Molecular diagnosis of rejection in renal transplant biopsies: new insights into outcomes

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Disclosures:

- My presentation is not influenced by any financial considerations
- I have had research, consulting, or clinical trials agreements with Astellas, Novartis, and One Lambda
- I hold shares in TSI, a University spinoff company
- My presentation does not mention off-label or investigational use of drugs

The problem with biopsies

Conventional phenotyping (histology-C4d-HLA antibody) is not good enough

The promise

- adding molecular phenotyping will make assessment quantitative, reproducible, and standardized, particularly for prognosis and theranostics.
- This will improve outcomes (and aid discovery of biomarkers)
Approaching an unknowable truth:
the actual state of a tissue

**Do not expect**

**perfect agreement**

<table>
<thead>
<tr>
<th>True disease state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular phenotype</td>
</tr>
<tr>
<td>Body fluid “biomarkers”</td>
</tr>
</tbody>
</table>

Histology phenotype, DSA

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7 papers recently published outline the Molecular Microscope approach

<table>
<thead>
<tr>
<th>Journal</th>
<th>Title</th>
</tr>
</thead>
</table>

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The problem and the solution

- Limitations of conventional biopsy diagnostics
- Developing the Reference Set
- Development of the Molecular Microscope
- Case studies
- Implications for nephrology
The problem and the solution

- **Limitations of conventional biopsy diagnostics:**
  - The example of antibody-mediated rejection (ABMR)

- Developing the Reference Set
- Development of the Molecular Microscope
- Case studies
- Implications for nephrology

**The Significance of the Anti-Cl Class I Antibody Response**

**Transplantation 49:85-91, 1990**

**Description of antibody mediated rejection:**

- Microcirculation lesions with DSA
- Distinct from T cell-mediated lesions

**Antibody-mediated rejection (ABMR)**

- Discovered as microcirculation lesions plus DSA
  - Subtle lesions: hard to diagnose
- Complement factor C4d staining emerged later
- ABMR definition: histology lesions, DSA, C4d staining
  - Type 1: early-presensitized
    - Type 2: late-de novo, usually after one year
- "Active" or "inactive" e.g. double contours, scarring
- Difficult to diagnose: all 3 features have problems with intra- and inter-center variation, standardization
  - C4d staining has two methods, much local variation, false +ve, -ve
  - DSA measurement platforms: limited standardization, not quantitative; often present with no phenotype
UNMET NEED

Biopsy diagnostic systems are not enough for evidence-based medicine

Precision diagnostics:
An idea whose time has come: new dimensions, data-driven

- Centralised high throughput platforms are beginning to impact cancer management in breast and colon cancer to extend opinion-based assessment
- Compare the unknown biopsy to a Reference Set
  - Oncotype DX, Mammaprint
- Main objective: prognosis and guidance for therapy

The problem and the solution

- Limitations of conventional biopsy diagnostics
- Developing the Reference Set
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- Implications for nephrology
INTERCOM and INTERCOM2: prospective studies to calibrate and validate the molecular diagnostic tools developed in the Genome Canada study

ClinicalTrials.gov NCT01299168

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Roche Molecular Systems, Roche Canada
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Muttart Chair in Clinical Immunology

Special thanks to our clinical collaborators
Special thanks to our patients

 approach to molecular phenotyping biopsy

- Focus on indication biopsies: phenotype
- Granularity: clinical, lab, histology, molecular
- Annotate the molecules: pathogenesis-based transcript sets
  - http://atagc.med.ualberta.ca/
- Correct the conventional classification: a new reference standard pathology classification
  - http://atagc.med.ualberta.ca/
- Discover the molecular classes - crossvalidate
- Validate in new biopsy set
- Engineer the reporting system
- Calibrate the readouts: real time clinical meaning
Histology-microarray comparisons: two prospective consented studies of indication kidney transplant biopsies

Genome Canada: 2005 to present: 403 biopsies ("BFC403"), 315 patients
INTERCOM: 2011-present: 300 biopsies (INT300) in 264 patients

Combined: 703 biopsies in 579 patients, 122 failures
(Beginning: INTERCOM2 2014)

Plus many other biopsies
- Paris collaboration (Alexandre Loupy, Carmen Lefaucheur):
  150 presensitized pts: type 1 ABMR
- Hannover: 105 6w protocol biopsies
- Edmonton heart transplants: 106 biopsies
- One hour biopsies to predict renal function

The Genome Canada and INTERCOM studies taught us much about the conventional phenotype

For example
- Principal cause of loss is ABMR
- Much of ABMR is C4d negative
- Result: a new Reference Standard biopsy classification
- Non-adherence is common after one year

Antibody-Mediated Microcirculation Injury Is the Major Cause of Late Kidney Transplant Failure


AJT 9:2520-2531, 2009: Many kidneys that fail with ABMR are C4d negative and are not diagnosed (or treated)
Unmet need: pathologists struggle with the diagnosis of ABMR in excellent centers

<table>
<thead>
<tr>
<th>Histology-DSA diagnosis</th>
<th>N</th>
<th>Clear statement in the final diagnosis</th>
<th>Statement of suspicion in the final diagnosis</th>
<th>Features noted only in the text</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ABMR or mixed</td>
<td>46</td>
<td>16</td>
<td>9</td>
<td>19</td>
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<tr>
<td>C4d+ABMR</td>
<td>13</td>
<td>9</td>
<td>1</td>
<td>3</td>
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<tr>
<td>C4d-ABMR</td>
<td>27</td>
<td>8</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Mixed</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>1</td>
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<tr>
<td>TCMR</td>
<td>32</td>
<td>29</td>
<td>1</td>
<td>2</td>
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</tbody>
</table>


Censored at 3 years post-bx

In 703 unselected prospective indication biopsies, when C4d-ve and +ve ABMR are included, ABMR and mixed rejection are the highest risk for death censored graft loss

In 703 indication biopsies, most ABMR is diagnosed in late biopsies (>1y)
Combined INT/BFC. 703 biopsies, 562 patients, 164 biopsies leading to failures (all biopsies shown here). 127 patients had kidneys that failed.

Some ABMR survives much later than 3 years: remission?

Red = ABMR
X=failure

Adherent 52%
Non-adherent 48%

ABMR 47%
Probable ABMR 13%
Mixed 5%
GN 13%
Medical conditions 10%
PVN 7%
Missing Data 5%

65% ABMR, probable ABMR, or mixed

Attribution of causes to failures (N=60 failures)


Graft survival after indication biopsy is severely affected by non adherence

Einecke et al manuscript in preparation Nov 2013
The problem and the solution

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The molecular microscope

A central diagnostic system that uses microarrays to measure transcript expression in a biopsy and compare it with the Reference set

Uses predefined measurements:
- T cell-mediated rejection (TCMR) score
- Antibody-mediated rejection (ABMR) score
- Atrophy-scarring score: extent of chronic damage
- Prognosis: Risk score: progression to failure
- Acute kidney injury (AKI) score: -(AKI Score = Risk Score)
- Probability of non-adherence: classifier under development

Emergence of the Risk Score

The molecules induced reflect parenchymal injury, not fibrosis
The classifier uses mainly the molecules of parenchymal injury: e.g. ITGB6, VCAN, NNMT.

New concept: progression is due to injury induced by disease ("nephron distress"), not relentless fibrosis.

Risk Score: predictor of future risk trained on all failures: molecular signal: nephron distress from disease.

Low risk score

High risk score

Emergence of the AKI Score

The molecules induced in acute kidney injury are also expressed in progressing chronic kidney disease and strongly predict survival.
Defining the acute injury signal in the biopsy
“The response to wounding”

The transcripts associated with nephron distress:
- e.g. ITGB6, VCAN, NNMT

Some AKI “biomarkers” e.g. HAVCR1 (“KIM1”)

Life is a little more complex with AKI

Histology cannot detect the injury response

All patients (n=315)

IRRAT score median = 0.323

Low Risk Score
Low AKI

High AKI
High Risk Score

The AKI signal predicts progression: “the response to wounding”, including “inflammation”

The TCMR Score

Correlates with inflammation (i-), tubulitis (t-) lesions
Agrees with most histology TCMR diagnoses
Changes diagnosis in 25% of biopsies, mostly in situations where histology is weak
- inflammation due to tissue injury: histology false +ve
- scarring: histology false +ve
- BK virus nephropathy: histology false -ve

No effect of TCMR on graft survival
### Top 30 molecules selected in 1000 class comparisons in the TCMR classifier algorithm

<table>
<thead>
<tr>
<th>Description</th>
<th>TCMR</th>
<th>Left out</th>
<th>Others</th>
<th>Proportion of times used by classifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD96 antigen</td>
<td>34</td>
<td>21</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>signal-regulatory protein beta 2</td>
<td>77</td>
<td>38</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>tumor necrosis factor (ligand) superfamily, member 8</td>
<td>73</td>
<td>41</td>
<td>31</td>
<td>27</td>
</tr>
<tr>
<td>B and T lymphocyte associated</td>
<td>54</td>
<td>29</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>Similar to Olfactory receptor 2I2 (LOC346170)</td>
<td>118</td>
<td>72</td>
<td>53</td>
<td>46</td>
</tr>
<tr>
<td>interleukin 21 receptor</td>
<td>50</td>
<td>28</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>CD28 antigen</td>
<td>27</td>
<td>16</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Transcribed sequences</td>
<td>61</td>
<td>35</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>gamma interferon (human)</td>
<td>66</td>
<td>36</td>
<td>26</td>
<td>23</td>
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<tr>
<td>protein tyrosine phosphatase, non-receptor type 7</td>
<td>131</td>
<td>81</td>
<td>63</td>
<td>56</td>
</tr>
<tr>
<td>interleukin 6 receptor</td>
<td>53</td>
<td>28</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Src-like-adaptor</td>
<td>152</td>
<td>90</td>
<td>69</td>
<td>62</td>
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<tr>
<td>B and T lymphocyte associated</td>
<td>58</td>
<td>32</td>
<td>26</td>
<td>24</td>
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<tr>
<td>TOX high mobility group box family member 2</td>
<td>184</td>
<td>118</td>
<td>88</td>
<td>69</td>
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<tr>
<td>interleukin 12 receptor, beta 1</td>
<td>78</td>
<td>49</td>
<td>37</td>
<td>27</td>
</tr>
<tr>
<td>programmed cell death 1 ligand 1</td>
<td>143</td>
<td>79</td>
<td>53</td>
<td>34</td>
</tr>
<tr>
<td>CD8 antigen, alpha polypeptide (p32)</td>
<td>426</td>
<td>211</td>
<td>109</td>
<td>65</td>
</tr>
<tr>
<td>phospholipase A2, group IID</td>
<td>84</td>
<td>52</td>
<td>44</td>
<td>43</td>
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<tr>
<td>thymocyte selection pathway associated</td>
<td>25</td>
<td>15</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Src-like-adaptor</td>
<td>252</td>
<td>148</td>
<td>106</td>
<td>86</td>
</tr>
</tbody>
</table>

TCMR landscape in the 403 set:
top TCMR transcripts (red) in relationship to IFNG effects, the inflammasome, and parenchymal injury and dedifferentiation changes

The TCMR hierarchy mapped in 403 biopsies is preserved in the INT300 validation set
The ABMR score correlates with microcirculation lesions in the 403 indication biopsies

<table>
<thead>
<tr>
<th>Median time of biopsy post transplant (months)</th>
<th>0.47***</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Mean histologic lesion scores</th>
<th>ABMR score</th>
<th>ABMR score</th>
<th>Gamma statisticB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median time of biopsy post transplant (months)</td>
<td>0.47***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABMR related lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peritubular capillaritis ptc-score</td>
<td>1.41</td>
<td>0.23</td>
<td>0.87***</td>
</tr>
<tr>
<td>Glomerular g-score</td>
<td>0.84</td>
<td>0.16</td>
<td>0.77***</td>
</tr>
<tr>
<td>Tubulointerstitial u-score</td>
<td>1.15</td>
<td>0.18</td>
<td>0.80***</td>
</tr>
<tr>
<td>TCMR/ABMR related lesion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intimal arteritis v-score</td>
<td>0.16</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td>TCMR related lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intimal fibrous cv-score</td>
<td>1.20</td>
<td>1.09</td>
<td>0.36***</td>
</tr>
<tr>
<td>Tubular atrophy t-score</td>
<td>1.72</td>
<td>1.15</td>
<td>0.52***</td>
</tr>
<tr>
<td>Intimal fibrous cv-score</td>
<td>1.28</td>
<td>1.11</td>
<td>0.22**</td>
</tr>
<tr>
<td>Arteriole hyaline ah-score</td>
<td>1.41</td>
<td>0.86</td>
<td>0.30***</td>
</tr>
</tbody>
</table>

Understanding outliers

- TCMR Score +ve Histology -ve
  - Histo false -ve due to scarring
  - Histo false -ve in PVN
  - (Potential TCMR score false +ve?)
- TCMR Score -ve Histology +ve
  - Histo false +ve:
    - t-, i-scores caused by AKI or renal diseases
    - Some "isolated" v-lesions
- All borderlines

The ABMR Score

Molecular signal: Microcirculation remodelling, NK cells, IFNG effects (but not IFNG expression)
More subtle than TCMR
Correlates with g-, ptc-, cg-lesions (and ci)
Correlates with DSA and diagnosis of ABMR
Similar in C4d+ve and C4d-ve
Strongly predicts future failure
Some discrepancies e.g. some in GN, IFTA
  - False positives or missed diagnoses?

The signal in ABMR is endothelial cell injury response, plus NK and IFNG –inducible transcripts
The ABMR score correlates with microcirculation lesions in the 403 indication biopsies

<table>
<thead>
<tr>
<th>Mean histologic lesion scores</th>
<th>ABMR score &gt; 0.2 [n=90]</th>
<th>ABMR score &lt;0.2 [n=313]</th>
<th>Gamma statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portulacaria Tipo: pt-score</td>
<td>1.41</td>
<td>0.23</td>
<td>0.89***</td>
</tr>
<tr>
<td>Glomerular: g-score</td>
<td>0.84</td>
<td>0.16</td>
<td>0.77***</td>
</tr>
<tr>
<td>Transplant glomerulopathy: t-score</td>
<td>1.15</td>
<td>0.18</td>
<td>0.60***</td>
</tr>
<tr>
<td>Intestinal inflammation: i-score</td>
<td>0.55</td>
<td>0.43</td>
<td>0.27</td>
</tr>
<tr>
<td>Tubular: t-score</td>
<td>0.66</td>
<td>0.63</td>
<td>0.15</td>
</tr>
<tr>
<td>Tubular atrophy: a-score</td>
<td>0.16</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>Interstitial fibrosis: ci-score</td>
<td>1.72</td>
<td>1.00</td>
<td>0.50***</td>
</tr>
<tr>
<td>Tubular atrophy: ct-score</td>
<td>1.72</td>
<td>1.15</td>
<td>0.32***</td>
</tr>
<tr>
<td>Interstitial fibrous: cv-score</td>
<td>1.38</td>
<td>1.11</td>
<td>0.22</td>
</tr>
<tr>
<td>Intimal arteritis: v-score</td>
<td>1.41</td>
<td>0.86</td>
<td>0.30***</td>
</tr>
</tbody>
</table>

J. Reeve and P. F. Halloran, unpublished analysis of 703 biopsy population plus controls.
The ABMR Score strongly predicts survival after indication biopsies

If score is low, no failure in first year

More details Saturday afternoon at 3:00-3:30
See also abstract 3807 Saturday 5:18 Rm 206

ABMR score: Powerful predictor of future failure
Even down to 0.05

J. Reeve, Andre Periera, and P F Halloran, unpublished analysis

N=597 (BK and GN excluded)

ABMR Score vs. Conventional diagnosis of ABMR
Losses in first year
Conventional variables except eGFR, proteinuria drop out and are replaced by molecular measurements AKI Score and ABMR score

Multivariable analysis of survival

Variables included:
Multivariable Cox regression for death censored graft survival in 703 indication biopsies, one random biopsy per patient (no biopsy exclusions)

<table>
<thead>
<tr>
<th>Variables included</th>
<th>N(all) = 562</th>
<th>N(late) = 342</th>
</tr>
</thead>
<tbody>
<tr>
<td>log10(Time post Tx)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRRATs</td>
<td></td>
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</tr>
<tr>
<td>ABMRprobB</td>
<td></td>
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<tr>
<td>GFR (CG)</td>
<td>i 6 6</td>
<td></td>
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<tr>
<td>ci</td>
<td>c 20 6</td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>t 7 6</td>
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<td>cl</td>
<td>20 6</td>
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<td>g</td>
<td>7 5</td>
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<tr>
<td>cg</td>
<td>9 5</td>
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<tr>
<td>v</td>
<td>v 22 14</td>
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<td>cv</td>
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<td>mm</td>
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<td>ptc</td>
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<td>GFR (CG)</td>
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<tr>
<td>ptc</td>
<td>ptc 20 18</td>
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</tbody>
</table>

Backward elimination
All (from 703, 1 random per)

<table>
<thead>
<tr>
<th>n=562, f=118</th>
<th>Exp (coef)</th>
<th>lower</th>
<th>upper</th>
<th>P-value</th>
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<tbody>
<tr>
<td>log10(Time post Tx)</td>
<td>2.85</td>
<td>2.11</td>
<td>3.85</td>
<td>7.7E-12</td>
</tr>
<tr>
<td>AKI signal (IRRAT)</td>
<td>2.29</td>
<td>1.73</td>
<td>3.01</td>
<td>4.7E-09</td>
</tr>
<tr>
<td>ABMR Score (ProbB)</td>
<td>4.33</td>
<td>2.17</td>
<td>9.44</td>
<td>5.5E-05</td>
</tr>
<tr>
<td>eGFR (C-Gault)</td>
<td>0.98</td>
<td>0.97</td>
<td>0.99</td>
<td>5.6E-03</td>
</tr>
</tbody>
</table>

Late (from 703, 1 random per)

<table>
<thead>
<tr>
<th>n=323, f=103</th>
<th>Exp (coef)</th>
<th>lower</th>
<th>upper</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis (ci score)</td>
<td>1.36</td>
<td>1.11</td>
<td>1.67</td>
<td>5.7E-03</td>
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<tr>
<td>AKI signal (IRRAT)</td>
<td>2.40</td>
<td>1.72</td>
<td>3.32</td>
<td>2.8E-10</td>
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<tr>
<td>ABMR Score (ProbB)</td>
<td>3.12</td>
<td>1.78</td>
<td>5.37</td>
<td>1.4E-03</td>
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<tr>
<td>eGFR (C-Gault)</td>
<td>0.98</td>
<td>0.97</td>
<td>0.99</td>
<td>2.2E-03</td>
</tr>
</tbody>
</table>

Proteinuria was not included because of missing values

(Jeff Reeve et al, unpublished, Nov 2013)
The problem and the solution

- Limitations of conventional biopsy diagnostics
- Developing the Reference Set
- Development of the Molecular Microscope

**Case studies**
- Implications for nephrology

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**Case: Illustrates a typical non-adherence triggered ABMR**

- Young male 5y post tx, non-adherent last year
- Now probably adherent but increasing proteinuria
- Saveable kidney: but what to do?

---

The problem and the solution

- Limitations of conventional biopsy diagnostics
- Developing the Reference Set
- Development of the Molecular Microscope
- Case studies

**Implications for nephrology**
- Experience with glomerulonephritis in transplants
The ABMR Score and AKI score strongly predict survival in indication biopsies with GN

Score correlates with presence of cg and g lesions

See abstract poster A Pereira et al Friday Morning

The ABMR Score is associated with impaired survival in GN

Proposal

• Incorporate the MM into diagnosis as an add-on to conventional tests
• Calibrate the classifier equations against real-time clinical features
• Eventually develop a decision tree that determines which tests (conventional, molecular) can be eliminated to reduce costs
The problem and the solution

• Limitations of conventional biopsy diagnostics
• Developing the Reference Set
• Development of the Molecular Microscope
• Case studies
• Implications for nephrology