Management of Kidney Transplant Recipients Using TruGraf® Testing

M. Roy First, MD, FAST
Chief Medical Officer
Transplant Genomics Inc.

June 4, 2018
The promise of precision medicine will only be achieved when molecular diagnostics detect actionable differences operating in individual patients, that can inform management and change clinical outcomes.

Daniel R. Salomon, MD
TGI Co-Founder
1953-2016
TruGraf Test Overview

• Urgent need: non-invasive test for early signs of inadequate immunosuppression
  - alternative/ complement to indiscriminate surveillance biopsies
  - early intervention may lead to improved longer term outcomes

• TruGraf family of blood tests

• Results reported:
  - v1 confirms the presence of immune quiescence (TX)
  - v2 confirms absence of subAR in patients with stable renal function
  - v1 or v2 interpretation can be generated from any sample
Evolution of the TruGraf Test

Sample processing from blood draw to final gene expression data is identical for all versions of TruGraf. All versions were derived from paired samples (blood and biopsy). The only differences between the various versions are clinical phenotypes used in discovery and validation phases, bioinformatics methodology, models and thresholds.

- **TruGraf v1.0** – Discriminates TX from not-TX: with TX as the positive result. Random Forests bioinformatics. Different probability thresholds.
- **TruGraf v1.3** – Discriminates TX from not-TX: with not-TX as the positive result. Random Forests bioinformatics.
- **TruGraf v2.0** – TX vs. subAR: only patients with stable renal function. Discovery (CTOT-08) and validation. Random Forests bioinformatics.
- **cAR vs. ADNR vs. TX**: small sample size; discovery only.
Interpretation of Results

**TruGraf v1 (TX/not-TX)**
*First generation*

- TX indicates with a high degree of probability that the patient is indeed stable.
- not-TX reflects an inability to confirm immune quiescence, and does not suggest any particular phenotype.

**TruGraf v2 (Pos/Neg)**
*Second generation*

- A negative result in a patient with stable renal function correlates with a phenotype of TX and indicates a low risk of harboring subAR.
- A positive result (expected in low % of patients) correlates with a phenotype of subAR and identifies those who should be followed more closely.

A non-invasive alternative/complement to indiscriminate use of surveillance biopsies to detect subAR
TruGraf v1 Performance: Early Access Program (EAP)  
Stable Patients (3 sites)

<table>
<thead>
<tr>
<th></th>
<th>Biopsy/Clinical not-TX</th>
<th>Biopsy/Clinical Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood not-TX</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>True Positive</td>
<td>False Positive</td>
</tr>
<tr>
<td>Blood TX</td>
<td>4</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>False Negative</td>
<td>True Negative</td>
</tr>
</tbody>
</table>

Accuracy 73/103 (71%)
False negative rate 4/103 (4%)
Accuracy of TruGraf TX result 58/62 (94%)
NPV = 91%
PPV = 45%
Sensitivity = 79%
Specificity = 69%
How is TruGraf Different?

- **Stable Kidney Function**
- **Graft Dysfunction**

- **TruGraf**
  - Tissue Gene Expression
  - Cell free DNA

- **Invasive**
- **Noninvasive**
Agenda

• Investigators from 3 EAPs will discuss their experience with the clinical utility of TruGraf v1 in managing transplant patients in their facility.

• We shall then share breaking news regarding TruGraf v2, which we believe has the potential to change the SOC in KTR management.
Value of TruGraf Testing in Sites Not Performing Surveillance Biopsies

V. Ram Peddi, M.D.
Kimi Ueda, Pharm D
California Pacific Medical Center
San Francisco, CA
Patients return to clinic for 3 month post-transplant follow up visit

Screened by research coordinator and enrolled in the EAP

Coordinator mails patient TruGraf kit and instructions for collection at local Quest Patient Service Center, along with routine labs

Quest collects TruGraf samples and ships to TGI for analysis

Compare TruGraf results with serum creatinine and clinical impressions to assess concordance

n = 55
High Concordance with Clinical Assessment

n = 55

not-TX: Positive result
TX: Negative result

<table>
<thead>
<tr>
<th></th>
<th>Clinical Assessment not-TX</th>
<th>Clinical Assessment TX</th>
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</thead>
<tbody>
<tr>
<td>TruGraf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>not-TX (21/55)</td>
<td>7 True Positive</td>
<td>14 False Positive</td>
</tr>
<tr>
<td>TruGraf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TX (34/55)</td>
<td>1 False Negative</td>
<td>33 True Negative</td>
</tr>
</tbody>
</table>

Accuracy 40/55 (73%)
False negative rate 1/55 (2%)
NPV = 95%
PPV = 49%
Sensitivity = 88%
Specificity = 70%
Value of TruGraf Testing at CPMC

TruGraf offers a noninvasive test with high NPV for TX result in stable patients, which supports diagnosis of immune quiescence.

Patients with not-TX could be followed more closely, and re-tested over time. If not-TX persists then consider biopsy.

Provides a valuable indicator of adequacy of immunosuppression for centers that do not perform surveillance biopsies.
Correlation of the TruGraf test and renal biopsy result

Richard J. Knight, MD, FACS
Houston Methodist Hospital, Houston, TX
TruGraf Use and Protocol Design

Patients screened by research coordinator the week of scheduled biopsy visit

**Inclusion Criteria:**
- male and female recipients of all races, ≥ 18 years of age
- primary or subsequent deceased-donor or living donor kidney transplantation
- at least 60 days post-transplant
- able to provide consent

TruGraf collected immediately prior to the scheduled biopsy

- **High Risk**
  - n = 11

- **Surveillance Biopsy**
  - n = 8

- **For-Cause Biopsy**
  - n = 4

Compare TruGraf results with histology readings and clinical impressions to assess concordance, n = 23
TruGraf Showed High Concordance with Histology Across All Patients

n = 23

<table>
<thead>
<tr>
<th></th>
<th>Biopsy not-TX</th>
<th>Biopsy TX</th>
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<tbody>
<tr>
<td>TruGraf not-TX</td>
<td>6 True Positive</td>
<td>4 False Positive</td>
</tr>
<tr>
<td>TruGraf TX</td>
<td>1 False Negative</td>
<td>12 True Negative</td>
</tr>
</tbody>
</table>

Accuracy 18/23 (78%)
False negative rate 1/23 (4%)
NPV = 94%
PPV = 53%
Sensitivity = 86%
Specificity = 75%

Note: The accuracy of the test was concordant in the 3 subgroups, because of small numbers, the data was pooled.
Potential Impact of Using TruGraf at Houston

- In stable patients receiving surveillance biopsy at 1 year post-transplant:
  - High correlation provides opportunity to reduce unnecessary surveillance biopsies
- In patients receiving for-cause biopsy:
  - Could be used when a biopsy is contraindicated, or to confirm ambiguous histology
- In high risk patients:
  - Potential to reduce surveillance biopsies and/or test more frequently
Potential for the TruGraf Test to Reduce Surveillance Biopsies

Roslyn B. Mannon, MD, FASN, FAST
Professor of Medicine and Surgery
Director of Research, Comprehensive Transplant Institute
Birmingham, AL
TruGraf Use and Protocol Design

Patients screened by nurse coordinator the week of scheduled visit
IRB-170113002

Stable patients coming in to clinic for surveillance biopsy scheduled at 6 months post-transplant

TruGraf, SCr, tissue collected on same day
n = 25

Normal Histology
n = 21

Abnormal Histology
n = 4

TruGraf compared with histology to assess concordance
High NPV Confirms Immune Quiescence

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<tr>
<th></th>
<th>Biopsy not-TX</th>
<th>Biopsy TX</th>
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<tbody>
<tr>
<td>TruGraf not-TX:</td>
<td>2 True Positive</td>
<td>8 False Positive</td>
</tr>
<tr>
<td></td>
<td>- BRL rejection (1)</td>
<td>- No rejection (6)</td>
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<tr>
<td></td>
<td>- Banff 2A (1)</td>
<td>- Thin GBM (1)</td>
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<tr>
<td></td>
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<td>- TAC toxicity (1)</td>
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<tr>
<td>TruGraf TX:</td>
<td>2 False Negative</td>
<td>13 True Negative</td>
</tr>
<tr>
<td></td>
<td>- BRL Rejection (2)</td>
<td>- Normal Bx (13)</td>
</tr>
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n = 25

High NPV Confirms Immune Quiescence
High NPV Confirms Immune Quiescence

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<td>2 True Positive</td>
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<td></td>
<td>▪ BRL Rejection (2)</td>
<td>▪ Normal Bx (13)</td>
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Accuracy 15/25 (60%)
False negative rate 2/25 (8%)
NPV = 80%
PPV = 30%
Sensitivity = 50%
Specificity = 62%
SUMMARY

TruGraf version 1 was designed to maximize true negative calls (TX), thereby avoiding unnecessary surveillance biopsies.

- A TruGraf result of TX was accurate in 13/15 (87%) instances.
- A false negative TruGraf result occurred in 2/25 (8%); both subjects had Banff borderline rejection.
- False positives were called in 8/25 (32%); however, under our current standard of care, all of these subjects would have had a surveillance biopsy.
- TruGraf accurately classified patients as TX (true negative: highly likely to be immune quiescent) in 13/25 (52%) cases, all of which represent surveillance biopsies that could have been avoided.
Potential Real-World Applications

- Reducing reliance on surveillance biopsies
- As a monitoring tool following treatment of subclinical TCMR
- May eventually provide confidence to reduce IS
DEVELOPMENT OF TRUGRAF VERSION 2.0

John Friedewald, MD

June 2018
Development of TruGraf Version 2.0

- Based on data from the CTOT 08 trial
- Focused on detecting *subclinical* rejection
  - In patients with *stable* graft function
  - Benchmarked against surveillance protocol biopsies
  - Intended to be used in conjunction with available clinical tools (HLA antibody testing, BK polyoma virus testing) not to replace them
  - Screening tool to identify patients are higher or lower risk of harboring subclinical acute rejection (subAR)
**CTOT 08 Trial** - 24-month Multi-Center Observational Study – 5 Centers - Surveillance Biopsies at 2-6, 12 and 24 months

<table>
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<th>Months</th>
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<th>9</th>
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<td>SubAR</td>
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**SubAR**: histology on a surveillance biopsy
- acute rejection (≥ Banff borderline cellular rejection and/or antibody mediated rejection)
- AND stable renal function,
  - serum creatinine <2.3 mg/dl and <20% increase in creatinine compared to a minimum of 2-3 prior values over a mean period and range of 132 and 75-187 days, respectively

**Transplant eXcellence (TX)**: normal histology on surveillance biopsy
- (no evidence of rejection - Banff i=0 and t=0, g=0, ptc=0; ci=0 or 1 and ct=0 or 1)
- AND stable renal function as defined above. Surveillance biopsies were performed on all subjects at 2-6, 12 and 24 months following transplantation.
Biomarker Discovery

530 CTOT08 Samples Gene Expression (GE) Data

ComBat Adjustment By Phenotype

Log2(Expression Distribution)

530 and 138 blood samples all paired with centrally read biopsies were used for discovery and validation respectively.

Gene Expression Profile (GEP): 57 Genes (61 probe sets) from RF Model
-7 genes linked to top 10 IPA immune/inflammatory pathways

Detailed in Abstract #250724
72-75% of patients would have a negative test and could therefore be spared a surveillance biopsy by ruling out the presence of subAR with 78-88% NPV.

The remaining 25-28% would have a positive test and would therefore be at higher risk harboring subAR with 47-61% PPV.
Clinical Endpoints in CTOT 08

• **Clinical Composite Endpoint (CCE):**
  - 24-month biopsy (central read) with chronic injury – Interstitial Fibrosis/Tubular Atrophy (IFTA) (Banff ≥ Grade II IFTA [ci ≥ 2 or ct ≥ 2]; OR
  - Biopsy-proven acute rejection (BPAR) on any ‘for-cause biopsy’ (central read); OR
  - a decrease in estimated glomerular filtration rate (∆eGFR) by >10ml/min/1.73m² (CKD-EPI) between 4 - 24 months post-transplant

• *De novo* DSA: Class I and/or II (local determination)
Clinical Validity – Clinical Phenotype

Based on biopsy results only

- Association of Clinical Phenotypes with 24 month Clinical Endpoints
- Association between dnDSA with Clinical Phenotype within 1 year Post Transplant
- Association of Clinical Phenotypes with dnDSA anytime Post Transplant
Clinical Validity - Gene Expression Profile

Based on GEP Biomarker Results only

Association of Gene Expression Profile (GEP) with 24 month Outcomes

Association of GEP with dnDSA anytime Post Transplant
Panel 1

Association between dnDSA and GEP within 1 year
Panel 2
SubAR Treatment Follow up - Clinical Utility

<50% of 8-week follow up biopsies after subAR treatment show histological improvement!!
Case Study

• 62 yo WF, Post strep GN
• Now 2.5 years post a DCD deceased donor renal transplant – dialysis for 5 years prior to transplant
• 3 month post transplant protocol biopsy – no rejection
• Prior to 12 month biopsy, Trugraf 1.3 assay “non-TX”. Patient requested repeat assay - ~4wks later again ”non-TX”. Protocol biopsy done with borderline subclinical rejection. Bolus steroids and maintenance immunosuppression (tac + mmf) increased. Creatinine remained stable 0.7 mg/dl
Case Continued

- During the second year, developed BK viruria, no viremia
- MMF dose slightly reduced, BK cleared
- Patient further “self-tapered” tacrolimus due to symptoms
- 2 year mark, TruGraf 1.3 sent – non-TX
- Protocol biopsy reveals subclinical borderline rejection –
  Immunosuppression increased, prednisone at low dose added
Case Continued

• 8 weeks later, TruGraf 2.0 sent – “TX”
• No repeat biopsy, maintain current IS
• Plan to repeat TruGraf every 3 months this year
Summary

• TruGraf 2.0 was developed based on a large, multi-center observational NIH-funded clinical trial
• All patients underwent protocol kidney biopsies
  • Patients meeting criteria for stable function used for analysis
• Biomarker developed to distinguish subclinical rejection from normal
• In CTOT 08, 72-75% of patients had normal (TX) result with 78-88% NPV
• 25-28% had a positive test and would therefore be at higher risk harboring subclinical AR (subAR) with 47-61% PPV
Summary

- TruGraf v 2.0 is intended to be used as a screening tool for **stable kidney transplant recipients** to identify those with a low likelihood of subclinical rejection (no need for surveillance biopsies) vs. those with a greater likelihood of harboring subclinical rejection (need closer monitoring that may include a surveillance or TruGraf informed biopsy).

- Positive TruGraf blood test is independently associated with worse transplant outcomes (higher risk of clinical acute rejection, *dnDSA* formation, trend towards more graft fibrosis and decrease in eGFR).
How is TruGraf Different?

- Stable Kidney Function
  - TruGraf
  - Tissue Gene Expression
  - Cell free DNA

- Graft Dysfunction
  - Invasive
  - Noninvasive
Proposed Clinical Algorithm
Sub-clinical Rejection Detected by Both Surveillance Biopsy and a Peripheral Blood Molecular Biomarker Correlates with \textit{de novo} Donor Specific Antibody following Kidney Transplant


Mayo Clinic, Northwestern Medicine, Scripps Health, Cleveland Clinic, Medical University of South Carolina, National Institute of Allergy and Infectious Diseases, Rho Federal
Disclosures

• JJJ, SMK, TCW and MMA paid consults
• JJJ, SMK and MMA have equity interests in Transplant Genomics, Inc
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Background

• Kidney transplant rejection can be associated with de novo DSA (dnDSA), which in turn is associated with graft loss. Currently only invasive surveillance biopsies can detect subclinical acute rejection (subAR)

• The objective of this study is to determine the correlation between subAR and the development of dnDSA
Methods

• 307 kidney transplant recipients prospectively enrolled between 2011 and 2014 at 5 US centers followed for 24 mths

• Surveillance biopsies at 2-4, 12 and 24 months

• Precise clinical phenotypes (PCP) were used to define subAR:
  • (SubAR) Banff > borderline changes and/or antibody mediated rejection)
  • (TX) The control group consisted of patients with normal surveillance biopsies: Banff i=0 and t=0, g=0, ptc=0; ci=0 or 1 and ct=0 or 1
  • All patients had stable renal function
Methods

• Peripheral blood paired with surveillance biopsies was used to generate a gene expression profile (GEP) predictive of subAR

• Based on serial assessments of PCP or GEP, patients were assigned to 3 groups:
  • Group 1- subAR only
  • Group 2- >1 instance of subAR
  • Group 3- no subAR
Results

• 136 (24.7%) of protocol biopsies were classified as SubAR (79% ‘borderline changes’, 21% ≥1A rejection)

• Tables and Figures illustrate the correlation of PCP and GEP with development of dnDSA

• dnDSA occurrence is shown for anytime during the 24 month observation period or only during the first post-transplant year
Distribution of PCP and GEP between Groups

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (SubAR only)</th>
<th>Group 2 (≥ 1 SubAR)</th>
<th>Group 3 (No SubAR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precise Clinical Profile</td>
<td>12%</td>
<td>51%</td>
<td>37%</td>
</tr>
<tr>
<td>Gene Expression Profile</td>
<td>11%</td>
<td>41%</td>
<td>48%</td>
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</table>
### Association between de novo DSA development and the Clinical Phenotype

<table>
<thead>
<tr>
<th>Clinical Phenotype at any time post-tx</th>
<th>subAR only (N=33)</th>
<th>TX only (N=146)</th>
<th>p-value</th>
<th>≥1 subAR (N=107)</th>
<th>TX only (N=146)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSA Class 1</td>
<td>18.2%</td>
<td>4.1%</td>
<td>0.01</td>
<td>8.4%</td>
<td>4.1%</td>
<td>0.15</td>
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<tr>
<td>DSA Class 2</td>
<td>21.2%</td>
<td>5.5%</td>
<td>0.008</td>
<td>19.6%</td>
<td>5.5%</td>
<td>0.0005</td>
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</table>

<table>
<thead>
<tr>
<th>Clinical Phenotype within Year 1</th>
<th>subAR only (N=35)</th>
<th>TX only (N=162)</th>
<th>p-value</th>
<th>≥1 subAR (N=81)</th>
<th>TX only (N=162)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>DSA Class 1</td>
<td>17.1%</td>
<td>3.7%</td>
<td>0.009</td>
<td>11.1%</td>
<td>3.7%</td>
<td>0.02</td>
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<tr>
<td>DSA Class 2</td>
<td>20.0%</td>
<td>6.8%</td>
<td>0.02</td>
<td>19.8%</td>
<td>6.8%</td>
<td>0.002</td>
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# Association between de novo DSA development and the GEP

<table>
<thead>
<tr>
<th>GEP at any time post-tx</th>
<th>subAR only (N=32)</th>
<th>TX only (N=134)</th>
<th>p-value</th>
<th>≥1 subAR (N=116)</th>
<th>TX only (N=134)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>DSA Class 1</td>
<td>18.8%</td>
<td>4.5%</td>
<td>0.01</td>
<td>7.8%</td>
<td>4.5%</td>
<td>0.28</td>
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<tr>
<td>DSA Class 2</td>
<td>18.8%</td>
<td>6.7%</td>
<td>0.04</td>
<td>17.2%</td>
<td>6.7%</td>
<td>0.01</td>
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<table>
<thead>
<tr>
<th>GEP within Year 1</th>
<th>subAR only (N=34)</th>
<th>TX only (N=148)</th>
<th>p-value</th>
<th>≥1 subAR (N=91)</th>
<th>TX only (N=148)</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>DSA Class 1</td>
<td>14.7%</td>
<td>4.1%</td>
<td>0.03</td>
<td>8.8%</td>
<td>4.1%</td>
<td>0.13</td>
</tr>
<tr>
<td>DSA Class 2</td>
<td>17.7%</td>
<td>9.5%</td>
<td>0.22</td>
<td>13.2%</td>
<td>9.5%</td>
<td>0.37</td>
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</table>
Association of Clinical Phenotypes with dnDSA anytime Post Transplant

% of subjects

0.008
0.01
0.0005

0 5 10 15 20 25

Tx only (n=146) ≥1 subAR (n=107) subAR only (n=33)

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MAYO CLINIC
Association between dnDSA with Clinical Phenotype within 1 year

% of Subjects

Tx only (n=162) ≥1 subAR (n=81) subAR only (n=35)

DSA class I DSA class II

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Association of GEP with dnDSA anytime Post Transplant

% of subjects

0.04
0.01
0.01

Tx only (n=134)  ≥1 subAR (n=116)  subAR only (n=34)

DSA class I  DSA class II

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Association between dnDSA and GEP within 1 year

% of subjects

0.03

Tx only (n=148) ≥1 subAR (n=91) subAR only (n=34)

DSA class I DSA class II
Conclusions

• During the 24-month observation study, both the clinical phenotype and peripheral blood gene expression profile for subAR (which includes Banff borderline changes) were found to be associated with the development of dnDSA
Acknowledgements

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  • John Friedewald
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  • David Ikle
  • Brian Armstrong
Discussion & Questions
THANK YOU

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