Neutrophil CD64: Therapeutic Target for Sustained Remission during Infliximab Maintenance

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Waning Infliximab Durability

• >80% primary response to infliximab induction
• 50% will require dose intensification during maintenance infliximab to maintain response
• Probability of remaining on infliximab
  - 1 year, 81-93%
  - 3 years, 60-69%
  - 5 years, 48-59%

Hyams et al. IBD 2009
De Bie et al. APT 2011
Church et al. IBD 2014
Grossi et al. CGH 2015
Monitoring Infliximab Durability

- Patient-specific factors that affect infliximab durability
  - High inflammatory burden
  - Individual pharmacogenetics/pharmacokinetics
  - Drug levels and/or neutralizing antibodies to drug

- Can we combine these known factors to improve durability during maintenance?

Treat-to-Target
Inflammatory biomarker & drug levels
Potential Target: Neutrophil CD64

Fc³ Receptor I (CD64)
• High affinity surface receptor IgG1 & IgG3 (activating)
• Expressed monocytes/macrophages
• Upregulated by IFN³ (STAT1) on PMN’s
  – Phagocytosis, ROS, clearance of immune complexes

Previous investigation
• Significant up-regulation of Neutrophil CD64 (peripheral blood) in endoscopically active pediatric Crohn’s

Minar et al. IBD 2014
Hypothesis:

- Elevations in Neutrophil CD64 during clinical remission will be associated with a higher probability of clinical relapse
Primary outcome:
• Clinical relapse over 52 weeks (stratified by baseline Neutrophil CD64)

Inclusion criteria:
• Crohn’s disease subjects receiving infliximab maintenance therapy (e4 doses) in clinical remission
  - Short PCDAI <15
  - Not receiving prednisone
  - Stable infliximab dose (x2 infusions)
  - Monotherapy/dual therapy included
Testing Methods

• Clinical relapse
  – Short PCDAI e15 on 2 consecutive infusions
  – Crohn’s related hospitalization/surgery/abscess
  – Steroids started
  – Infliximab discontinued (+ drug antibodies)

• Neutrophil CD64 index\(^1\)
  – Peripheral blood
  – Flow cytometry (MFI & bead calibration) \(<\)1 hour prep to results
  – CD64 index \(\geq\)1 predictor for endoscopic activity\(^2\)

• Infliximab trough, µg/ml (ELISA)\(^3\)
  – Study entry, at relapse, at week 52

• Antibody to infliximab\(^4\)
  – Clinician-driven
  – Drug level (µg/ml); antibody (ng/ml)

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1. Leuko64. Trillium Diagnostics, LLC
2. Minar et al. IBD 2014
3. IDKmonitor®, Immundiagnostik AG
4. Esoterix Laboratory Services, INC. (ECLIA)
### Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Remission (=22)</th>
<th>Relapse (n=15)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>10 (46%)</td>
<td>5 (33%)</td>
<td>0.51</td>
</tr>
<tr>
<td>Age at diagnosis (years, mean)</td>
<td>11 (4)</td>
<td>12 (5)</td>
<td>0.26</td>
</tr>
<tr>
<td>Age at study entry (years, mean)</td>
<td>15 (4)</td>
<td>15 (5)</td>
<td>0.65</td>
</tr>
<tr>
<td>No. of infusions prior to entry (mean)</td>
<td>14 (8)</td>
<td>13 (9)</td>
<td>0.66</td>
</tr>
<tr>
<td>Time on IFX, prior to entry (months, mean)</td>
<td>25 (18)</td>
<td>18 (16)</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>Dose at entry (mg/kg, median)</strong></td>
<td>4.9 (3-12.5)</td>
<td>7.7 (3.7-11.6)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ileum (L1)</td>
<td>4</td>
<td>1</td>
<td>0.63</td>
</tr>
<tr>
<td>Colonic (L2)</td>
<td>0</td>
<td>2</td>
<td>0.16</td>
</tr>
<tr>
<td>Ileocolonic (L3)</td>
<td>18</td>
<td>12</td>
<td>1.00</td>
</tr>
<tr>
<td>Perianal location (p)</td>
<td>6</td>
<td>2</td>
<td>0.43</td>
</tr>
<tr>
<td>Crohn’s behavior</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory (B1)</td>
<td>13</td>
<td>9</td>
<td>1.00</td>
</tr>
<tr>
<td>Stricturing (B2)</td>
<td>2</td>
<td>3</td>
<td>0.38</td>
</tr>
<tr>
<td>Penetrating (B3)</td>
<td>7</td>
<td>2</td>
<td>0.26</td>
</tr>
<tr>
<td>Both penetrating/stricturing</td>
<td>0</td>
<td>1</td>
<td>0.41</td>
</tr>
<tr>
<td>Concomitant medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-mercaptopurine</td>
<td>1</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>2</td>
<td>1</td>
<td>1.00</td>
</tr>
<tr>
<td>5-ASA</td>
<td>6</td>
<td>1</td>
<td>0.20</td>
</tr>
<tr>
<td>Biomarker</td>
<td>Remission (n=22)</td>
<td>Relapsed (n=15)</td>
<td>p</td>
</tr>
<tr>
<td>-------------------------------</td>
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</tr>
<tr>
<td>Neutrophil CD64 index</td>
<td>0.64 (0.38-1.16)</td>
<td>1.04 (0.5-4.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ESR (mm/hr.)</td>
<td>10 (2-16)</td>
<td>13 (2-81)</td>
<td>0.2</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.29 (0.29-2.6)</td>
<td>0.31 (0.29-2.9)</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Albumin (gm/dL)</strong></td>
<td><strong>4 (0.32)</strong></td>
<td><strong>3.5 (0.3)</strong></td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Infliximab trough (µg/ml)</td>
<td>4.0 (3.8)</td>
<td>2.8 (1.8)</td>
<td>0.32</td>
</tr>
</tbody>
</table>

CD64, ESR, CRP shown as median (min-max); Albumin, infliximab trough shown as mean (sd)
**Cause for Relapse**

1. Short PCDAI e15 at 2 consecutive infusions (n=9)
2. Surgery/abscess (n=2)
3. IV steroids (n=1)
4. Antibody to infliximab/discontinued infliximab (n=3)
<table>
<thead>
<tr>
<th></th>
<th>CD64 &lt;1 (n=25)</th>
<th>CD64 ≥1 (n=12)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Received infliximab intensification</td>
<td>9/25 (36%)</td>
<td>4/12 (33%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Infliximab dose (mg/kg, mean)</td>
<td>6.4 (2.7)</td>
<td>7.5 (2.8)</td>
<td>0.26</td>
</tr>
<tr>
<td>Infliximab level μg/ml, mean</td>
<td>4 (3.6)</td>
<td>2.3 (1.8)</td>
<td>0.15</td>
</tr>
<tr>
<td>Antibody to infliximab*</td>
<td>9 (36%)</td>
<td>5 (42%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Albumin (g/dL, mean)</td>
<td>4 (0.32)</td>
<td>3.5 (0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/dL, median)</td>
<td>0.3 (0.3-2.6)</td>
<td>0.5 (0.3-2.9)</td>
<td>0.003</td>
</tr>
<tr>
<td>ESR (mm/hr., median)</td>
<td>10 (2-36)</td>
<td>15 (11-81)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*11/14 antibody to infliximab <100 ng/ml
ESR ≥10 mm/hour; log-rank = 0.09
CD64 Correlates with Relapse, Drug levels

Mean time to relapse 119(65) days

Spearman $r = -0.66$, $p = 0.007$

**ELISA**

- $r = -0.36$
- $p = 0.04$

**Esoterix**

- $r = -0.42$
- $p = 0.04$
## Multivariable Cox Regression Analysis

<table>
<thead>
<tr>
<th>Covariate (study entry)</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMN CD64 &gt;1</td>
<td>7.8</td>
<td>2.2-27</td>
<td>0.0013</td>
</tr>
<tr>
<td>Albumin &lt;3.7 gm/dL</td>
<td>3.7</td>
<td>0.94-15</td>
<td>0.06</td>
</tr>
<tr>
<td>Infliximab trough &lt; 1 μg/ml</td>
<td>3.1</td>
<td>0.61-15.7</td>
<td>0.17</td>
</tr>
</tbody>
</table>

All covariates met proportional hazards assumption
Conclusions

• Elevations in Neutrophil CD64 expression in asymptomatic patients is a significant risk factor for clinical relapse during infliximab maintenance

• Larger studies are needed, however, Neutrophil CD64 should be considered a potential Treat-to-Target biomarker in pediatric Crohn’s disease
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