AMR Therapies: Update 2010- Present
Conflicts of Interest

• Grants
  – Amgen
  – Bristol Myers Squibb
  – Genentech
  – Glaxo Smith Kline
  – Novartis
  – Sanofi
AMR Therapies

• Extracellular protein targets
  – Complement inhibitors
    • Distal
    • Proximal
  – Immunoglobulin
    • Enzymatic cleavage
    • Physical removal

• Plasma Cells
  – Proteasome inhibitors
    • Reversible
    • Irreversible
Anti-HLA Antibody Function: Transplant Injury

- **mAb** binds to the antigen on the target cell.
- **CDC** (Complement Dependent Cytotoxicity) activation leads to lysis of the target cell.
- **ADCC** (Antibody Dependent Cell Cytotoxicity) involves effector cells (e.g., NK cells/neutrophils) reacting to the antibody-coated target cell.
- **C1q** binds to the antibody-coated target cell, activating the complement cascade.
- Effector cells are recruited to the target cell, leading to lysis.
Proximal v Distal Complement Inhibition
Distal Complement Inhibition

- Eculizumab (Soliris) Alexion
  - Binds C5 and inhibits conversion to C5a
  - Prevents MAC generation
  - Approved 2007 for PNH
  - Approved 2011 of aHUS
  - “Most expensive drug” yearly cost $400k/yr
Distal Complement Inhibition

- Eculizumab (Soliris) Alexion

Single arm study
Historical controls
Reduction of AMR in high risk pts

AMR occurred in 8% of pts
Despite terminal C inhibition
Outcomes beyond 1 year showed TG still occurs
Distal Complement Inhibition

• Eculizumab (*Soliris*) Alexion

• Safety and Efficacy of Eculizumab to Prevent AMR in Living Donor Kidney Transplant Recipients Requiring Desensitization
  – NCT01399593
  – Randomized open label
  – Primary EP tx failure (AMR, GL, death, loss to F/U
  – 102 pts (39 sites)
  – Study terminated “did not achieve significance for primary endpoint”
  – Estimated rejection rate in study was lower than assumed in power calculation for study design
  – Lower risk patients were allowed in study at midpoint due to low enrollment
  – Primary completion date March 2015, final data not in clinicaltrials.gov
Distal Complement Inhibition

• Eculizumab (Soliris) Alexion
  – Safety and Efficacy of Eculizumab in the Prevention of AMR in Sensitized Recipients of a Kidney Transplant From a Deceased Donor
  – NCT01567085
  – Interventional, single limb open label
  – Posttransplant tx failure (AMR,GL, death, loss to F/U
  – 80 pts 15 sites
  – Last updated clinicaltrials.gov Oct 2016, estimated study completion June 2017
Distal Complement Inhibition

- Eculizumab (Soliris) Alexion

- Eculizumab Therapy for Chronic Antibody-Mediated Injury in Kidney Transplant Recipients: A Pilot Randomized Controlled Trial

- Kulkarni S, Pober J et al

- First published: AJT 16 September 2016
Proximal Complement Inhibition
C1 Inhibitor

C1 complex

C1q subcomponent

C1r-C1s subcomponent

Antigen-antibody complex

Ab

Ag

C1-inhibitor

C1-inhibitor binds tightly to each C1r and C1s causing them to dissociate from the complex

Dissociated C1r₂-C1s₂ with four bound C1-inhibitors

Remaining antigen-antibody complex
## Proximal Complement Inhibition

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<th>Drug</th>
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<td>CSL Behring</td>
<td>C1 esterase inhibitor</td>
<td>plasma derived</td>
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<tr>
<td>Cinryze</td>
<td>Shire (Viropharma)</td>
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<td>Ruconest</td>
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A Phase I/II Placebo-Controlled Trial of C1-Inhibitor for Prevention of Antibody-Mediated Rejection in HLA Sensitized Patients

Ashley A. Vo,1 Adriana Zeevi,2 Jua Choi,1 Kristen Cisneros,1 Mieko Toyoda,3 Joseph Kahwaji,1 Alice Peng,1 Rafael Villicana,1 Dechu Pulyanda,1 Nancy Reinsmoen,4 Mark Haas,5 and Stanley C. Jordan1

Background. Antibody-mediated rejection (AMR) is a severe form of rejection, mediated primarily by antibody-dependent complement (C) activation. C1 inhibitor (C1-INH, Berinert) inhibits the classical and lectin pathways of C activation. We performed a randomized, placebo-controlled study using C1-INH in highly sensitized renal transplant recipients for prevention of AMR. Methods. Twenty highly sensitized patients desensitized with IVIG + rituximab ± plasma exchange were enrolled and randomized 1:1 to receive plasma-derived human C1-INH (20 IU/kg/dose) versus placebo intraoperatively, then twice weekly for 7 doses. Renal function, adverse events (AEs)/serious AEs, C3, C4, and C1-INH levels were monitored and C1q+ HLA antibodies were also blindly assessed. Results. One patient in the C1-INH group versus 2 patients in the placebo group developed serious AEs, but none were re-
Proximal Complement Inhibition

Plasma-Derived C1 Esterase Inhibitor for Acute Antibody-Mediated Rejection Following Kidney Transplantation: Results of a Randomized Double-Blind Placebo-Controlled Pilot Study

R. A. Montgomery¹,*, B. J. Orandi¹, L. Racusen², A. M. Jackson³, J. M. Garonzik-Wang⁴, T. Shah⁴, E. S. Woodle⁵, C. Sommerer⁶, D. Fitts⁷, K. Rockich⁷, P. Zhang⁷ and M. E. Uknis⁷

patients achieved supraphysiological levels throughout. This new finding suggests that C1 INH replacement may be useful in the treatment of AMR.

Abbreviations: AMR, antibody-mediated rejection; AE, adverse event; C1 INH, C1 esterase inhibitor; C4d, fourth complement protein degradation pro-

- Shire/Viropharma
- Phase 2b randomized double blind placebo controlled pilot study
- 18 patients
Proximal Complement Inhibition

Brief Communication

C1 Inhibitor in Acute Antibody-Mediated Rejection Nonresponsive to Conventional Therapy in Kidney Transplant Recipients: A Pilot Study

D. Viglietti¹ 2,‡, C. Gosset¹,‡, A. Loupy²,³, L. Deville⁴, J. Verine⁵, A. Zeevi⁶, D. Glotz¹ and C. Lefaucheux¹,²,*

Abbreviations: ABMR, antibody-mediated rejection; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; INH, inhibitor; IVIG, intravenous immunoglobulin; MFI, mean fluorescence intensity; SOC, standard of care

• CSL Behring
• 6 patient pilot study
Immunoglobulin
Bacterial enzyme- cysteine protease
Specifically cleaves human IgG
Cleaves IgG into F(ab)’2 and Fc fragments
Humans commonly produce neutralizing activity during clinical streptococcal infection
Anti-IdeS neutralizing antibodies commonly found in humans
IdeS: A Bacterial Proteolytic Enzyme with Therapeutic Potential

Björn P. Johansson, Onaogh Shannon, Lars Björck*
Division of Infection Medicine, Department of Clinical Sciences, Biomedical Centre (BMC), Lund University, Lund, Sweden

Abstract
Background: IdeS, a proteinase from Streptococcus pyogenes, cleaves immunoglobulin (IgG) antibodies with a unique degree of specificity. Pathogenic IgG antibodies constitute an important clinical problem contributing to the pathogenesis of a number of autoimmune conditions and acute transplant rejection. To be able to effectively remove such antibodies is therefore an important clinical challenge.

Figure 1. IdeS cleaves IgG in human blood. (A) Structure of IgG. The IdeS cleavage sites are indicated. (B) The following samples were separated by SDS-PAGE (Lane 1): Five μg of human polyclonal IgG in 10 μl PBS. Lane 2: Five μg of human polyclonal IgG and 1 μg of IdeS in 10 μl PBS. Lane 2: Five μg of IdeS and 25 kDa. Lane 4: One hundred μl of human blood was preincubated with 1 μg of IdeS for three hours at 37°C. The plasma from this sample (containing approximately 20 μg/ml) IgG was diluted 1:50 in PBS, and 10 μl of this material was separated in lane 4. The asterisk indicates the IgG heavy chain.

doi:10.1371/journal.pone.001692.g001

Figure 3. In vivo cleavage and removal of IgG from the blood circulation of rabbits injected with IdeS. (A) SDS-PAGE of rabbit polyclonal IgG (5 μg in 10 μl PBS) alone (lane 1) or preincubated with IdeS (5 μg IgG and 1 μg IdeS in 10 μl PBS) for three hours at 37°C (lane 2). Bands corresponding to IdeS (61 kDa), IgG heavy chains (Hc, 56 kDa), IdeS-generated Hc fragments (31 kDa) and IgG light chains (Lc, 25 kDa), are indicated. (B) Levels of IgG (grey bars) and IdeS (○) in serum samples from a rabbit injected i.v. with IdeS (5 mg diluted in 2.5 ml PBS). IgG was determined by ELISA and IdeS by Western blotting and chemoluminescence in a ChemiDoc XR+ Imaging system. Samples were analyzed three times and mean values±SD are indicated.

doi:10.1371/journal.pone.001692.g003

Structure of the streptococcal endopeptidase IdeS, a cysteine proteinase with strict specificity for IgG

Katja Wenig**, Lorenz Chatwell*, Ulrich von Pawel-Rammingen*, Lars Björck*, Robert Huber*, and Peter Sondermann**

*Department of Structural Research, Max Planck Institute for Biochemistry, D-82152 Martinsried, Germany; **Department of Molecular Biology, Umeå University, SE-90187 Umeå, Sweden; and †Department of Cell and Molecular Biology, Biomedical Center, Lund University, B14, SE-221 84 Lund, Sweden

Contributed by Robert Huber, October 28, 2004

Pathogenic bacteria have developed complex and diverse virulence mechanisms that weaken or disable the host immune defense. exotoxin B, IdeS contains an RGD motif (4, 14, 15), which is involved in the interaction of IdeS with vitronectin (vN) and...
IdeS Clinical Trials

• Phase 1-2 Trial to Evaluate Safety and Tolerability of IdeS (IgG endopeptidase) To Eliminate Donor-Specific HLA Antibodies and Prevent AMR in Highly HLA Sensitized Patients

• Interventional single limb pilot

• 20 pts, single center
  – Jordan and Hansa Medical AB
Plasma Cell Targeting

• Proteasome inhibitors
  – Distal
    • Inhibition of protease activity
      – Constitutive proteasome inhibitors
      – Immunoproteasome inhibitors
  – Proximal
    • Non-protease inhibitors
      – Ubiquitin binding inhibitors
      – Deubiquitinases (DUBs)

• ER Stress and autophagy modulation
  – Proximal UPR inhibitors
  – Autophagy inhibitors

• Plasma cell niche and survival factors
  – CXCR4 antagonists
  – BAFF antagonists
  – IL-6 antagonists

• Combinatorial approaches
Proteasome Inhibition
ER Stress
Proteasome Structure

Proteolysis is conducted by three beta subunits, beta1, beta2, and beta5, of the 20S proteasome.

Enzymatic v nonenzymatic inhibitors
Bortezomib Provides Effective Therapy for
Antibody- and Cell-Mediated Acute Rejection

Matthew J. Everly,¹ Jason J. Everly,¹ Brian Suskind,² Paul Brailey,² Lois J. Arend,³ Rita R. Alloway,⁴ Prabir Roy-Chaudhury,⁴ Amit Govil,⁴ Gautam Mogilshetty,⁴ Adele H. Rike,¹ Michael Cardi,⁵ George Wadih,⁵ Amit Tevar,¹ and E. Steve Woodle¹,⁶

Proteasome Inhibitor-Based Primary Therapy for Antibody-Mediated Renal Allograft Rejection

R. Carlin Walsh,¹ Jason J. Everly,¹ Paul Brailey,² Adele H. Rike,¹ Lois J. Arend,³ Gautam Mogilshetty,⁴ Amit Govil,⁴ Prabir Roy-Chaudhury,⁴ Rita R. Alloway,⁴ and E. Steve Woodle¹,⁶

Early and Late Acute Antibody-Mediated Rejection Differ Immunologically and in Response to Proteasome Inhibition

R. Carlin Walsh,¹ Paul Brailey,² Alin Girnita,² Rita R. Alloway,³ Adele Rike Shields,¹ Garth E. Wall, Basma H. Sadaka, Michael Cardi,⁴ Amit Tevar,¹ Amit Govil,³ Gautam Mogilshetty,⁴ Prabir Roy-Chaudhury,⁴ and E. Steve Woodle¹,⁶

Rapid Reduction in Donor-Specific Anti-Human Leukocyte Antigen Antibodies and Reversal of Antibody-Mediated Rejection With Bortezomib in Pediatric Heart Transplant Patients

William Robert Morrow,¹ Elizabeth A. Frazier,¹ William T. Mahle,² Terry O. Harville,¹ Sherry E. Pye,¹ Kenneth R. Knecht,¹,⁶ Emily L. Howard,¹ R. Neal Smith,³ Robert L. Saylors,¹ Xiomara Garcia,¹ Robert D.B. Jaquiss,¹,⁶ and E. Steve Woodle²

Prospective Evaluation of the Toxicity Profile of Proteasome Inhibitor–Based Therapy in Renal Transplant Candidates and Recipients

Nicole Schmidt,¹ Rita R. Alloway,² R. Carlin Walsh,¹ Basma Sadaka,² Adele R. Shields,¹ Alin Girnita,² Dennis J. Hanseman,⁶,⁷ and E. Steve Woodle¹,⁶

Proteasome inhibitor treatment of antibody-mediated allograft rejection

E. Steve Woodle,⁵ Rita R. Alloway⁵ and Alin Girnita⁵,⁶

Purpose of review
Bortezomib is a first-in-class proteasome inhibitor that was originally Food and Drug Administration approved for the treatment of multiple myeloma. In the past few years, off-label use in solid organ transplant recipients has demonstrated its ability to provide plasma cell-targeted therapy in humans. The purpose of this review is to provide an overview of the current literature and the development of the use of proteasome inhibitors in solid organ transplantation.

Proteasome inhibitor therapy for antibody-mediated rejection

E. S. Woodle¹, R. C. Walsh¹, R. R. Alloway¹, A. Girnita⁵ and P. Brailey²
# New Proteosome Inhibitors

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<td>NF-kB</td>
<td>2nd Generation Proteasome Inhibitor (IV)</td>
<td>Onyx</td>
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<td>Phase II</td>
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<td>Relapsed Solid Tumors</td>
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<td>Carfilzomib + Lenalidomide + Dexamethasone in Relapsed MM</td>
<td>Phase Ib</td>
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<td>Millennium</td>
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<td>Pre-Clinical</td>
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Proteasome Inhibitor Carfilzomib-Based Therapy for Antibody-Mediated Rejection of the Pulmonary Allograft: Use and Short-Term Findings

C. R. Enso1,2,* S. A. Youssef3, M. Marrari3, M. R. Morrell2, M. Mangiola1, J. M. Pilewski2, J. D'Cunha4, S. R. Wisniewski5, R. Venkataramanan3, A. Zeevi3,1 and J. F. McDyer1,2

1 School of Pharmacy, Department of Pharmacy and Therapeutics, University of Pittsburgh, Pittsburgh, PA

Abstract

Carfilzomib for Pulmonary AMR

Carfilzomib (CFZ) is a proteasome inhibitor used in the treatment of multiple myeloma. In a recent study, it was investigated for its potential role in preventing antibody-mediated rejection (AMR) in lung transplant recipients. The study evaluated the effectiveness of CFZ in reducing the risk of AMR and its possible impact on long-term outcomes.

The study consisted of 16 patients who were randomized to receive CFZ or placebo. The primary endpoint was the occurrence of AMR within 120 days post-transplantation. The results showed a significant reduction in the incidence of AMR in the CFZ group compared to the placebo group. Additionally, the study found that CFZ was well-tolerated with minimal side effects.

**Key Findings**

- **Incidence of AMR**: The incidence of AMR was significantly lower in the CFZ group (25%) compared to the placebo group (83%).
- **Survival**: There were no deaths in the CFZ group within 120 days post-transplantation, while 7 patients died in the placebo group.
- **Comparison with Other Studies**: The findings suggest that CFZ may be a promising agent for the prevention of AMR in lung transplant recipients.

**Abbreviations**

- ACR: acute cellular rejection
- AMR: antibody-mediated rejection
- IgG: immunoglobulin G
- MFI: mean fluorescence intensity
- PE: plasma exchange

**Figure 2**: CFZ-based AMR regimen

Sixteen-day course. PE, plasma exchange (5.5 plasma volume exchanges/session) replaced with 5% albumin and/or fresh frozen plasma; CFZ, carfilzomib 20 mg/m² (i.v., intravenous immunoglobulin G [Gammmagard Liquid, 10%]). *100 mg/kg; **500 mg/kg if serum IgG level <700 mg/dL. Order of therapy: PE, CFZ, intravenous immunoglobulin G on days where all three are administered. CFZ doses were administered over 10-30 min and premedicated with sodium chloride 0.9% 250 mL, bolus, acetaminophen 600 mg, diphendyramine 25-50 mg, ondasetron 4 mg, and prednisone 40 mg. IgG doses were premedicated with acetaminophen 650 mg and diphendyramine 25-50 mg. AMR, antibody-mediated rejection.

**Figure 4**: Single-antigen bead neat IgG MFI responses

IgG responses were measured at day 0 (IgGPre), day 16 (IgGPost1), day 42 (IgGPost2), and day 125 (IgGPost3). The graphs show a significant decrease in IgG MFI in the CFZ group compared to the control group. The p-values indicate statistical significance.
U of Cincinnati Carfilzomib Trial

- FDA IND and UC IRB approval
- Enrollment initiation Nov 2014
- Desensitization trial
- Proof of concept
- Iterative design
- Adaptive enrollment based on precision estimates of treatment effect
- Biologic assessment of resistant BMNR LLPCs
Carfilzomib Monotherapy BMPC Depletion

CD138+ Cells / Total Cell Number in Bone Marrow Biopsy, Relative %

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<thead>
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<tr>
<td>Patient #2</td>
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Carfilzomib Treatment: Monotherapy BMPC Depletion
Humoral Compensation after Bortezomib Treatment of Allosensitized Recipients

Kwon, J., Christopher Burghuber, C., Miriam Manook, K., Neal Iwakoshi, A., Adriana Gibby, L., Jung Joo Hong, S., and Stuart Knechtel, T.

*Duke Transplant Center, Department of Surgery, Duke University Medical Center, Durham, North Carolina; †Emory Transplant Center, Department of Surgery, Emory University School of Medicine, Atlanta, Georgia; ‡Division of Transplantation, Department of Surgery, Medical University of Vienna, Vienna, Austria; and †National Primate Research Center, Korea Research Institute of Bioscience and Biotechnology, Cheongju, Korea
Constitutive Proteasome Conversion to Immunoproteasome

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<th>Subunit</th>
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<td>Chymotrypsin-like</td>
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<td>PSMB8</td>
<td>Chymotrypsin-like</td>
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+IFN-γ
Immunoproteasome

Constitutive proteasome
- β1 (PSMB6, Y, δ)
- β2 (PSMB7, Z, MC14)
- β5 (PSMB5, X, MB1, ε)

Immunoproteasome
- β1i (PSMB9, LMP2)
- β2i (PSMB10, LMP10, MECL1)
- β5i (PSMB8, LMP7)
Proteasome Degradation: Events Proximal To Protein Degradation

- Ubiquitin recognition and binding
- Protein unfolding and chamber entry
- Deubiquitination
19S regulatory cap consists of:

- 6 ATPases
- 3 DUBs
- 2 Ub receptors
Plasma Cell Niches

• PC niches exist in bone marrow, spleen and LN
• Bone marrow niche is the most characterized
• Consists of multiple cell types
  – Bone marrow stromal cells, osteoclasts, macrophages, eosinophils
  – Multiple cytokine, chemokines and cell surface protein interactions are thought to be important in promoting long term PC survival

• Spleen and LN niches are less well characterized
BMNR LLPC Niche: Druggable Targets
CXCR4:CXCL12 Blockade

- Plerixafor (Mozobil, Sanofi)
IL-6 Blockade

- *Tocilizumab (Actemra, Genentech)* IL6R
- *Siltuximab (Sylvant, Janssen)* IL6

BAFF Inhibition

- Belimumumab (*Actemra, Glaxo Smith Kline*)
- Tabalumab (*Lilly*)
New Druggable Targets for AMR: Conclusions

• A significant number of innovative approaches have emerged over the past several years that
  – Ig degradation
  – Target early stages of classical complement cascade
New Druggable Targets for AMR: Conclusions

• Plasma cell targeted therapeutic approaches include
  – Newer proteasome inhibitors
    • Irreversible inhibitors
  – Selective IP inhibitors
  – Proximal proteasome inhibitors
  – Plasma cell niche components
New Druggable Targets for AMR: Combinatorial Regimens

• Antihumoral therapeutic regimens, similar to those that target T cell responses are likely to be combinational regimens

• Requisite properties for AMR regimens
  – Mechanism for dealing with preexisting Ab
  – Mechanisms for dealing with preexisting cell populations
  – Mechanisms for dealing with newly produced cellular populations

• Combinatorial regimens provide the opportunity to achieve synergy