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

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Medicines and Healthcare Products Regulatory Agency

TGN1412 Phase I Trial disaster

LIVE | BBC NEWS CHANNEL

Last Updated: Wednesday, 15 March 2006, 09:52 GMT

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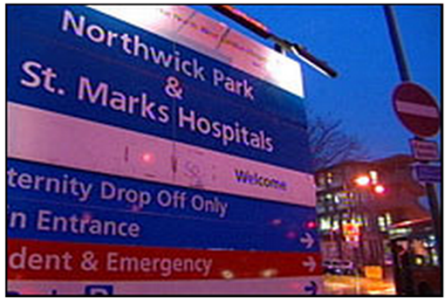
Six taken ill after drug trials

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



The six are being treated at Northwick Park hospital

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.

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First for Breaking News

Drug Trial Victim Set To Lose Fingers

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8:59pm UK, Sunday April 16, 2006

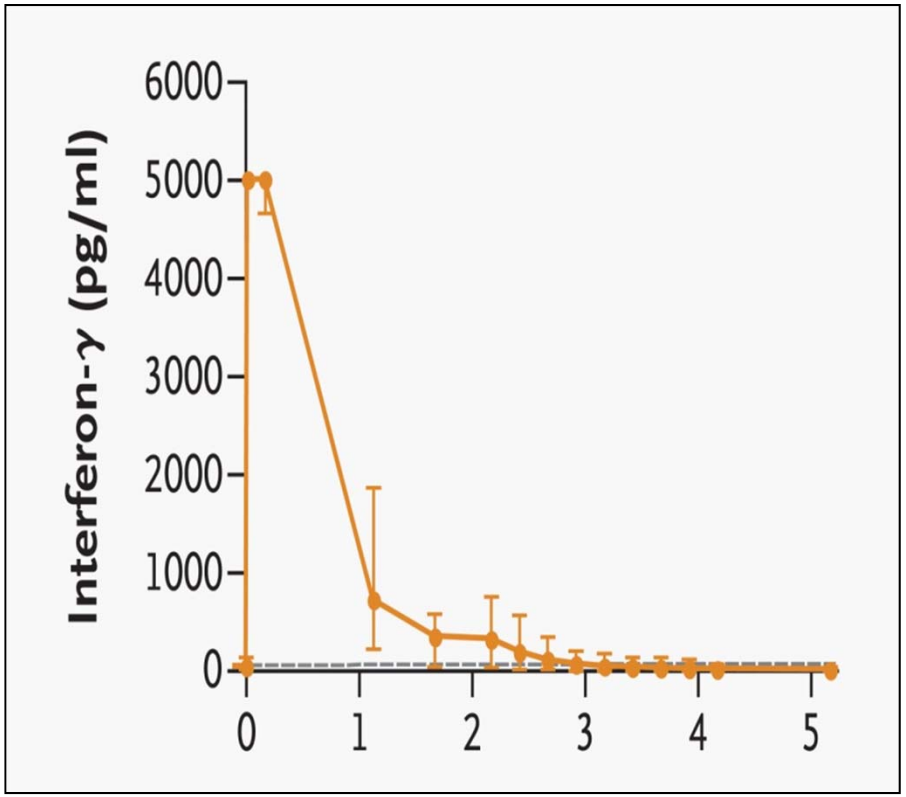
A 20-year-old man who suffered horrific side-effects during a drug trial says he is to lose his fingers and toes.Ryan Wilson, of London, was one of six previously healthy men whose heads and bodies swelled up after taking the drug last month.

Medics did not expect him to wake up when he slipped into a coma but he has now returned to consciousness.

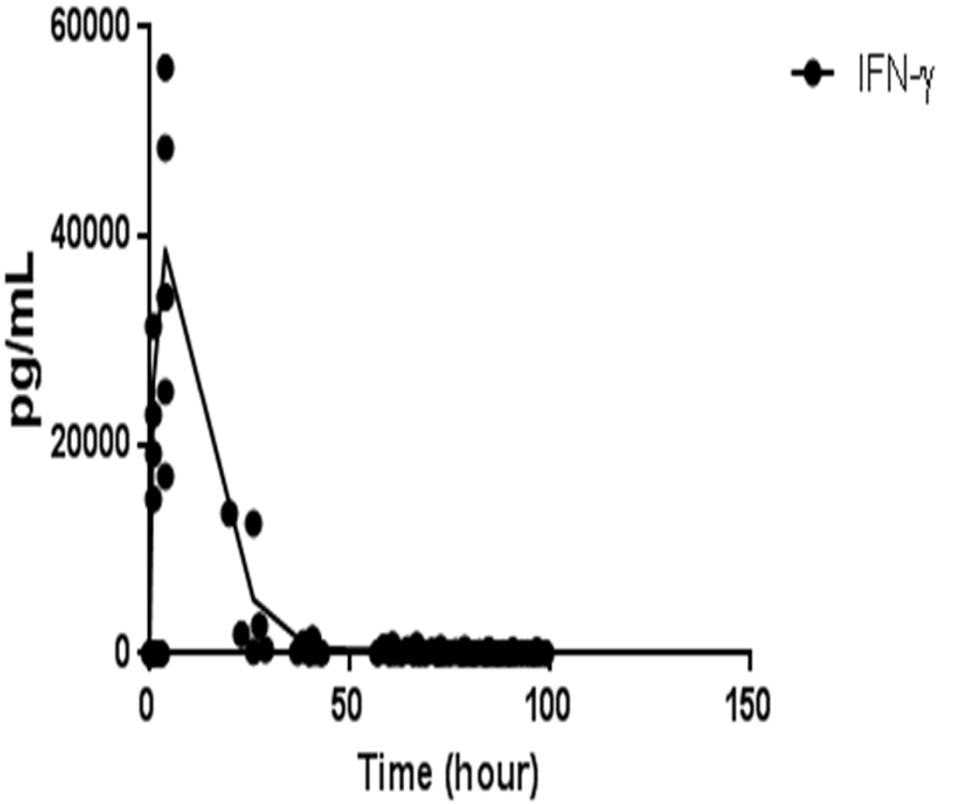
He said he endured heart failure, kidney failure, pneumonia, septicaemia, liver failure and was ventilated on 99% oxygen.

He told the News of the World from his hospital bed that he had awakened to find his fingers and toes swollen and rotting.

TGN1412 caused a cytokine release in volunteers



Sunthralingham et al 2006



TGN1412 investigation

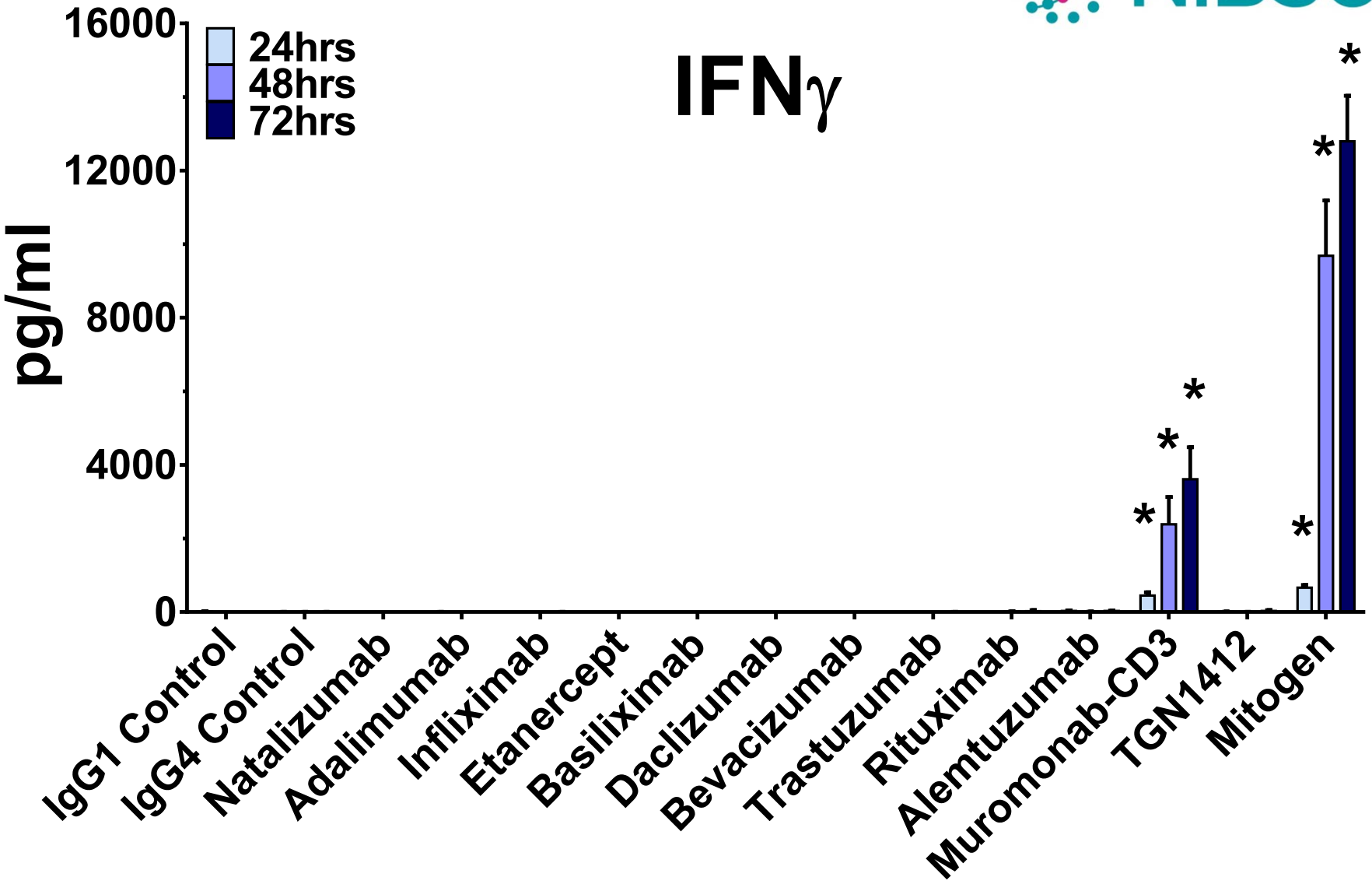
- **NIBSC (UK OMCL) received the trial material for testing**
  - **Not Contaminated and correctly formulated**
  - **Not Proinflammatory using standard *in vitro* tests**
  - **No Immunotoxicity in macaques (50 - 0.1 mg/kg)**

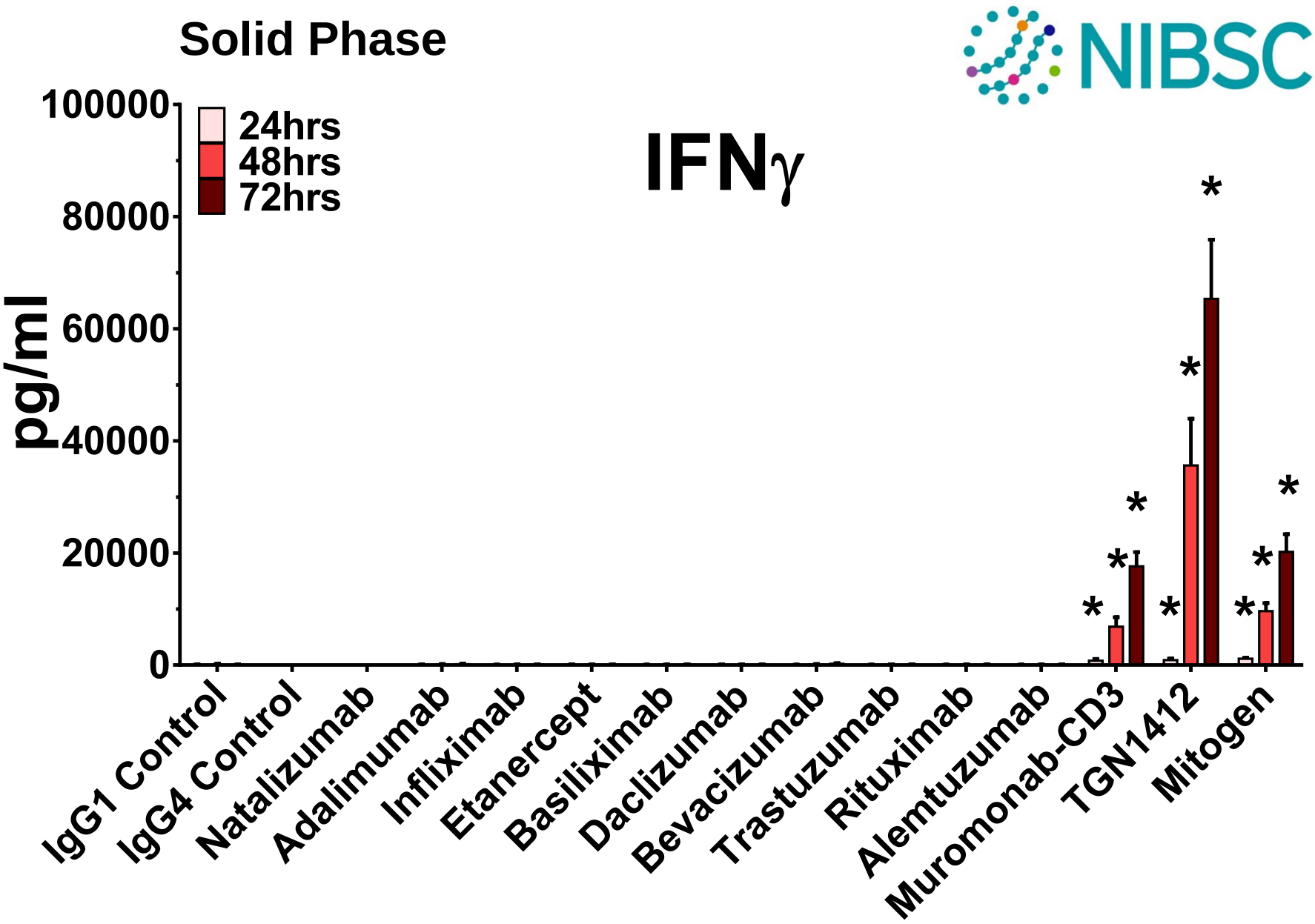
- **In vitro tests developed at NIBSC based upon immobilisation, that would have predicted the adverse response to TGN1412 in man.**

Current Assay Format

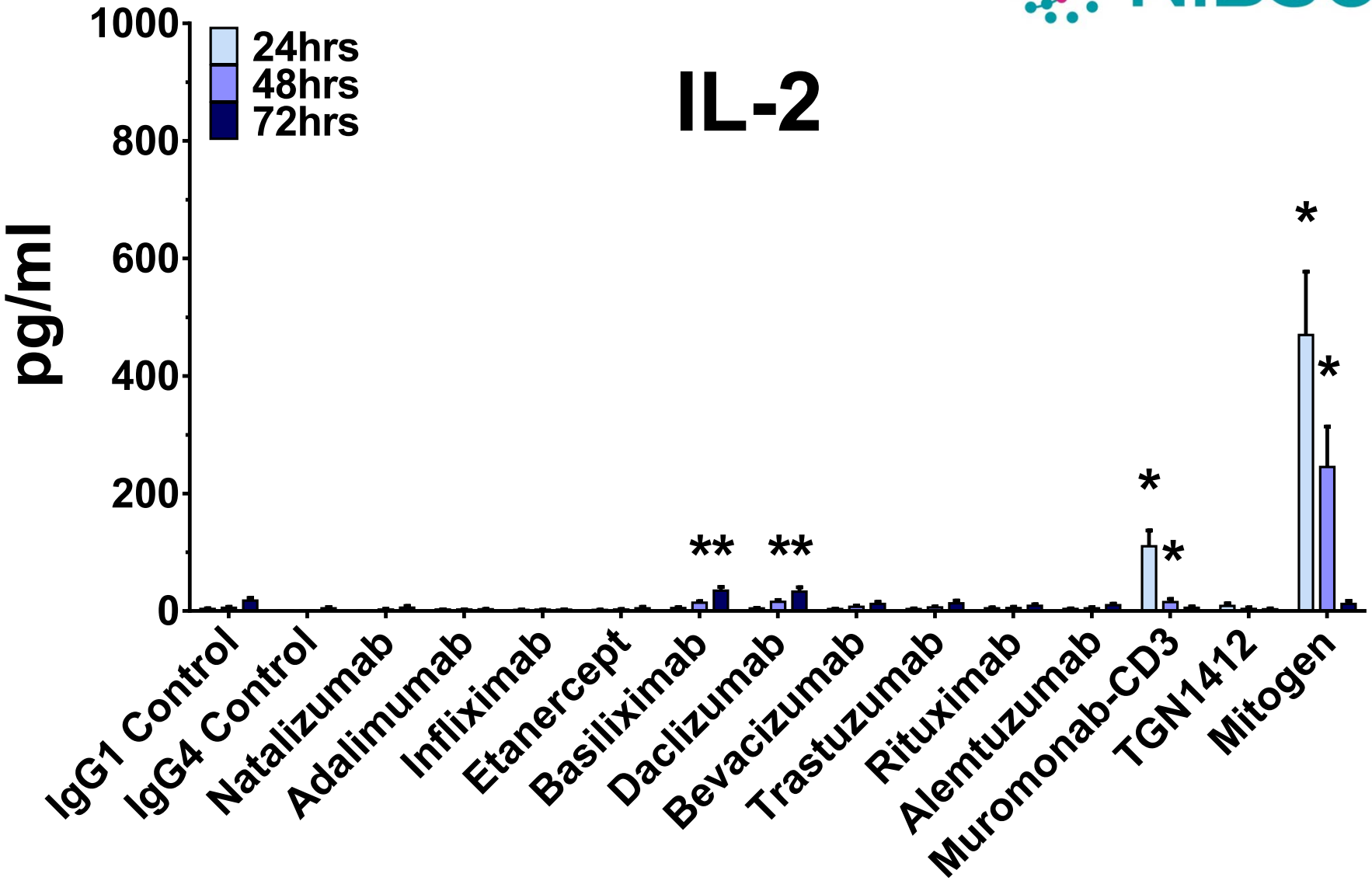
- PBMC based assay, non-tissue culture treated 96 well polypropylene microtitre plates, coated for 1 hour with 1 µg well^{-1} therapeutic mAb
- Negative controls: Human IgG1 and IgG4 Isotype controls, non-CRS inducing therapeutic mAbs
- Positive controls: Alemtuzumab, Muromonab-CD3, TGN1412 (weak, intermediate,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

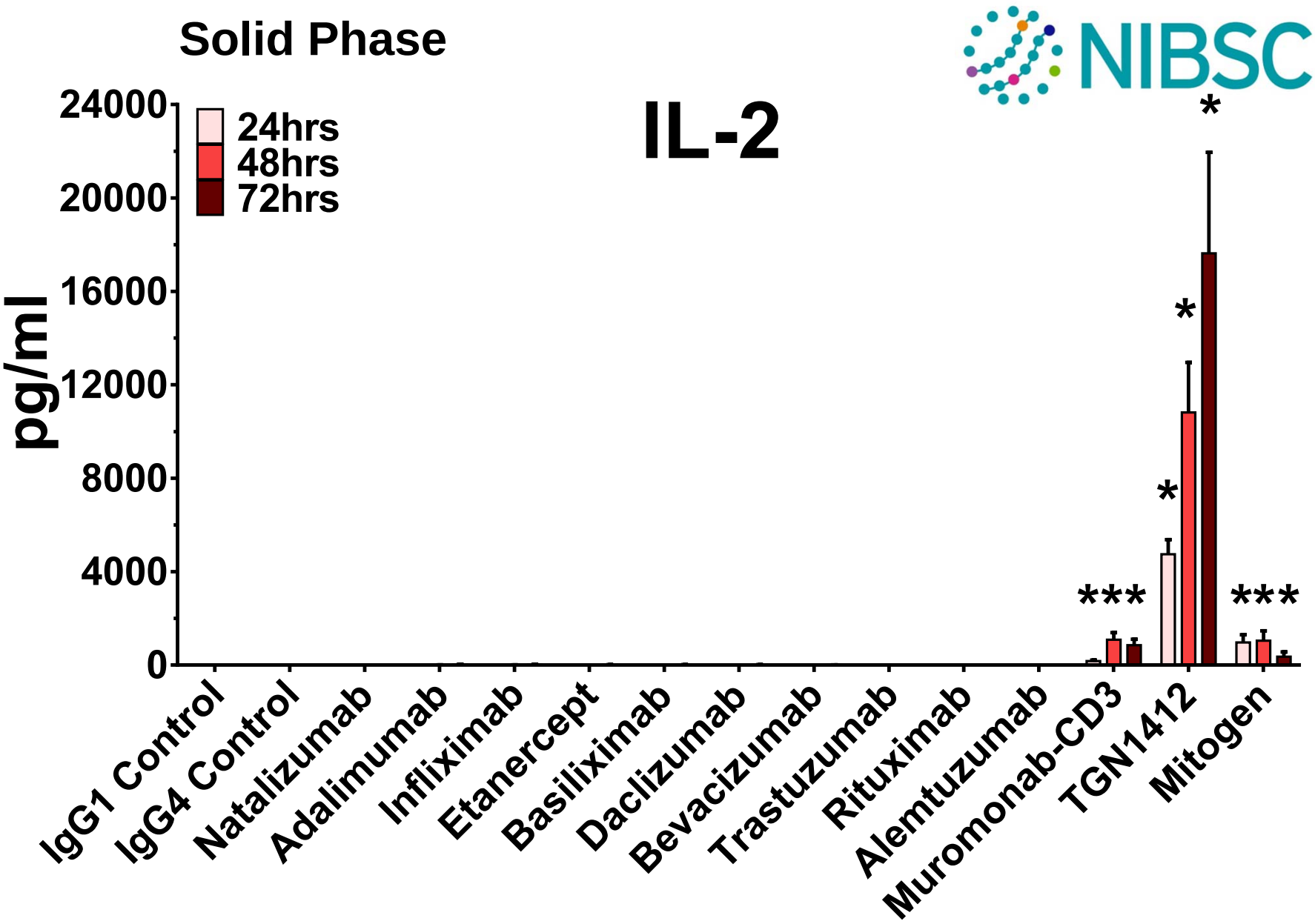
Aqueous Phase




Solid Phase
NIBSC
IFNγ
24hrs
48hrs
72hrs
pg/ml
100000
80000
60000
40000
20000
0
IgG1 Control
IgG4 Control
Natalizumab
Adalimumab
Infliximab
Etanercept
Basiliximab
Daclizumab
Bevacizumab
Trastuzumab
Rituximab
Alemtuzumab
Muromonab-CD3
TGN1412
Mitogen

Aqueous Phase




Solid Phase
IL-2
24hrs
48hrs
72hrs
pg/ml
24000
20000
16000
12000
8000
4000
0
IgG1 Control
IgG4 Control
Natalizumab
Adalimumab
Infliximab
Etanercept
Basiliximab
Daclizumab
Bevacizumab
Trastuzumab
Rituximab
Alemtuzumab
Muromonab-CD3
TGN1412
Mitogen
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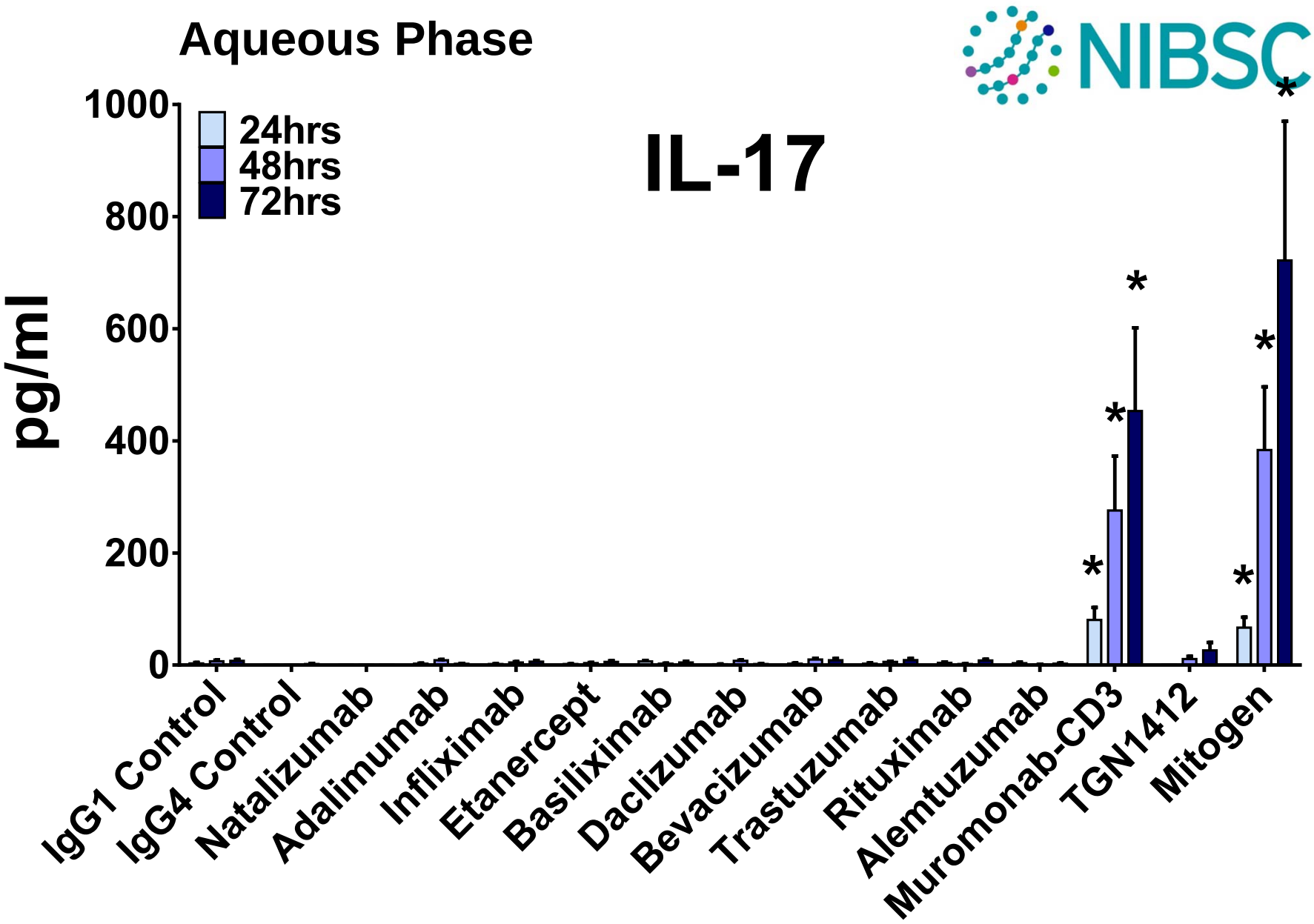
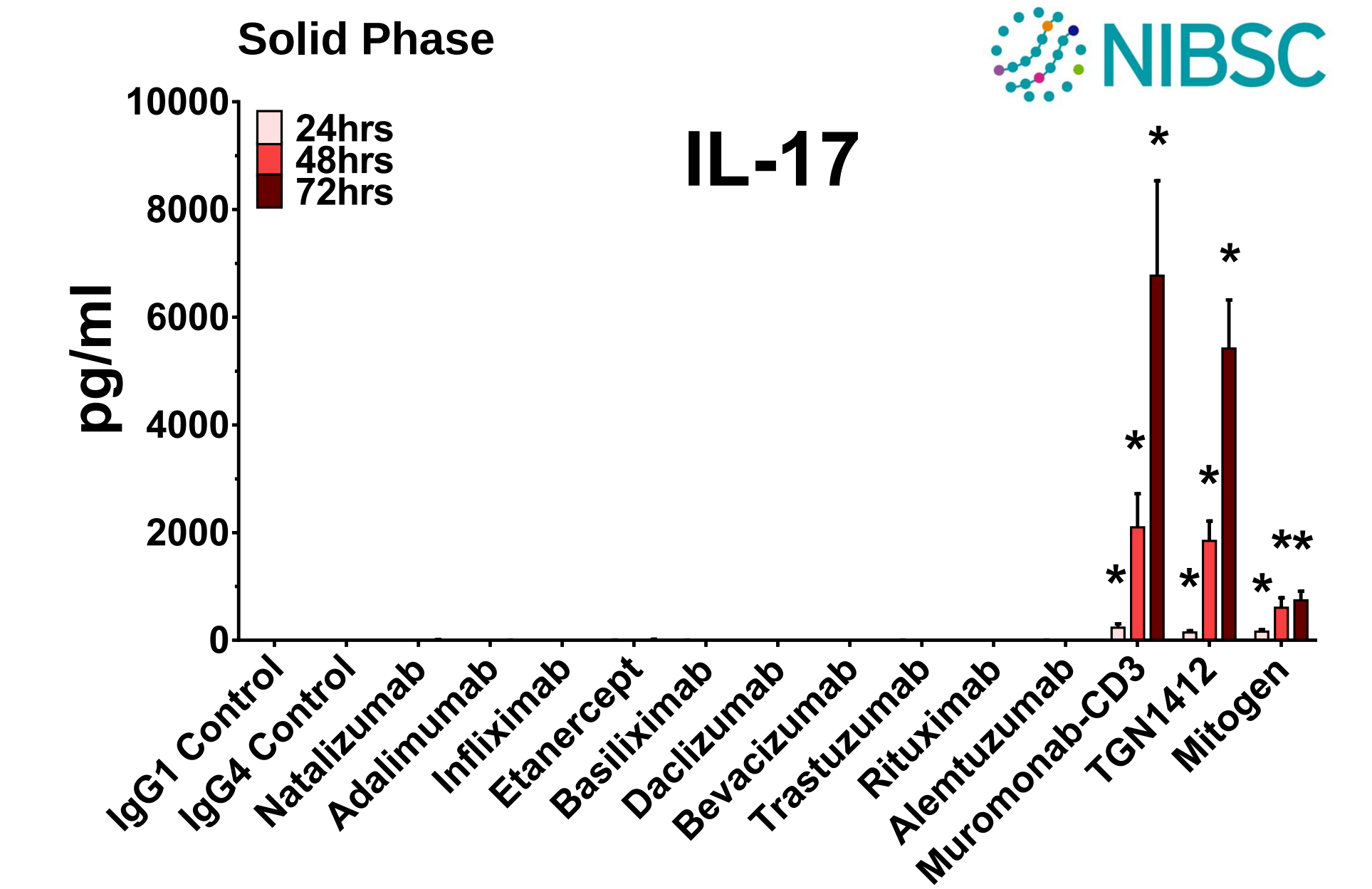
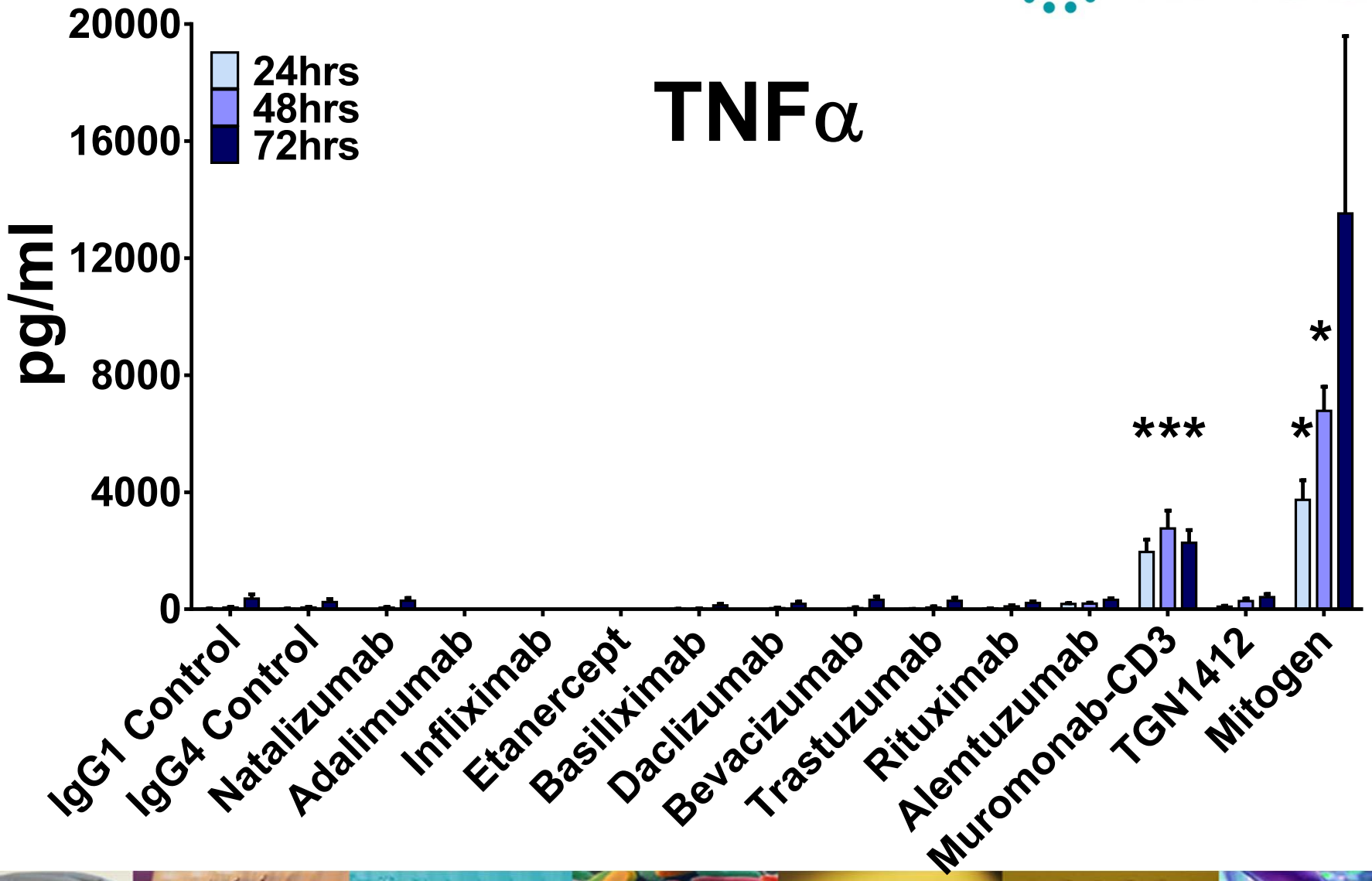
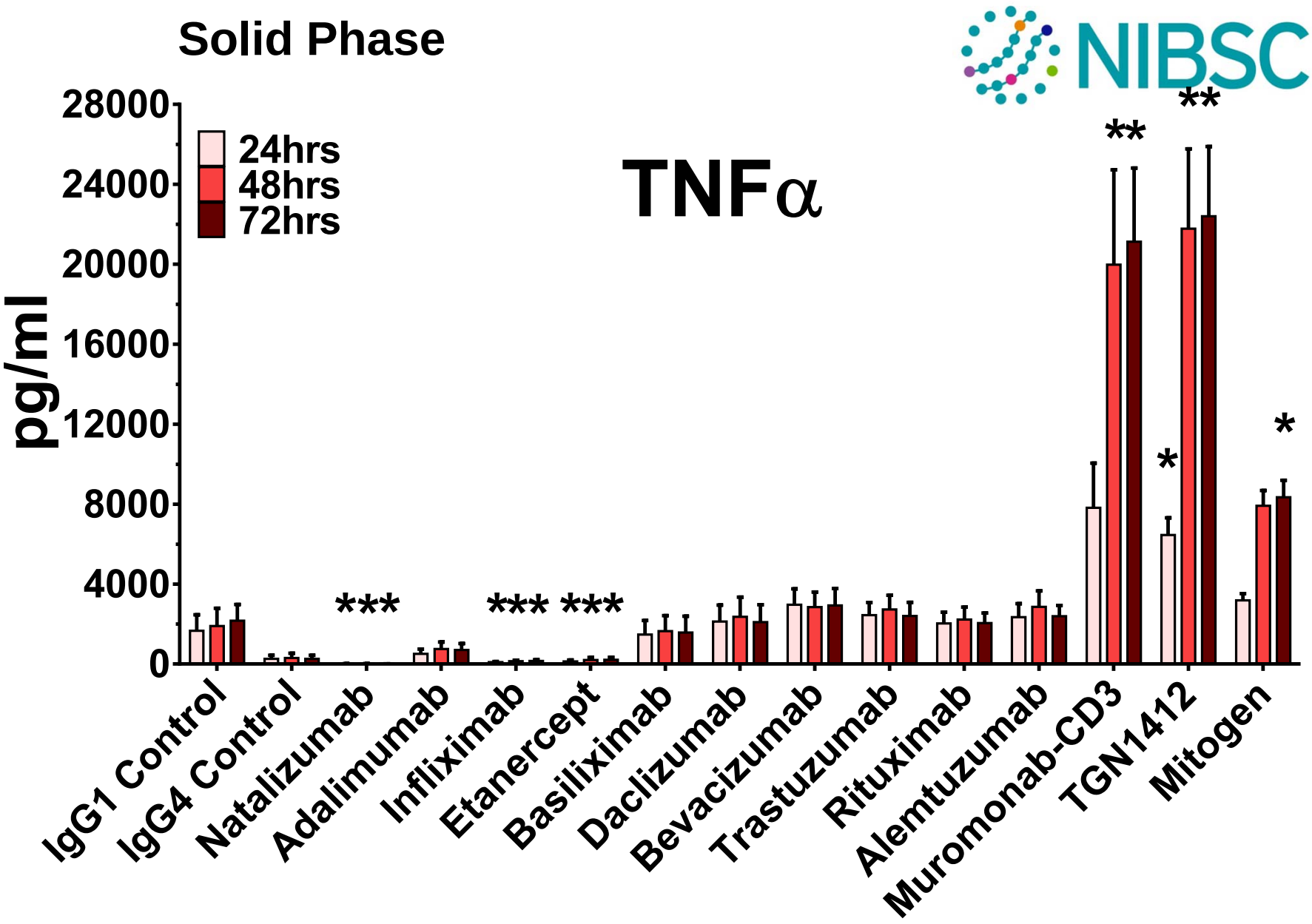
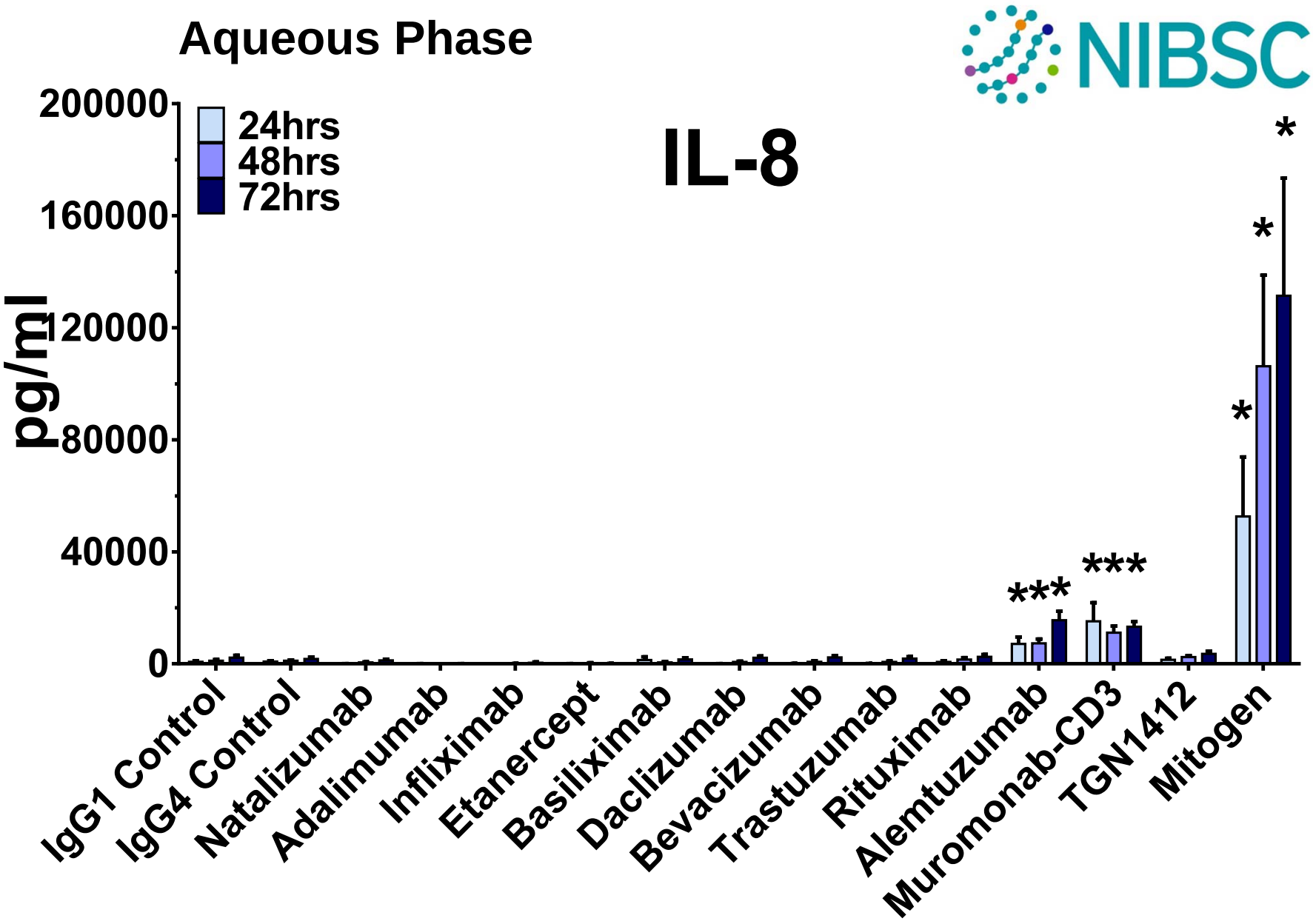

Aqueous Phase
NIBSC
IL-17
24hrs
48hrs
72hrs
pg/ml
1000
800
600
400
200
0
IgG1 Control
IgG4 Control
Natalizumab
Adalimumab
Infliximab
Etanercept
Basiliximab
Daclizumab
Bevacizumab
Trastuzumab
Rituximab
Alemtuzumab
Muromonab-CD3
TGN1412
Mitogen

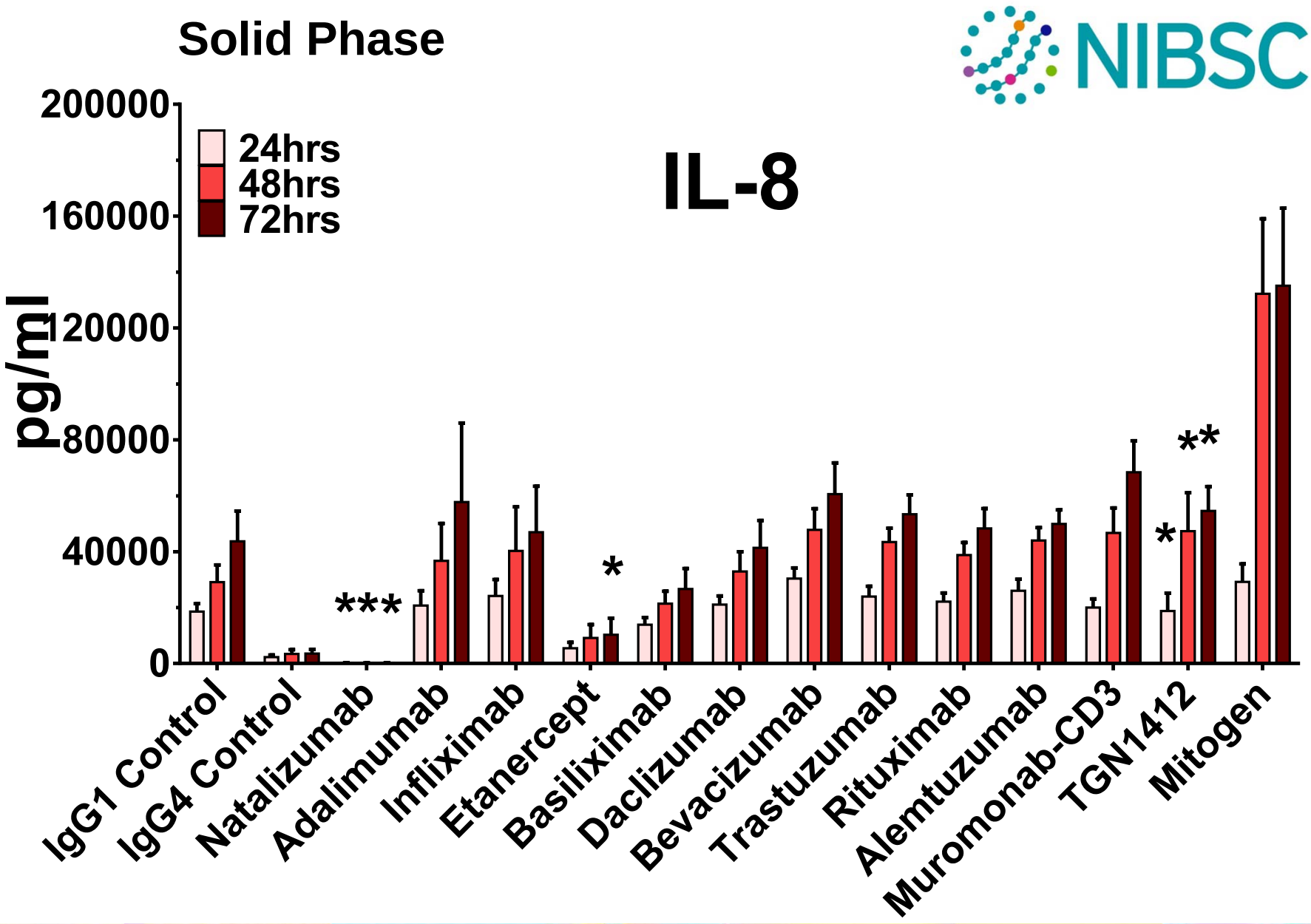

Solid Phase
NIBSC
IL-17
24hrs
48hrs
72hrs
pg/ml
10000
8000
6000
4000
2000
0
IgG1 Control
IgG4 Control
Natalizumab
Adalimumab
Infliximab
Etanercept
Basiliximab
Daclizumab
Bevacizumab
Trastuzumab
Rituximab
Alemtuzumab
Muromonab-CD3
TGN1412
Mitogen

Aqueous Phase




Solid Phase
NIBSC
TNFα
24hrs
48hrs
72hrs
pg/ml
28000
24000
20000
16000
12000
8000
4000
0
***
***
***
**
**
*
*
IgG1 Control
IgG4 Control
Natalizumab
Adalimumab
Infliximab
Etanercept
Basiliximab
Daclizumab
Bevacizumab
Trastuzumab
Rituximab
Alemtuzumab
Muromonab-CD3
TGN1412
Mitogen


Aqueous Phase
NIBSC
IL-8
24hrs
48hrs
72hrs
pg/ml
200000
160000
120000
80000
40000
0
***
***
*
*
*
IgG1 Control
IgG4 Control
Natalizumab
Adalimumab
Infliximab
Etanercept
Basiliximab
Daclizumab
Bevacizumab
Trastuzumab
Rituximab
Alemtuzumab
Muromonab-CD3
TGN1412
Mitogen

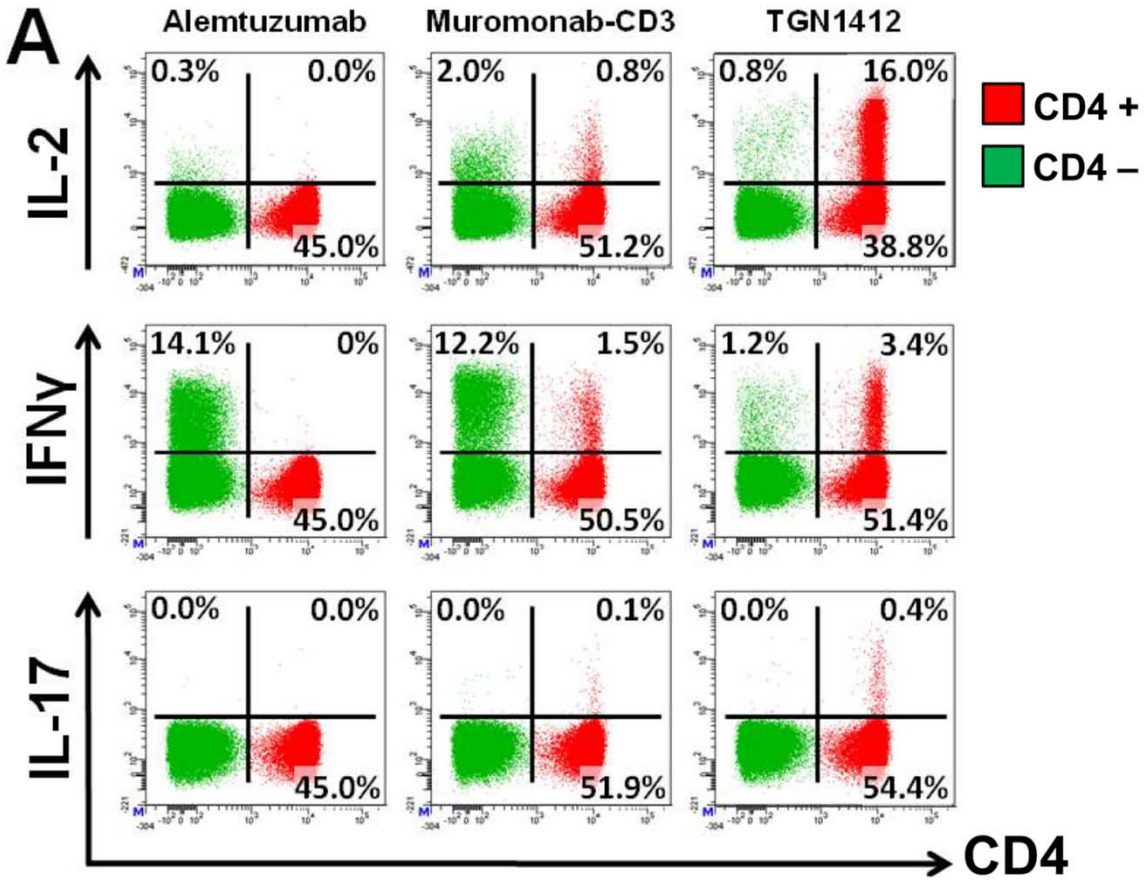

Solid Phase
NIBSC
IL-8
24hrs
48hrs
72hrs
pg/ml
200000
160000
120000
80000
40000
0
***
*
*
**
IgG1 Control
IgG4 Control
Natalizumab
Adalimumab
Infliximab
Etanercept
Basiliximab
Daclizumab
Bevacizumab
Trastuzumab
Rituximab
Alemtuzumab
Muromonab-CD3
TGN1412
Mitogen

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

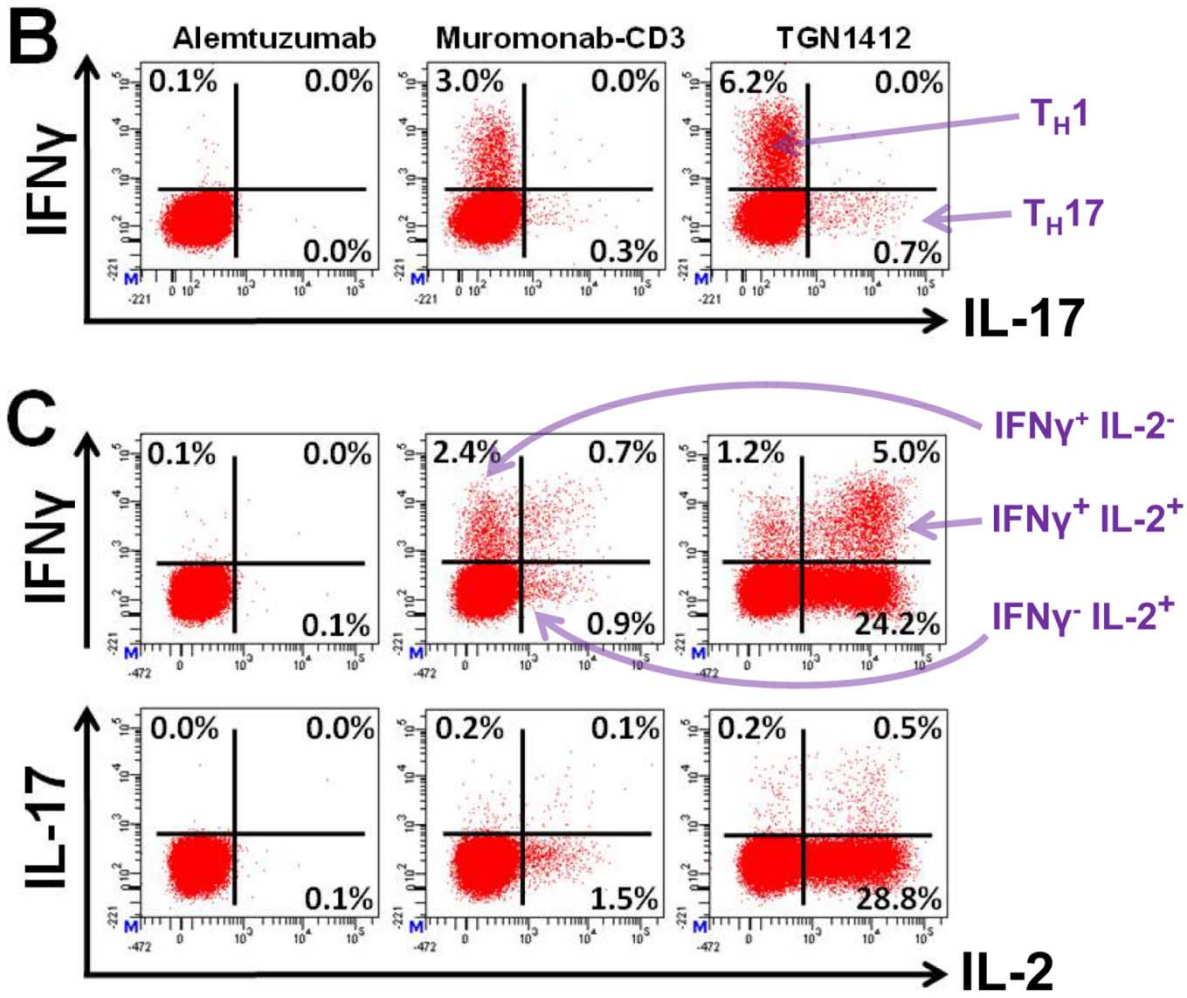
MSD TH1/TH2 10-plex assay

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

Acknowledgements

- David Eastwood, Dr Sandrine Vessillier, Christopher Bird

- Dr Robin Thorpe and Dr Stephen Poole (both now retired)

- NIBSC staff for blood donations

- Eastwood et al (2013) Brit. J. Clin. Pharmacol. 76:299-315.