (US)</td>
<td>Efficacy and safety of peginesatide noninferior to epoetin</td>
</tr>
<tr>
<td>EMERALD 2</td>
<td>Maintenance study: peginesatide vs epoetin alfa or beta in dialysis patients (IV/SC)</td>
<td>~540 vs 270 (US and Europe)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: EMERALD, Hematide Injection for Anemia in Chronic Hemodialysis Patients; HR, hazard ratio; IV, intravenous; PEARL, Safety and Efficacy of Hematide for the Correction of Anemia in Patients With Chronic Renal Failure; SC, subcutaneous; US, United States.
Mean Hb Profiles Over Time: Dialysis Population

**EMERALD 1**

- **Peginesatide** N = 524, 518, 507, 492, 480, 467, 459, 447, 440, 436, 422, 410, 400, 393, 390, 384, 362, 311, 251, 198, 149, 114, 83, 69, 44, 32, 16

**Evaluation Period** Median Dose
- **Peginesatide**: 5.7 mg/injection
- **Epoetin**: 9900 U/week

**EMERALD 2**

- **Peginesatide** N = 542, 533, 523, 516, 509, 502, 493, 485, 482, 476, 467, 460, 456, 451, 443, 391, 303, 231, 185, 134, 102, 75, 69, 47, 40, 21
- **Epoetin** N = 269, 267, 256, 250, 248, 246, 246, 239, 233, 226, 227, 223, 218, 211, 210, 197, 156, 115, 95, 70, 55, 42, 30, 29, 24, 13

**Evaluation Period** Median Dose
- **Peginesatide**: 4.8 mg/injection
- **Epoetin**: 6805 U/week
Primary CSE Analysis: Dialysis and Nondialysis Combined

![Graph showing comparison between Peginesatide and Comparator ESA](image)

**HR (95% CI):** 1.06 (0.89, 1.26)

<table>
<thead>
<tr>
<th></th>
<th>Peginesatide (N=1722)</th>
<th>Comparator ESA [Epoetin/Darbepoetin] (N=869)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with CSE events</td>
<td>384 (22%)</td>
<td>188 (22%)</td>
</tr>
</tbody>
</table>
Prespecified CSE Analysis: Dialysis Population

HR (95% CI): 0.95 (0.77, 1.17)

<table>
<thead>
<tr>
<th></th>
<th>Peginesatide (N=1066)</th>
<th>Epoetin (N=542)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with CSE events</td>
<td>243 (23%)</td>
<td>132 (24%)</td>
</tr>
</tbody>
</table>

Note: In the nondialysis population, the HR (95% CI) was: 1.32 (0.97, 1.81).
Prespecified CSE Analysis: Non-Dialysis Population

HR (95% CI): 1.32 (0.97, 1.81)

<table>
<thead>
<tr>
<th>Patients with CSE events</th>
<th>Peginesatide (N=656)</th>
<th>Darbepoetin (N=327)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>141 (22%)</td>
<td>56 (17%)</td>
</tr>
</tbody>
</table>

Patients Without Events (%)

Days to First CSE Event
## Future Erythropoiesis – Stimulating Agents

**Table 2. Future Erythropoiesis-Stimulating Agents**

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<td>Phase 2</td>
</tr>
</tbody>
</table>

Abbreviations: *EPO*, erythropoietin; GATA-2, GATA-binding protein 2; HIF, hypoxia inducible factor.
Regulation of hypoxia inducible factor (HIF) activity

(i) Normal conditions (normoxia) -- HIF is degraded

(ii) Hypoxic conditions / inhibition of prolyl hydroxylase -- HIF is stabilized

Regulation of EPO (erythropoietin) gene expression

Figure 2. Regulation of EPO (erythropoietin) gene expression, showing transcriptional factors that suppress the EPO promoter or activate the EPO enhancer. Abbreviations: -ve, negative; +ve, positive; HIF, hypoxia inducible factor; NF-κB, nuclear factor κB.
FG-2216 Dose-Related Induction of Endogenous Serum EPO

No Desensitization in EPO Response Following repeated Dosing with FG-2216
Phase 2a (rHuEPO-naïve): Individual Hemoglobin Response to FG-2216 vs. Placebo – Change from Baseline

6 mg/kg group (first cohort, rHuEPO-naïve, predialysis patients)

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mean Baseline Hb (g/dL)</th>
<th>Mean change from Baseline Hb (g/dL) Day 42* (or last value carried forward)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FG-2216 (n=5)</td>
<td>9.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Placebo (n=3)</td>
<td>9.8</td>
<td>-0.35</td>
</tr>
</tbody>
</table>

*Difference between treatment and placebo group is statistically significant (Mann–Whitney rank sum test), p = 0.036

Wiecek A. et al. XLII ERA-EDTA Congress, Istanbul 2005
Phase 2a (rHuEPO-treated): Individual Hemoglobin Response to FG-2216 vs Placebo – Change from Baseline

6 mg/kg group (first cohort, rHuEPO-treated, predialysis patients)

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mean Baseline Hb (g/dL)</th>
<th>Mean change from Baseline Hb (g/dL) Day 42* (or last value carried forward)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FG-2216 (n=6)</td>
<td>11.7</td>
<td>-0.9</td>
</tr>
<tr>
<td>Placebo (n=3)</td>
<td>11.5</td>
<td>-1.5</td>
</tr>
</tbody>
</table>

Wiecek A. et al. XLII ERA-EDTA Congress, Istanbul 2005
FG-2216 increases plasma-EPO levels in healthy controls and in HD patients with and without remaining renal tissue

24-hour kinetics of plasma EPO levels after a single dose of FG-2216

FG-4592 Oral Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitor Corrects Anemia in Nondialysis CKD Patients without IV Iron

Anatole Besarab, Robert Provenzano, Steven Fishbane, Chao H. Sun, Diogo S. Belo, Thomas B. Neff, Tyson T. Lee, Marietta Franco, Robert Leong, Kin-Hung Peony Yu. Henry Ford Hosp., Detroit, MI; St. Clair Specialty Physicians, Detroit, MI; Winthrop Dialysis Ctr., Mineola, NY; California Inst. of Renal Research, Chula Vista, CA; FibroGen, Inc., San Francisco, CA; Apex Research, Riverside, CA

Background: We report data from an ongoing phase 2b, open-label study of FG-4592 to correct anemia in adult nondialysis CKD patients

Methods: 96 patients (Hb ≤10.5 g/dL at baseline) who had not received ESAs for the prior 12 weeks were randomized to 4 equal cohorts. Cohorts A and B: 16 weeks initial tiered, weight-adjusted doses of 60-140 mg FG-4592 thrice weekly; Cohorts C and D: 24 weeks initial fixed doses of 50 and 100 mg FG-4592 thrice weekly, respectively. Cohort-dependent dose adjustment was allowed every 4 weeks. IV iron was not permitted
ASN - Kidney Week 2011 – PO364

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<table>
<thead>
<tr>
<th>Cohort</th>
<th>Starting dose (mg)</th>
<th>n</th>
<th>4</th>
<th>8</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3x weekly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>60-140</td>
<td>24</td>
<td>1.6 ± 1.1</td>
<td>2.4 ± 1.2</td>
<td>2.3 ± 1.2</td>
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<tr>
<td>B</td>
<td>60-140</td>
<td>24</td>
<td>1.1 ± 0.9</td>
<td>1.8 ± 1.0</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>23</td>
<td>0.6 ± 0.7</td>
<td>1.0 ± 0.9</td>
<td>NA</td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td>24</td>
<td>1.5 ± 1.0</td>
<td>2.1 ± 1.1</td>
<td>NA</td>
</tr>
</tbody>
</table>

Conclusions: Treatment with initial weight-adjusted or fixed oral FG-4592 doses corrected anemia in CKD patients in the absence of IV iron repletion
Medical need for HIF-stabilizers - *pro*

- **HIF inhibitors are orally - active** (could provide in the future a more convenient and cost-effective therapy for anemia of chronic kidney disease than protein-based therapeutics)
- **They may stimulate erythropoiesis with much lower EPO levels** (given some concerns about clinical outcomes arising from use of ESAs that cause supraphysiologic circulating EPO levels, use of agents producing transient, more physiologic increases in endogenous EPO may have significant benefit in the treatment anemia in patients with CKD)
Medical need for HIF-stabilizers - *contra*

- **Potential and unpredictable adverse effects** (rare development of severe liver toxicity)
- **Several other (hundreds?) genes are also activated** (e.g. VEGF)
- **Larger and long-term clinical trials are needed** (in order to document the safety profile and final clinical benefits of these new compounds)
# Future Erythropoiesis – Stimulating Agents

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Abbreviations: EPO, erythropoietin; GATA-2, GATA-binding protein 2; HIF, hypoxia inducible factor.

Renal anemia of inflammation: Role of hepcidin

- Hypoferremia
- Parenteral iron treatment
- ↓ Duodenal iron absorption
- ↓ Iron release from macrophages
- ↑ Hepcidin production
- ESA treatment
- ↓ Inflammation
- Relative EPO deficiency and reduced GFR
- Anemia/hypoxia
- Chronic kidney disease

Background – Hepcidin in Anemia of Inflammation

- Hepcidin is the master regulator of iron homeostasis via its effect on ferroportin, the only known iron export protein.
- Cytokine-induced synthesis of hepcidin plays a crucial role in macrophage iron retention, which underlies the anemia of inflammation by limiting the availability of iron for erythroid progenitor cells, “functional iron deficiency.”
- Patients with anemia of inflammation display an impaired response to erythropoietin (EPO).

Hepcidin – a potential target for future anaemia therapies?

Antihepcidin antibody treatment modulates iron metabolism and is effective in a mouse model of inflammation-induced anemia

Barbra J. Sasu,1 Keegan S. Cooke,1 Tara L. Arvedson,1 Cherylene Plewa,2 Aaron R. Ellison,2 Jackie Sheng,2 Aaron Winters,2 Todd Juan,2 Hongyan Li,3 C. Glenn Begley,1 and Graham Molineux1

Departments of 1Hematology/Oncology, 2Protein Sciences, and 3Pharmacokinetics and Drug Metabolism, Amgen Inc, Thousand Oaks, CA

Iron maldistribution has been implicated in multiple diseases, including the anemia of inflammation (AI), atherosclerosis, diabetes, and neurodegenerative disorders. Iron metabolism is controlled by hepcidin, a 25-amino acid peptide. Hepcidin is induced by inflammation, causes iron to be sequestered, and thus, potentially contributes to AI. Human hepcidin (hHepc) overexpression in mice caused an iron-deficient phenotype, including stunted growth, hair loss, and iron-deficient erythropoiesis. It also caused resistance to supraphysiologic levels of erythropoiesis-stimulating agent, supporting the hypothesis that hepcidin may influence response to treatment in AI. To explore the role of hepcidin in inflammatory anemia, a mouse AI model was developed with heat-killed Brucella abortus treatment. Suppression of hepcidin mRNA was a successful anemia treatment in this model. High-affinity antibodies specific for hHepc were generated, and hHepc knock-in mice were produced to enable antibody testing. Antibody treatment neutralized hHepc in vitro and in vivo and facilitated anemia treatment in hHepc knock-in mice with AI. These data indicate that antihepcidin antibodies may be an effective treatment for patients with inflammatory anemia. The ability to manipulate iron metabolism in vivo may also allow investigation of the role of iron in a number of other pathologic conditions. (Blood. 2010;115(17):3616-3624)
MAb against hepcidin was effective in combination therapy for anaemia of inflammation.

Ab 2.7 restored response to ESA treatment in hHepc knock in AI mice.

# Hepcidin Inhibitor NOX-H94

<table>
<thead>
<tr>
<th>Target</th>
<th>Hepcidin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
<td>44-nucleotide L-RNA oligonucleotide / 40 KDa PEG</td>
</tr>
<tr>
<td><strong>$K_D$ (plate-based competition assay)</strong></td>
<td>3.9 nM</td>
</tr>
<tr>
<td>Stage of Development</td>
<td>Pre-clinical</td>
</tr>
<tr>
<td>Administration</td>
<td>i.v. and s.c.</td>
</tr>
<tr>
<td>Pharmacokinetics</td>
<td>Half life similar to other Spiegelmers in development</td>
</tr>
<tr>
<td>Pharmacodynamics</td>
<td>Inhibition of IL-6 induced anemia in monkeys</td>
</tr>
<tr>
<td>Target Indications</td>
<td>Anemia of inflammation</td>
</tr>
<tr>
<td>Licensing status</td>
<td>Un-partnered</td>
</tr>
</tbody>
</table>
Daily s.c. human IL-6 injections induce anemia through *endogenous* hepcidin; cynomolgus monkey model:

**Effect of NOX-H94 on Hemoglobin in IL-6 Induced Anemia**

Data:
- IL-6 every other day, 0.3 µg/kg
- mean+SEM, n=4
- baseline corrected for the individual animals
## Future Erythropoiesis – Stimulating Agents

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Abbreviations: EPO, erythropoietin; GATA-2, GATA-binding protein 2; HIF, hypoxia inducible factor.

Regulation of EPO (erythropoietin) gene expression

**Figure 2.** Regulation of EPO (erythropoietin) gene expression, showing transcriptional factors that suppress the EPO promoter or activate the EPO enhancer. Abbreviations: -ve, negative; +ve, positive; HIF, hypoxia inducible factor; NF-κB, nuclear factor κB.

# Future Erythropoiesis – Stimulating Agents

<table>
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<tr>
<th>Agent</th>
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<th>Manufacturing Process</th>
<th>Stage of Development</th>
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Biopumps for gene therapy

- a) Harvest microorgans
- b) Transfer to processing station
- c) Vector carrying gene for desired protein P
- d) Process each microorgan into Biopump
- e) Biopump producing protein P

- f) Cryo storage
- g) Measure daily protein P production per Biopump dosing
- h) Wash for several days to remove vector
- i) Reimplant Biopumps SC per dosing
- j) Steady sustained delivery of fresh protein P
Sustained Erythropoiesis (6-30 Months) by the EPODURE Biopump in Patients with CKD: Further Results of Phase I/II Proof of Concept Trial

Anatole Besarab, Michal Elhalel, Doron Schwartz, Ehud Shoshani, Andrew L. Pearlman, Baruch Stern, Philip Ng, Allen R. Nissenson. Henry Ford Hospital, Detroit, MI; Hadassah Hospital, Jerusalem, Israel; Sourasky Medical Center, Tel Aviv, Israel; Medgenics, Inc, Misgav, Israel; Baylor College of Medicine, Houston, TX; DaVita, El Segundo, CA

**Background:** Sustained delivery of EPO maintaining levels 1-5 fold of normal could reduce risks of Hb variability yet achieve recommended Hb targets, avoid supra-physiologic EPO concentrations, and increase patient compliance. The goal of EPODURE is to provide >6 months of sustained EPO delivery from a single treatment using autologous 30 mm x 2 mm dermis core biopsies excised from the patient's skin under local anesthetic and converted in days into “biopump” EPO production units by introducing the EPO gene into cells of the intact explant

**Methods:** 16 CKD patients (8 EPO-naïve, 8 EPO-dependent) treated 6-30 months by 20, 40, or 60 IU/kg/day EPODURE implanted dose in an open label, dose ranging Phase I-II study
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Results: No serious AEs were reported, EPO serum levels never exceeded 70 mU/ml, and tests for anti-EPO antibodies were negative. A single EPODURE administration elevated Hb levels for >3 months in 14/16 and >6 months in 10/16, maintained Hb between 10-12 g/dl in 14/16 for >3 months and 9/16 for >6 months, with longest >30 months. Where Hb declined it correlated with decreasing EPO levels, which peaked at 3 days post implantation. We suspect possible decline in EPO output in some biopumps, possibly due to suboptimal implantation.

Conclusions: EPODURE is safe at doses up to 65 IU/kg/day, a single administration in most patients can elevate Hb levels for 3-30 months and in appropriate dose maintain Hb in 10-12 g/dl range for up to 30 months
Summary of the lecture

- New (neglected) factors in the pathogenesis of anaemia in CKD male patients (low serum testosterone concentration)
- KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease *(Kidney Int., 2012)*
- Hyporesponsiveness to ESAs is a powerful predictor of CV risk and mortality in CKD patients
- New ESAs are close to the market (in USA peginesatide is approved by the FDA in HD patients, other are during the clinical trials)
- Biopomp for gene therapy – a novel concept of the anaemia management in patients with CKD
Thank you for your attention!

Andrzej Wiecek
Katowice