Treatment of antibody-mediated rejection with eculizumab

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Diagnostic criteria for AMR

**C4d-Positive ABMR**

- Serologic Evidence
  - DSA present

- Immunopathologic Evidence
  - IF: Diffuse-positive C4d in PTC
  - IHC: Diffuse- or focal-positive C4d in PTC

- Histopathologic Evidence ACUTE
  - ATN like changes, and/or
  - Peritubular capillaritis, and/or
  - Glomerulitis, and/or
  - Arterial fibroid necrosis, and
  - No evidence for chronic capillary injury (reduction and/or multilayering of glomerular and peritubular capillary basement membranes)

- Histopathologic Evidence CHRONIC
  - Transplant glomerulopathy, and/or
  - PTC basement membrane multilamination, and/or
  - IFTA, and/or
  - Fibrous intimal thickening of arteries
  - May be accompanied by glomerulitis and/or capillaritis

**C4d-Negative ABMR**

- Serologic Evidence
  - DSA present

- Immunopathologic Evidence
  - Negative C4d staining; and
  - Endothelial activation, detected by increased mRNA expression of endothelial genes, such as W/F, PECAM, SELE, etc; and/or
  - Evidence for glomerular and/or capillary endothelial cycling (CD31+Kl67+ cells lining the microcirculation)

- Histopathologic Evidence ACUTE
  - Peritubular capillaritis, and/or
  - Glomerulitis, and/or
  - Thrombotic microangiopathy, and/or
  - Arterial fibroid necrosis, and
  - No evidence for chronic capillary injury (reduction and/or multilayering of glomerular and peritubular capillary basement)

- Histopathologic Evidence CHRONIC
  - Transplant glomerulopathy, and/or
  - PTC basement membrane multilamination, and/or
  - Fibrous intimal thickening of arteries
  - May be accompanied by glomerulitis and/or capillaritis

Djamali et al, AJT 2014
Antibody-mediated allograft damage

Functional course 1
- Clinical acute AMR

Functional course 2
- Subclinical or late acute AMR

Chronic AMR
- Endothelial-associated transcripts
- Electron microscopy
- Fluctuating C4d status
- Persisting Microvascular Inflammation

Preformed
- DSA

Persisting – De novo
- Endothelial injury

Kidney Transplant

Complement cascade

- Classical pathway
  - C1q
  - C1r, C1s, C4, and C2
  - C4b2a (C3 convertase)

- Lectin pathway
  - MBL
  - MASP, C4, and C2
  - C4b2a (C3 convertase)

- Alternative pathway
  - C3(H2O) or C3b
  - FD, FB C3
  - C3bBb (C3 convertase)

- Amplification step
  - C3a
  - Opsonin
  - iC3b
  - C3dg

- C5a
  - C5b
  - C5b-9
  - Protectin CD59

- Vasodilation
  - Smooth muscle cell contraction
  - Chemoattractant for basophils, eosinophils
  - Mast cell activation
  - Phagocyte activation
  - Release of granule-based enzymes
  - Generation of oxygen radicals
  - T-cell activation and survival

Vieyra&Heeger, KI 2010
Complement can induce T-cell priming

C3aR and C5aR signalling skews the differentiation of naive CD4+ T cells towards TH1

→ Increases alloAg presentation

→ Increases expression of co-stimulatory molecules (CD80 & CD40)

Vieyra&Heeger, KI 2010
DSA can induce damage without complement activation

- Intermittent or low level DSA might lead to TG in absence of C4d deposition and low inflammation

- DSAs might cause damage independent of complement via direct activation of ECs promoting TG

Stegall M et al Nat Rev 2012
Signaling pathways regulating antibody-mediated activation of endothelial cells
DSA can induce damage without complement activation

Graft survival in RTRs tested for anti-HLA immunoglobulin subclasses (n=274; 2008)

A pathogenic view of AMR

• H-AMR:
  • Preformed antibodies against donor-antigens CDC+

• A-AMR:
  • Previous antigen exposure
  • Pre-transplant DSA or memory B-cell
  • Severe complement activation

• SC/C-AMR
  • DSA persistence of de novo
  • Low-intensity complement activation
  • Complement-independent DSA pathogenic mechanisms
Eculizumab in AMR

- a-AMR prophylaxis
- a-AMR treatment
- sc-AMR and c-AMR
Eculizumab (Soliris®)

Eculizumab está diseñado para reducir la inmunogenicidad y eliminar las funciones efectoras:

- Adición de regiones determinantes complementarias murinas entre las secuencias estructurales de las cadenas pesadas y ligeras humanas.
- Combinación de secuencias humanas de cadenas pesadas IgG2 e IgG4 para formar una región constante híbrida incapaz de unirse a receptores Fc o de activar la cascada del complemento.

Eculizumab tiene así una alta afinidad para C5 humano, bloqueando eficazmente su activación, la secuencia proinflamatoria y sus propiedades citolíticas.

C1q deficiency does not delay allograft rejection

C1q deficiency exacerbates pathology of rejection

Csencsits et al, AJT 2008
C1q deficiency accelerates production of donor-reactive IgG

Classical complement pathway is needed to graft acceptance

Csencsits et al, AJT 2008
C5 Blockade with Conventional Immunosuppression Induces Long-Term Graft Survival in Presensitized Recipients

Rother et al, AJT 2008
C3b formation may amplify B-cell activation

Vieyra & Heeger, KI 2010
Peripheral T cells are depleted by rATG in the presence of Eculizumab

Month 1 after TX

Month 3 after TX

Goh B K et al Tranplant Immunol 2012
Prevention of ABMR in Sensitized patients (POS BFXM) pre-TX >200 <450

(Comparison with an historical cohort group not treated with eculizumab)
Main Clinical Outcome

C5 inhibition prevented ABMR despite high levels of DSAs and C4d deposition in the allograft

2 patients with ABMR (day 7) despite eculizumab → C5-independent mechanism ??
Positive Crossmatch Kidney Transplant Recipients Treated With Eculizumab: Outcomes Beyond 1 Year

L. D. Cornell¹, C. A. Schinstock², M. J. Gandhi³, W. K. Kremers² and M. D. Stegall²,*

American Journal of Transplantation 2015; XX: 1–10
Wiley Periodicals Inc.

![Graph showing death-censored graft survival over years post-transplant for Control and EC groups. At risk numbers for each group are provided for 0 to 5 years post-transplant.](image)

Control | EC
---|---
0 | 0
1 | 1
2 | 2
3 | 3
4 | 4
5 | 5

P=0.26
Subclinical ABMR on protocol biopsies

A

Subclinical ABMR in Controls vs. Eculizumab

<table>
<thead>
<tr>
<th>Subclinical ABMR in Controls vs. Eculizumab</th>
<th>3-4 months</th>
<th>1 year</th>
<th>2 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>All EC</td>
<td>35.7% (10/28)</td>
<td>36.7% (11/30)</td>
<td>27.3% (6/22)</td>
</tr>
<tr>
<td>Control</td>
<td>46.2% (18/39)</td>
<td>36.8% (14/38)</td>
<td>15.1% (5/33)</td>
</tr>
<tr>
<td>p-value (EC vs. control)</td>
<td>P=0.46</td>
<td>P=1.0</td>
<td>P=0.32</td>
</tr>
</tbody>
</table>

B

Subclinical ABMR over time in Eculizumab group

<table>
<thead>
<tr>
<th>Subclinical ABMR over time in Eculizumab group</th>
<th>1 month</th>
<th>3-4 months</th>
<th>6 months</th>
<th>1 year</th>
<th>2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>All EC</td>
<td>21.4% (6/28)</td>
<td>35.7% (10/28)</td>
<td>48.3% (14/29)</td>
<td>36.7% (11/30)</td>
<td>27.3% (6/22)</td>
</tr>
<tr>
<td>Continued EC</td>
<td>21.4% (6/28)</td>
<td>66.7% (6/9)</td>
<td>66.7% (6/9)</td>
<td>25.0% (2/8)</td>
<td>NA</td>
</tr>
<tr>
<td>Discontinued EC</td>
<td>NA</td>
<td>21.1% (4/19)</td>
<td>40.0% (8/20)</td>
<td>40.9% (9/22)</td>
<td>NA</td>
</tr>
<tr>
<td>p-value</td>
<td>NA</td>
<td>P=0.03</td>
<td>P=0.26</td>
<td>P=0.67</td>
<td>NA</td>
</tr>
</tbody>
</table>

Cornell LD, et al AJT 215
Moderate-to-severe peritubular capillaritis on protocol biopsies

**A**

<table>
<thead>
<tr>
<th></th>
<th>3 months</th>
<th>1 year</th>
<th>2 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>All EC</td>
<td>25.0% (7/28)</td>
<td>60.0% (18/30)</td>
<td>45.4% (10/22)</td>
</tr>
<tr>
<td>Control</td>
<td>34.1% (14/41)</td>
<td>60.0% (21/35)</td>
<td>60.0% (15/25)</td>
</tr>
</tbody>
</table>

**B**

<table>
<thead>
<tr>
<th></th>
<th>1 month</th>
<th>3-4 months</th>
<th>6 months</th>
<th>1 year</th>
<th>2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>All EC</td>
<td>10% (3/30)</td>
<td>25.0% (7/28)</td>
<td>48.3% (14/29)</td>
<td>60% (18/30)</td>
<td>45.4% (10/22)</td>
</tr>
<tr>
<td>Continued EC</td>
<td>10% (3/30)</td>
<td>44.4% (4/9)</td>
<td>77.7% (7/9)</td>
<td>87.5% (7/8)</td>
<td>NA</td>
</tr>
<tr>
<td>Discontinued EC</td>
<td>NA</td>
<td>15.9% (3/19)</td>
<td>35.0% (7/20)</td>
<td>50.0% (11/22)</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Moderate-to-Severe Peritubular capillaritis in Controls vs. Eculizumab**

<table>
<thead>
<tr>
<th></th>
<th>3-4 months</th>
<th>1 year</th>
<th>2 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>All EC</td>
<td>P=0.59</td>
<td>P=1.00</td>
<td>P=0.39</td>
</tr>
<tr>
<td>Control</td>
<td>P=0.59</td>
<td>P=1.00</td>
<td>P=0.39</td>
</tr>
</tbody>
</table>

**Moderate-to-Severe Peritubular capillaritis over time in Eculizumab group**

<table>
<thead>
<tr>
<th></th>
<th>1 month</th>
<th>3-4 months</th>
<th>6 months</th>
<th>1 year</th>
<th>2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>All EC</td>
<td>P=0.17</td>
<td>P=0.05</td>
<td>P=0.10</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Continued EC</td>
<td>NA</td>
<td>P=0.17</td>
<td>P=0.05</td>
<td>P=0.10</td>
<td>NA</td>
</tr>
<tr>
<td>Discontinued EC</td>
<td>NA</td>
<td>P=0.17</td>
<td>P=0.05</td>
<td>P=0.10</td>
<td>NA</td>
</tr>
</tbody>
</table>

p-value (control vs. EC)
Transplant glomerulopathy (chronic ABMR) on protocol biopsies

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### Transplant Glomerulopathy in Controls vs. Eculizumab

<table>
<thead>
<tr>
<th></th>
<th>3-4 months</th>
<th>1 year</th>
<th>2 year</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All EC</strong></td>
<td>0% (0/28)</td>
<td>26.7% (8/30)</td>
<td>45.4% (10/22)</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>9.3% (4/43)</td>
<td>39.5% (15/38)</td>
<td>63.6% (21/33)</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>P=0.15</td>
<td>P=0.31</td>
<td>P=0.27</td>
</tr>
</tbody>
</table>

### Transplant glomerulopathy over time in Eculizumab group

<table>
<thead>
<tr>
<th></th>
<th>1 month</th>
<th>3-4 months</th>
<th>6 months</th>
<th>1 year</th>
<th>2 years</th>
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</thead>
<tbody>
<tr>
<td><strong>All EC</strong></td>
<td>0% (0/30)</td>
<td>0% (0/28)</td>
<td>10.3% (3/29)</td>
<td>26.7% (8/30)</td>
<td>45.4% (10/22)</td>
</tr>
<tr>
<td><strong>Continued EC</strong></td>
<td>0% (0/30)</td>
<td>0% (0/9)</td>
<td>22.2% (2/9)</td>
<td>50.0% (4/8)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Discontinued EC</strong></td>
<td>NA</td>
<td>0% (0/19)</td>
<td>5.0% (1/20)</td>
<td>18.1% (4/22)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>NA</td>
<td>P=1.0</td>
<td>P=0.22</td>
<td>P=0.16</td>
<td>NA</td>
</tr>
</tbody>
</table>

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On-going trials with Eculizumab for Prevention of ABMR in Sensitized Kidney Transplantant patients

1. Open-Label, Single-arm, Phase II Study for Prevention of AMR in Sensitized Recipients of Deceased Donor Kidney Transplant (NCT01567085)

   Objective ➔ Safety and potential efficacy of eculizumab to prevent ABMR in sensitized recipients

2. Open-Label, Randomized, Phase II Study to Prevent AMR in Living Donor Kidney Transplant Recipients Requiring Desensitization (NCT01399593)

   Objective ➔ Safety and potential efficacy of eculizumab to prevent ABMR in sensitized recipients

Eculizumab dosing:

- 1200mg prior to kidney allograft reperfusion (Day 0)
- 900mg on postoperative days 1, 7, 14, 21 and 28
- 1200mg on weeks 5, 7 and 9
Eculizumab in AMR

• a-AMR prophylaxis

• a-AMR treatment

• sc-AMR and c-AMR
The Bellvitge’s protocol for acute ABMR

Cruzado et al, AJT 2009
Locke et al, AJT 2008
<table>
<thead>
<tr>
<th>Authors</th>
<th>DSA Class</th>
<th>Desensitization</th>
<th>Rejection</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td>Locke et al, AJT 2009</td>
<td>Pre-TX I&amp;II</td>
<td>Desensitization</td>
<td>ABMR</td>
<td>Eculizumab + Rxmab</td>
</tr>
<tr>
<td>Lonze et al, AJT 2010</td>
<td>Pre-TX I&amp;II</td>
<td>Desensitization</td>
<td>ABMR</td>
<td>6-month Eculizumab</td>
</tr>
<tr>
<td>Biglarnia et al, Transpl Int 2011</td>
<td>ABOi &amp; Pre-TX I&amp;II</td>
<td>Desensitization</td>
<td>ABO Rejection</td>
<td>IA + 2 dose Eculizumab</td>
</tr>
<tr>
<td>Chandran et al, Transpl Proc 2011</td>
<td>Pre-TX DSA</td>
<td>Desensitization</td>
<td>ABMR / MAT</td>
<td>2 doses Eculizumab</td>
</tr>
<tr>
<td>Noone et al, AJT 2012</td>
<td>Pre-TX DSA and aHUS</td>
<td>Desensitization</td>
<td>ABMR / MAT</td>
<td>Eculizumab + Rxmab</td>
</tr>
<tr>
<td>Glez-Roncero et al, Transpl Proc 2012</td>
<td>Pre-TX DSA</td>
<td>no desensitization</td>
<td>ABMR</td>
<td>Eculizumab + Rxmab</td>
</tr>
<tr>
<td></td>
<td>Pre-TX no DSA</td>
<td>no desensitization</td>
<td>ABMR / MAT</td>
<td>Eculizumab + Rxmab</td>
</tr>
</tbody>
</table>

Excellent Short-term clinical follow-up
<table>
<thead>
<tr>
<th>Case</th>
<th>Yr</th>
<th>PreTx DSA</th>
<th>aAMR</th>
<th>Therapy</th>
<th>Re bx</th>
<th>Eculizumab</th>
<th>outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2010</td>
<td>DSA DR03 MFI&gt;10000 Negative CDC</td>
<td>11 d DSA</td>
<td>rATG PF IvIg</td>
<td>40 d aAMR persistence</td>
<td>600 mg Single dose</td>
<td>Recovery sCr 130 No uProt</td>
</tr>
<tr>
<td>2</td>
<td>2012</td>
<td>DSA DR07 MFI&gt;19000 Negative CDC</td>
<td>3d DSA</td>
<td>rATG PF IvIg</td>
<td>17 d aAMR persistence</td>
<td>600 mg + 600 mg after 3wks</td>
<td>Partial recovery eGFR 26 GS 16 m</td>
</tr>
<tr>
<td>3</td>
<td>2013</td>
<td>No DSA</td>
<td>17d DSA A23 MFI 4600 A24 MFI 2890 B44 MFI 2500</td>
<td>Eculizumab 900 mg Single dose</td>
<td>6 m protocol DSA A23 MFI 6500 A24 MFI 4283 scAMR Cd4 neg</td>
<td>-</td>
<td>Recovery sCr 120 No uProt</td>
</tr>
</tbody>
</table>
Report of the Inefficacy of Eculizumab in Two Cases of Severe Antibody-Mediated Rejection of Renal Grafts

Maren Burbach,\textsuperscript{1} Caroline Suberbielle,\textsuperscript{2} Isabelle Brochériou,\textsuperscript{3,4} Christophe Ridel,\textsuperscript{1} Laurent Mesnard,\textsuperscript{1,4} Karine Dahan,\textsuperscript{5} Eric Rondeau,\textsuperscript{1,4} and Alexandre Hertig\textsuperscript{1,4,6}

\textbf{Background.} Acute antibody-mediated rejection (AMR) is responsible for up to 20\% to 30\% of acute rejection after kidney transplantation. New therapeutic agents have recently emerged, such as eculizumab, an anticomplement protein-C5 monoclonal antibody. In the setting of renal transplantation, eculizumab has so far proved effective both for preventive and curative treatments of AMR in sensitized patients and patients diagnosed with severe AMR. Unsuccessful eculizumab treatment has only been reported once in the literature by Stegall et al. (Am J Transplant 2011; 11: 2405).

\textbf{Methods and Results.} We present two cases of AMR resistant to eculizumab after renal transplantation. One patient received the anti-C5 antibody curatively, and the other patient developed AMR while being treated with eculizumab after a relapse of atypical hemolytic uremic syndrome. The peculiarity of these two cases was the absence of C4d deposition in peritubular capillaries as well as the absence of C1q-binding donor-specific anti–human leukocyte antigen alloantibody, as determined retrospectively, suggesting that a complement-independent mechanism underlies the pathogenesis of these AMR.

\textbf{Conclusion.} The use of eculizumab in C4d-negative or C1q-negative AMR does not seem effective.

\textbf{Keywords:} Antibody-mediated rejection, Eculizumab, Complement, Kidney transplantation.

\textit{(Transplantation 2014;00: 00–00)}
Conclusions

• Eculizumab is useful to prevent aAMR in sensitized patients but seems to be insufficient to prevent cAMR

• Eculizumab is effective for aAMR treatment (first line and rescue therapy)