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Detection of Antibodies to AT1R, An Overview

Gordon Hill

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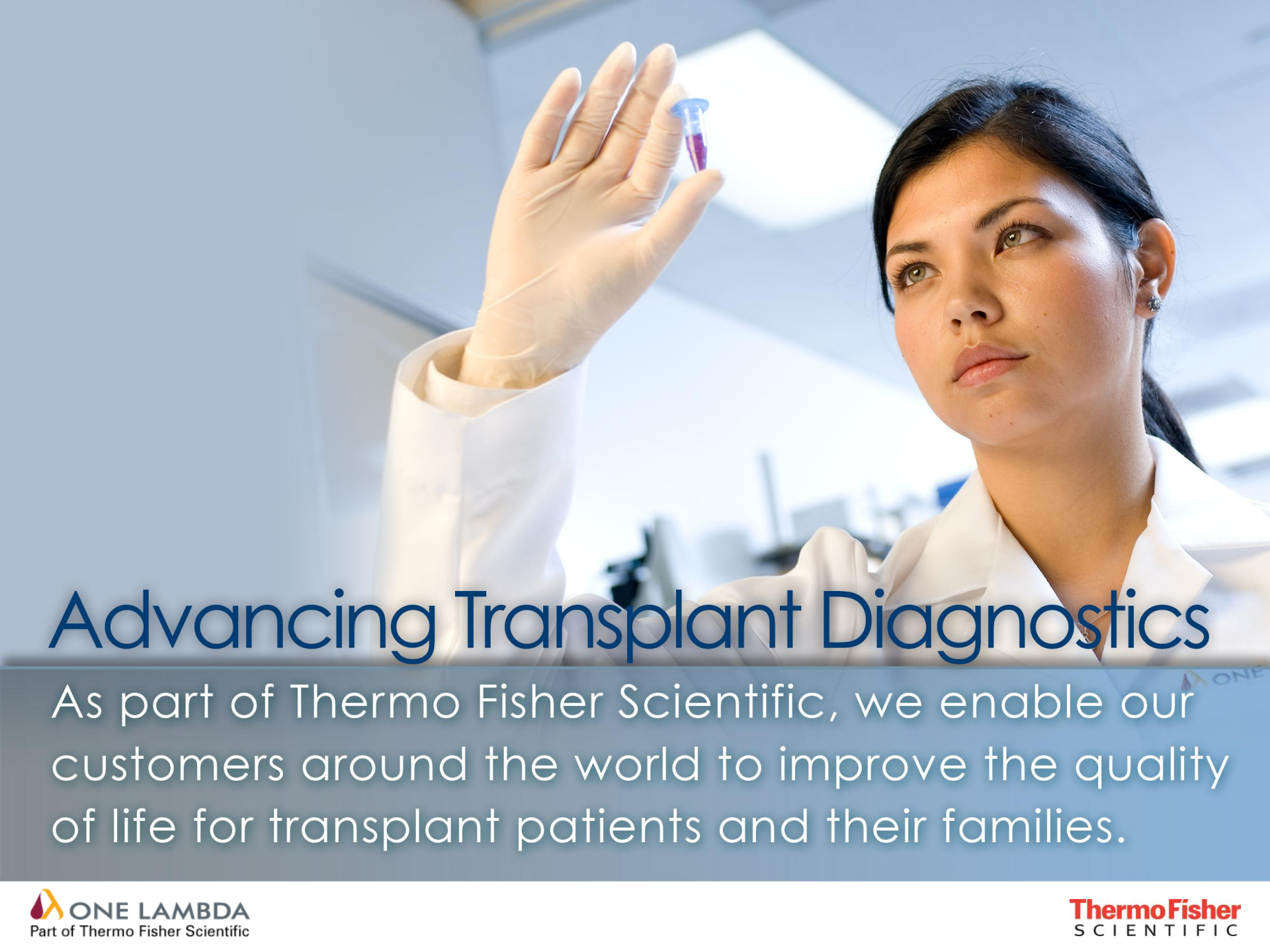

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Case 1 - Lunz, JG (UPMC)

- sensitized 34 year old male underwent a 5th renal transplant with an HLA-matched organ
- no HLA-DSA or MICA antibody was present pre-transplant
- CDC and Flow Xm's were negative
- following reperfusion, there was gross evidence of hyperacute AMR, no renal blood flow, and the kidney was removed after 1 day
- Post-transplant antibody testing again showed no HLA-DSA, MICA, and Xm's were still negative.

Case 2 – Eng, HS (Johns Hopkins)

- sensitized 30 year old male in KPD/desensitization program received a 2nd renal transplant
- HLA-DSA was present pre-transplant and the patient underwent pre- and post-transplant desensitization reducing the antibody from Flow positive to Flow negative Xm levels.
- Around 3-4 months post-transplant, the patient was admitted with increased creatinine level from baseline 1.3 mg/dl to 5.6 mg/dl.
- AMR was proven by biopsy, but HLA-DSA levels continued to be low. An endothelial cell Xm was also negative.
- The patient was given multiple rounds of IVIG/PP and despite some initial improvement, ultimately lost the graft at 8 months post-transplant.

Case 3 – Taniguchi (TFL)

- non-sensitized 38 year old male underwent 1st transplant with living related donor.
- no HLA-DSA or MICA antibody was present pre-transplant
- Serum creatinine (sCr) was stable, at 1.8-2.0 mg/dl, for 36 months post-transplant.
- sCr was still stable at 40 months (1.8 mg/dL) and did not rise until 48 months post-transplant. Patient was diagnosed with focal segmental glomerulosclerosis (FSGS).
- Nephrectomy was performed soon after due to chronic transplant vasculopathy.

AT1R Background

1) Angiotensin II is a peptide hormone which regulates blood pressure, water-salt balance, neuronal function and releases aldosterone from the adrenal cortex.

2) Angiotensin II Type 1-Receptors (AT1R) are expressed in multiple cells of the body, including vascular smooth muscle cells, heart, lung, brain, liver, kidney and others.

3) Auto-antibodies against the AT1R have demonstrated a correlated risk of antibody mediated rejection (AMR)

Formative Studies

Dragun et al. Angiotensin II Type 1-Receptor Activating Antibodies in Renal-Allograft Rejection. *N Engl J Med*; 2005; 352(6):558-69.

- Studied 33 renal transplant patients with refractory vascular rejection (13 had HLA-DSA and 20 did not).
- 16 of the 20 patients had severe vascular rejection and malignant hypertension, but no HLA-DSA. Antibodies targeting AT1R were found in all 16 of these patients.
- Passive infusion of AT1R receptor antibodies in a rat renal-transplantation model increased blood pressure and induced vascular changes. An AT1R antagonist blocked the effects of the anti-AT1R antibodies.
- Concluded that an AT1R-mediated pathway may contribute to rejection and that removal of anti-AT1R antibodies or blocking of AT1R may be of benefit to transplant recipients.

Formative Studies

Reinsmoen et al. Anti-angiotensin Type 1 Receptor Antibodies Associated With Antibody Mediated Rejection in Donor HLA Antibody Negative Patients. *Transplantation*; 2010; 90(12):1473-77

• reported that high levels of anti-AT1R antibodies were associated with AMR in 10% of patients (6/63) who did not have HLA-DSA or MICA antibodies.

• concluded that assessment of anti-AT1R antibodies in combination with HLA-DSA testing provides additional information for determining immunological risk and may aid in AMR diagnosis.

EIA-AT1R Assay

Straightforward ELISA procedure :
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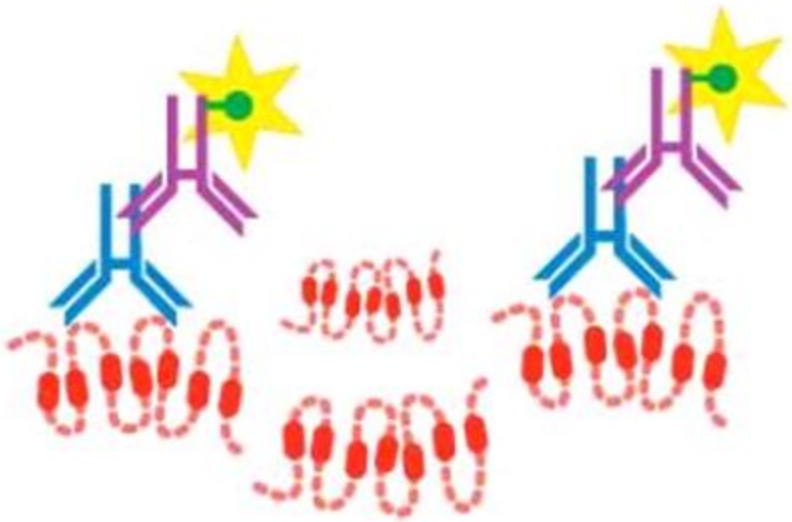
1) incubate diluted serum in plate coated with AT1R
2) detection by HRP labeled anti-human IgG antibody.
3) collect on standard ELISA reader



AT1R Analysis

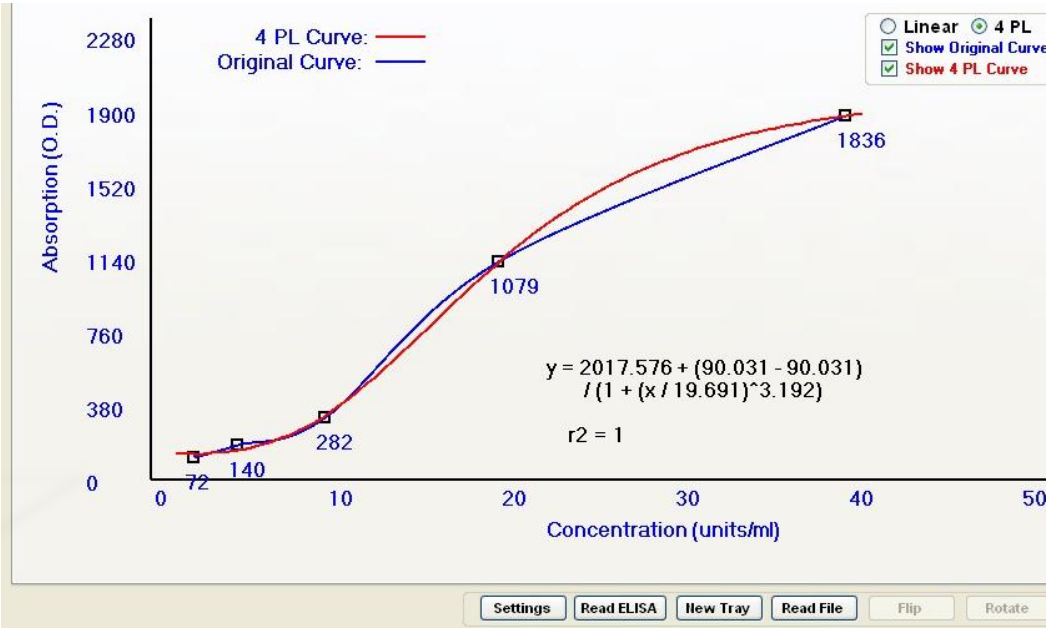
- 5 controls included to generate a standard curve (U/ml vs. O.D.), allows for quantitative results

- AT1R software for data collection and U/ml calculations

>17 U/ml = strong positive

10-17 U/ml = at risk

<10 U/ml = negative



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AT1R+

Pre-txp:
32 U/ml

Post-txp:
55 U/ml

Case 2 – Eng, HS (Johns Hopkins)

- sensitized 30 year old male in KPD/desensitization program received a 2nd renal transplant
- HLA-DSA was present pre-transplant and the patient underwent pre- and post-transplant desensitization reducing the antibody from Flow positive to Flow negative Xm levels.
- Around 4 months post-transplant, the patient was admitted with increased creatinine level from baseline 1.3 mg/dl to 5.6 mg/dl.
- AMR was proven by biopsy, but HLA-DSA levels continued to be low. An endothelial cell Xm was also negative.
- The patient was given multiple rounds of IVIG/PP, but ultimately lost the graft at 8 months Post-transplant.

Case 2 – Eng, HS (Johns Hopkins)

- Due to low DSA levels, inconsistent with persistent AMR, an anti-AT1R antibody test was also performed.
- The AT1R level at the time of the first positive biopsy was found to be at **> 40 U/ml**.
- Retrospective testing also showed that AT1R was positive one day pre-transplant.
- In addition to the IVIG/PP, Losartan, an AT1R antagonist, was administered as part of anti-rejection and hypertension treatment. Unfortunately, the creatinine remained >3 mg/dl and the graft was lost.

Case 3 – Taniguchi M (TFL)

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AT1R Post Transplant

1 month
10 U/ml

12 months
5 U/ml

38 months
12 U/ml

40 months
40 U/ml

Clinical Implications of AT1R

1. It is increasingly recognized that immune responses to both HLA and non-HLA targets act together in the pathogenesis of graft rejection.

2. AT1R antibodies have been associated with AMR in the absence of HLA-DSA. There also appear to be synergistic effects when both are present, decreasing graft survival.

3. Monitoring of anti-AT1R antibodies may allow more complete immunologic risk assessment pre- and post-transplant

4. Removal of AT1R antibodies or blocking of AT1R may become a new therapeutic target.

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Thank You

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