Severity of the TGN1412 trial disaster cytokine storm correlated with IL-2 release.

Richard Stebbings
Six men remain in intensive care after being taken ill during a clinical drugs trial in north-west London.

The healthy volunteers were testing an anti-inflammatory drug at a research unit based at Northwick Park Hospital when they suffered a reaction.

Relatives are with the patients, who suffered multiple organ failure. Two men are said to be critically ill.
TGN1412 caused a cytokine release in volunteers

Sunthralingham et al 2006
TGN1412 investigation

• NIBSC (UK OMCL) received the trial material for testing
  – Not Contaminated and correctly formulated
  – Not Proinflammatory using standard in vitro tests
  – No Immunotoxicity in macaques (50 - 0.1 mg/kg)

• In vitro tests developed at NIBSC based upon immobilisation, that would have predicted the adverse response to TGN1412 in man.
Current Assay Format

- PBMC based assay, non-tissue culture treated 96 well polypropylene microtitre plates, coated for 1 hour with 1 µg well$^{-1}$ therapeutic mAb
- Negative controls: Human IgG1 and IgG4 Isotype controls, non-CRS inducing therapeutic mAbs
- Positive controls: Alemtuzumab, Muromonab-CD3, TGN1412 (weak, intermediate, strong) & Mitogen
- Cytokine release assayed by ELISA and Multiplex assay (Meso Scale Discovery)
Solid Phase

IFNγ

pg/ml

24hrs 48hrs 72hrs

IgG1 Control IgG4 Control Natalizumab Adalimumab Infliximab Etanercept Basiliximab Daclizumab Bevacizumab Trastuzumab Rituximab Alemtuzumab-Muromonab-CD3 TGN1412 Mitogen
Aqueous Phase

IL-2

pg/ml

24hrs
48hrs
72hrs

IgG1 Control
IgG4 Control
Natalizumab
Adalimumab
Etanercept
Basiliximab
Daclizumab
Bevacizumab
Trastuzumab
Rituximab
Alemtuzumab
Muromonab-CD3
TGN1412
Mitogen

* * *
Aqueous Phase

pg/ml

IGG1 Control
IGG4 Control
Natalizumab
Adalimumab
Infliximab
Etanercept
Basiliximab
Daclizumab
Bevacizumab
Trastuzumab
Rituximab
Alemtuzumab
Muromonab-CD3
TGN1412
Mitogen

IL-17

24hrs
48hrs
72hrs

*
Aqueous Phase

pg/ml

24hrs  48hrs  72hrs

TNFα

IgG1 Control  IgG4 Control  Natalizumab  Adalimumab  Infliximab  Etanercept  Basiliximab  Daclizumab  Bevacizumab  Trastuzumab  Rituximab  Alemtuzumab  Muromonab-CD3  TGN1412  Mitogen

*  ***  **  *
Aqueous Phase

IL-8

pg/ml

24hrs
48hrs
72hrs

IgG1 Control
IgG4 Control
Natalizumab
Adalimumab
Infliximab
Etanercept
Basilizumab
Dacizumab
Bevacizumab
Trastuzumab
Rituximab
Alemtuzumab
Muromonab-CD3
TGN1412
Mitogen

* ***
Solid Phase

IL-8

pg/ml

24hrs
48hrs
72hrs

IgG1 Control
IgG4 Control
Natalizumab
Adalimumab
Infliximab
Etanercept
Basiliximab
Daclizumab
Bevacizumab
Trastuzumab
Rituximab
Alemtuzumab
Muromonab-CD3
TGN1412
Mitogen
## Induction of Th2 cytokines IL-5 and IL-13 by TGN1412

MSD TH1/TH2 10-plex assay

<table>
<thead>
<tr>
<th>Therapeutic mAb</th>
<th>IFNγ</th>
<th>TNFα</th>
<th>IL-1β</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1 Control</td>
<td>59.4 pg ml⁻¹ (35.2 – 100)</td>
<td>726 pg ml⁻¹ (494 – 1066)</td>
<td>30.9 pg ml⁻¹ (20.0 - 47.9)</td>
<td>41.8 pg ml⁻¹ (34.1 - 51.1)</td>
<td>9.8 pg ml⁻¹ (8.8 - 10.9)</td>
<td>11.4 pg ml⁻¹ (10.5 - 12.3)</td>
</tr>
<tr>
<td>IgG4 Control</td>
<td>3.1 pg ml⁻¹ (2.6 - 3.6)</td>
<td>25.4 pg ml⁻¹ (16.3 - 39.7)</td>
<td>1.9 pg ml⁻¹ (1.2 - 3.2)</td>
<td>9.5 pg ml⁻¹ (7.2 - 12.5)</td>
<td>3.4 pg ml⁻¹ (2.8 - 4.0)</td>
<td>3.7 pg ml⁻¹ (2.2 - 6.2)</td>
</tr>
<tr>
<td>Rituximab</td>
<td>75.3 pg ml⁻¹ (26.7 – 212)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>33.3 pg ml⁻¹ (26.5 - 41.9)</td>
<td>9.7 pg ml⁻¹ (7.9 - 11.8)</td>
<td>11.5 pg ml⁻¹ (9.7 - 13.6)</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>88.8 pg ml⁻¹ (43.3 – 182)</td>
<td>1972 pg ml⁻¹ (1097 – 3547)</td>
<td>107 pg ml⁻¹ (41.0 - 280)</td>
<td>41.7 pg ml⁻¹ (35.2 - 49.5)</td>
<td>13.8 pg ml⁻¹ (10.8 - 17.7)</td>
<td>14.6 pg ml⁻¹ (11.6 - 18.3)</td>
</tr>
<tr>
<td>Muromonab-CD3</td>
<td>18013 pg ml⁻¹ (13598 – 23861)</td>
<td>9855 pg ml⁻¹ (7939 – 12235)</td>
<td>392 pg ml⁻¹ (166 – 926)</td>
<td>2781 pg ml⁻¹ (1453 – 5325)</td>
<td>41.0 pg ml⁻¹ (31.1 - 54.2)</td>
<td>498 pg ml⁻¹ (146 – 1693)</td>
</tr>
<tr>
<td>TGN1412</td>
<td>30748 pg ml⁻¹ (20767 – 45527)</td>
<td>11314 pg ml⁻¹ (10123 – 12644)</td>
<td>148 pg ml⁻¹ (103 – 212)</td>
<td>8600 pg ml⁻¹ (5229 – 14144)</td>
<td>54.0 pg ml⁻¹ (46.6 - 62.6)</td>
<td>1904 pg ml⁻¹ (1450 – 2502)</td>
</tr>
<tr>
<td>Mitogen</td>
<td>19491 pg ml⁻¹ (16164 – 23503)</td>
<td>6322 pg ml⁻¹ (4953 – 8070)</td>
<td>289 pg ml⁻¹ (194 – 431)</td>
<td>113 pg ml⁻¹ (51.2 – 247)</td>
<td>35.9 pg ml⁻¹ (29.4 - 43.9)</td>
<td>225 pg ml⁻¹ (109 – 465)</td>
</tr>
</tbody>
</table>
TGN1412 stimulates more IL-2 producing T cells
TGN1412 induces co-release of IL-2 by different T-cell subsets
Conclusions

• The severity of the adverse response to TGN1412 correlates with IL-2 release

• Stimulation of PBMC with immobilised TGN1412 replicates massive cytokine release in man

• Aqueous phase assay better suited to assess IL-8 and TNFα release for non-TGN1412 mechanisms of action
Acknowledgements

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• NIBSC staff for blood donations