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

Richard Stebbings
TGN1412 Phase I Trial disaster

Six taken ill after drug trials

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

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Aqueous Phase

pg/ml

IL-2

24hrs
48hrs
72hrs

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

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Solid Phase

IL-17

pg/ml

24hrs  48hrs  72hrs

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

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Induction of Th2 cytokines IL-5 and IL-13 by TGN1412

MSD TH1/TH2 10-plex assay

| Therapeutic mAb   | IFNγ  | TNFα   | IL-1β  | IL-2     | IL-4     | IL-5     |
|-------------------|-------|--------|--------|----------|----------|----------|
| IgG1 Control      | 59.4 pg ml⁻¹ (35.2 – 100) | 726 pg ml⁻¹ (494 – 1066) | 30.9 pg ml⁻¹ (20.0 - 47.9) | 41.8 pg ml⁻¹ (34.1 - 51.1) | 9.8 pg ml⁻¹ (8.8 - 10.9) | 11.4 pg ml⁻¹ (10.5 - 12.3) |
| IgG4 Control      | 3.1 pg ml⁻¹ (2.6 - 3.6) | 25.4 pg ml⁻¹ (16.3 - 39.7) | 1.9 pg ml⁻¹ (1.2 - 3.2) | 9.5 pg ml⁻¹ (7.2 - 12.5) | 3.4 pg ml⁻¹ (2.8 - 4.0) | 3.7 pg ml⁻¹ (2.2 - 6.2) |
| Rituximab         | 75.3 pg ml⁻¹ (26.7 – 212) | n.d. | n.d. | 33.3 pg ml⁻¹ (26.5 - 41.9) | 9.7 pg ml⁻¹ (7.9 - 11.8) | 11.5 pg ml⁻¹ (9.7 - 13.6) |
| Alemtuzumab       | 88.8 pg ml⁻¹ (43.3 – 182) | 1972 pg ml⁻¹ (1097 – 3547) | 107 pg ml⁻¹ (41.0 – 280) | 41.7 pg ml⁻¹ (35.2 - 49.5) | 13.8 pg ml⁻¹ (10.8 - 17.7) | 14.6 pg ml⁻¹ (11.6 - 18.3) |
| Muromonab-CD3     | 18013 pg ml⁻¹ (13598 – 23861) | 9855 pg ml⁻¹ (7939 – 12235) | 392 pg ml⁻¹ (166 – 926) | 2781 pg ml⁻¹ (1453 – 5325) | 41.0 pg ml⁻¹ (31.1 - 54.2) | 498 pg ml⁻¹ (146 – 1693) |
| TGN1412           | 30748 pg ml⁻¹ (20767 – 45527) | 11314 pg ml⁻¹ (10123 – 12644) | 148 pg ml⁻¹ (103 – 212) | 8600 pg ml⁻¹ (5229 – 14144) | 54.0 pg ml⁻¹ (46.6 - 62.6) | 1904 pg ml⁻¹ (1450 – 2502) |
| Mitogen           | 19491 pg ml⁻¹ (16164 – 23503) | 6322 pg ml⁻¹ (4953 – 8070) | 289 pg ml⁻¹ (194 – 431) | 113 pg ml⁻¹ (51.2 – 247) | 35.9 pg ml⁻¹ (29.4 - 43.9) | 225 pg ml⁻¹ (109 – 465) |
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
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• Eastwood et al (2013) Brit. J. Clin. Pharmacol. 76:299-315.