Establishing risk of human experimentation with drugs: lessons from TGN1412

M J H Kenter, A F Cohen

Lancet 2006; 368: 1387–91

Central Committee on Research Involving Human Subjects, The Hague, Netherlands (M J H Kenter PhD, A F Cohen MD)**; and Centre for Human Drug Research, Leiden, Netherlands** (A F Cohen)

Correspondence to:
Dr A F Cohen,
CHDR, Zernikedreef 10,
2333 CL Leiden, Netherlands
ac@chdr.nl

Administration of a chemical or biological compound to a human being is never without risk. Although knowledge about risks increases during the development process, risks are still present even when a substance is marketed.[1] Particular care is necessary when a new drug is given to healthy volunteers without previous human testing. General principles for such research have been laid down in guidelines as early as 1983, and these were the basis for many current regulations.[2] Most drugs at that time were small molecules with fairly well characterised, classic, pharmacological mechanisms. Proposed primary objectives for studies in healthy people were therefore to show pharmacological action in man and the dose (or concentration) response curve. This approach was judged safe and was lent support by findings of available surveys.[3,4] Over time, the main objectives for these trials changed—perhaps owing to the perceived safety of new traditional (small-molecule) medicines—to general tolerance and safety.

The advent of increasingly potent and selective compounds for human-receptor systems led to situations in which predictability from animal data was diminishing. The first substances in this category were small molecules with fairly foreseeable pharmacokinetics, and any unexpected adverse events were mostly fully reversible. Biotechnology provided compounds with unique specificity for human targets, potentially further reducing the predictability of animal work. However, the deaths of two volunteers in clinical studies[5–7] led to the realisation that they could have been prevented by proper examination of existing data.

The serious adverse events that arose during the very first administration of TGN1412, the so-called CD28 superagonistic antibody, have led to immediate reactions from different regulators,[8,9] ranging from a moratorium on CD28 research to rules about how many individuals should receive a new compound at the same time.[10] A common theme was that special care should be given to ill-defined high-risk drugs. In this Viewpoint, we propose a set of factors facilitating rational risk analysis of all new substances to be administered to human beings (figure 1). We use TGN1412 as an illustration because it represents a new compound with a complex and novel mechanism.

Risk analysis of TGN1412

What is known about the mechanism of action of TGN1412? This molecule is a humanised version of the mouse antibody 5.11A1, which is an agonist of the CD28 antigen that activates T cells without specific engagement of the T-cell receptor with the antigen-presenting cell. Although the overall biology of this immunological interaction is fairly well understood,[11,12] the precise mechanism by which mitogenic anti-CD28 activate T cells is unknown.[13] Because the TGN1412 compound is novel, little published data exist for its specific mechanism and, therefore, risk for unexpected occurrences is enhanced.

The TGN1412 study was the first trial of this type of compound that was undertaken in man, so only a small amount of human data were available for risk analysis. Nevertheless, much can be learned from findings of similar clinical trials of antibodies, such as interference with the cytotoxic T lymphocyte-associated antigen (CTLA)-4 receptor. This somewhat distinct but related biological mechanism is at present being tested in cancer patients using the MDX-010 antibody.[14] This molecule is not a mitogenic antibody but it inhibits CTLA-4-mediated signals that turn off T-cell responses. It causes severe side-effects,[15,16] probably owing to activation of autoreactive T-cell clones. Additionally, several clinical studies of antibodies against the T-cell CD3 antigen have been done, in which massive systemic release of several cytokines and an array of toxic effects have been recorded.[17] The results of this trial led to modification of the Fc-receptor binding domain of anti-CD3.[18,19] In the TGN1412 clinical trial protocol, a cytokine burst was judged theoretical without any scientific consideration.[20] These two analogous mechanisms suggest that T cells can be triggered either by an agonist at an activating site (CD3) or by an antagonist on an inhibitory site (CTLA 4) and that such activation could produce serious toxic effects. Since the CTLA-4 receptor-mediated mechanism is closely related to that of CD28, these facts augment the risk profile of TGN1412 even further.

Our analysis focuses on the idea that the effects of an untested mechanism of action in man can be adequately predicted from work done in animal models or human cell systems. A prerequisite for this theory is that an analogous mechanism is in operation in the relevant animal species in-vitro systems, and human beings. The qualitative and quantitative response must be similar, which requires comparable receptor structure, expression, binding, and second-messenger effects.

The rhesus monkey (*Macaca mulatta*) tolerated large doses of TGN1412 without any serious side-effects, and the cynomolgus monkey (*Macaca fascicularis*) was used for final toxicology studies. According to the investigator's brochure,[21] 100% homology exists between the CD28 TGN1412 binding site in human beings and monkeys, restricted to the so-called C"D loop. However, no sequence comparison was included in the disclosed information.

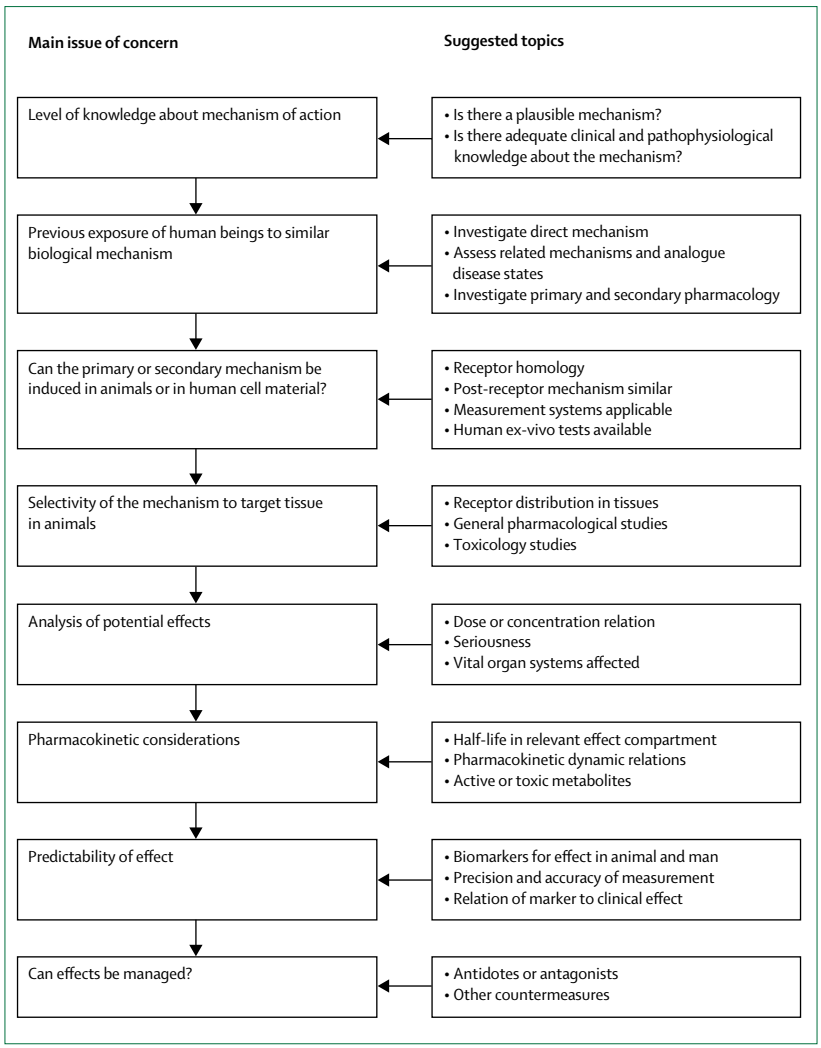

Figure 1: **Main of issues of concern to be included in a risk analysis of a new compound**
This analysis assumes acceptable and stable pharmaceutical and chemical quality.

Figure 2 compares the human and rhesus monkey CD28 aminoacid sequences, and clear differences can be seen. The potential importance of the sequence variation can be deduced from the crystal structure of the human CD28 molecule,[13] which indicates 14 contact residues with the parental antibody 5.11A1. A non-conservative change is noted at position 65 (Gly [G] to Glu [E]). Epitope mapping in a previous CD28 study recorded an identical aminoacid variation in the species (rat, mouse, and man) specificity of agonistic antibodies.[22] The human CD28 sequence is glycosylated at position 53 but not at this site in the rhesus monkey counterpart. A search of rhesus monkey CD28 aminoacid sequences in the National Center for Biotechnology Information (NCBI) database retrieved neither cynomolgus nor additional rhesus CD28 sequences (accessed April, 2006).[23]

The non-conservative variation at position 65 could lead to differences in binding characteristics of TGN1412 to the human and monkey CD28 molecule, which might result in varying amounts of T-cell activation in man and rhesus monkeys.[13] Unfortunately, the investigator's brochure only provides information on the affinity of TGN1412 to the human CD28 molecule ($1{\cdot}88\times10^{-9}$ mol/L), not for its monkey counterpart. In our risk analysis, these factors increase the risk category of the antibody and lead to further questions that can only be answered by preclinical experiments. Furthermore, rapid and fairly longlasting human T-cell depletion was noted in a mouse model with a human immune system after in-vivo administration of low doses of the parental antibody 5.11A1,[24] but these data were not included in the investigator's brochure. Also, this document did not provide results of a comparison of in-vitro activation of human and monkey peripheral blood mononuclear cells (PBMC) by TGN1412. Such findings could have provided insight into the similarity or otherwise of the activation of human and monkey T cells. In-vitro stimulation of human PBMC by the parental antibody 5.11A1 has been reported, showing its potent mitogenic capacity.[22]

The main proposed action of TGN1412 reported in the investigator's brochure[21] is activation of so-called regulatory T cells. However, specificity for a particular T-cell subpopulation is not expected because the human CD28 antigen is expressed on most CD4+ T cells and half of CD8+ T cells. Data also indicate a lack of specificity in activation of T-cell populations since both anti-inflammatory and pro-inflammatory cytokines are generated (table). Production of interleukin 10 and tumour growth factor β by activated regulatory T cells was not ascertained for the investigator's brochure. The claim in the clinical trial protocol[20] of preclinical evidence that TGN1412 inhibits pro-inflammatory cytokine production and activates regulatory T cells is not substantiated by in-vivo data. Moreover, published work reporting that the human CD28 antigen is also expressed on granulocytes[25,26] was not included in the investigator's brochure.

The mechanism of the antibody TGN1412 suggested that deleterious effects in man could not be ruled out conclusively from findings of animal experiments. There are two potential areas of concern. First, TGN1412 administration could lead to T-cell activation and massive cytokine release. Second, the antibody could result in a strong expansion of regulatory T cells and non-specific immunosuppression. Therefore, either possibility—activation or immunosuppression—could not be ruled out with the available data and, since these effects would have serious outcomes, the risk category should have been increased accordingly.

Neither activation nor immunosuppression was reported in non-human primate studies, and a starting dose of 0·1 mg/kg was selected for the clinical trial. This amount was ascertained by a fraction of the so-called no-adverse-effect dose concentration in the cynomolgus

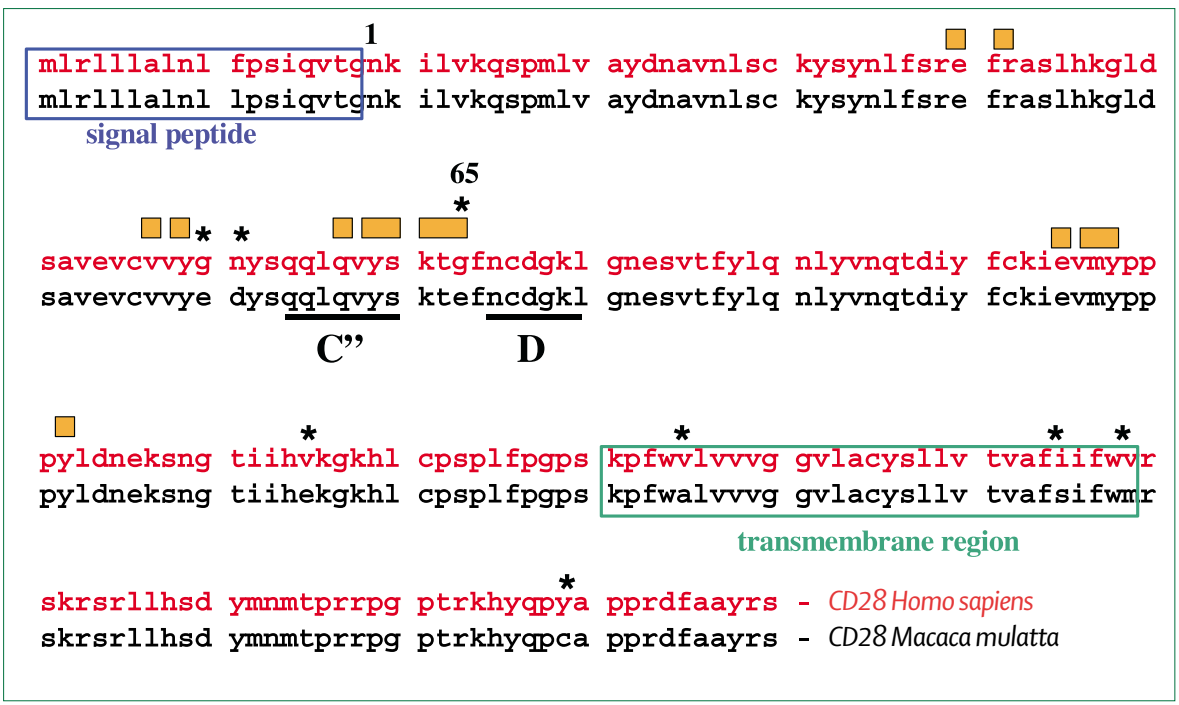

Figure 2: **Comparison of deduced human and rhesus monkey CD28 aminoacid sequences**
Human (*Homo sapiens*) accession NM 006139.1; rhesus monkey (*Macaca mulatta*) accession AF344855.1. Asterisks denote variations between the human and monkey sequences. Putative contact residues with the TGN1412 antibody are marked by orange boxes; the C'' D loop[13] is indicated by black bars.

monkey. However, cytokine release was already recorded at a low dose in this species (table). Therefore, a proper starting dose would most probably be much less than a 500th of the concentration causing effects in the monkey—even assuming the sensitivity of man and monkey to TGN1412 was equal.

Most monoclonal antibodies have long plasma half-lives, and animal data in the investigator's brochure[21] show that TGN1412 has a half life of about 8 days. Thus, full removal from the body would take about a month. This factor is an additional risk because any untoward effects would be equally longlasting.

The effect of the antibody TGN1412 could be expected to relate to dose or plasma concentration since the compound exerts its action by receptor binding. Post-receptor effects in the immune system could, however, be amplified easily by disturbing the delicate balance between several T-cell subpopulations, as seen in early anti-CD3 clinical trials. This fact makes the effects described above unpredictable with respect to dose or concentration dependency.

Individuals can have quite different reactions to a drug, and results of the experiment in which TGN1412 was added to human and animal blood ex vivo could have given information about cytokine release or T-cell expansion, as is typically done with inflammatory substances such as lipopolysaccharide.[27] According to the clinical trial protocol,[20] this test was done only with PBMC from patients with B-cell lymphatic leukaemia, and the results showed polyclonal expansion and activation of T lymphocytes. These standard experiments might have provided the data needed to predict effects in man.

In the investigator's brochure,[21] little guidance is given to doctors on how side-effects can be controlled and treated. Potential non-specific longlasting immunosuppressive effects would need particular care and instructions for the treating clinician and study participants—eg, in case of infections. Management of activation of autoreactive T-cell clones would require special long-term monitoring and, if necessary, treatment with high-dose corticosteroids. A clear strategy would also be needed for control of cytokine-release syndromes and rapid decline of T cells. In any case, these adverse reactions would most probably be serious and difficult to manage, again increasing the risk of administration of the antibody TGN1412.

	Inflammation type	Peak cytokine concentration (ng/L)		
		Control	Low dose (5 mg/kg)	High dose (50 mg/kg)
Interleukin 2	Pro-inflammatory	37 (20–60)	25 (0–84)	100 (25–211)
Interleukin 4	Anti-inflammatory	12 (0–18)	13 (8–18)	17 (0–40)
Interleukin 5	Anti-inflammatory	6 (3–7)	49 (6–139)	107 (11–458)
Interleukin 6	Pro-inflammatory	7 (0–22)	68 (32–101)	128 (24–390)
Tumour necrosis factor α	Pro-inflammatory	20 (11–26)	20 (15–27)	22 (19–26)
Interferon γ	Pro-inflammatory	18 (0–35)	23 (19–32)	33 (17–93)

Data are mean (range). Data taken from table 9 in the investigator's brochure.[21]

Table: **Cytokine production in cynomolgus monkey on administration of TGN1412**

Discussion

The above risk analysis, undertaken with data available in the research file and public domain before the TGN1412 trial started, shows that essential information was absent and the antibody was a high-risk compound unlikely to be suitable for administration to healthy people without additional preclinical experiments. A prerequisite for thorough assessment of the protocol and preclinical data for any clinical trial is that all parties involved have access to all necessary findings. The sponsor has main responsibility for making these results available and should include and discuss the data in the research file. Relevant new information that becomes available after submission should be added and discussed as soon as possible. This process is of special importance in the early and rapid development of a new medicine.

From the information that was disclosed, we conclude that the assessors did not receive all relevant findings. Even when all data are available, the different people who assess risk of a human study should communicate their findings in a consistent and orderly manner to boost the chance that the right questions are asked. Our proposed scheme will ensure that all parties cover the indicated points in a transparent and critical manner, followed by a synthesis. This approach can be used by investigators, regulators, research ethics committees, and for internal review in the clinical research unit.

In the UK, scientific assessment is done by the competent authority at the Medicines and Health Care Products Regulatory Agency (MHRA). The report of the TGN1412 trial by the MHRA includes three distinct subreports: a medical, a pharmaceutical, and a pharmaco-toxicology (safety) assessment that were finalised on separate dates.[28] The published MHRA document suggests that the subreports are the result of isolated assessments from different individuals without much interdisciplinary interaction. Moreover, the safety report contains several passages that seem to be copied from text that was supplied by the sponsor company in the investigator's brochure. This work does not suggest independent critical assessment.

The primary investigator takes scientific and medical responsibility for the participants, which requires full understanding of risks. This responsibility cannot be devolved to the employer, a governmental agency, or the research ethics committee. The TGN1412 trial was undertaken by two companies: a small venture-capital-driven company (ie, sponsor) and a clinical research organisation with a strong interest in the actual implementation of the study. Both relied heavily on the regulators to provide clearance for rapid undertaking of the trial.

Administration of high-risk interventions should be done in an institution at which adequate evaluation and monitoring can be done by in-house experts. For example, a university medical centre (in the case of TGN1412, with a clinical and research immunology department) with a well equipped and good clinical practice-compliant research unit.

The interim report of the expert scientific group on phase I clinical trials has now been published.[29] The document provides a thorough overview on the events of the TGN1412 trial based on information that was available to the expert scientific group, and it lists 22 recommendations to increase safety of volunteers in such trials that test a compound for the first time in man. Later in 2006, a final report is scheduled to be published, which will take account of opinions and comments on the interim report. We welcome most of the recommendations of the expert scientific group but regret that important data are still not in the public domain for an independent and rational assessment by the scientific community. As an example, the group conclude that "Sequence analysis of the extracellular domain revealed a 100% amino acid homology to the human counterpart, thereby confirming identical binding characteristics of the TGN1412 to human and cynomolgus monkey CD28 [p 13]",[29] but (again) no sequence comparison and functional analysis is given to substantiate this claim.

We believe that thorough analysis of human, rhesus monkey, and cynomolgus monkey complete and functional CD28 molecules may be important for our understanding of the adverse events that severely affected six healthy volunteers. Our comparison with publicly available CD28 sequence data shows that variation in aminoacids between man and rhesus monkey might account (in part) for the different outcome of administration of TGN1412 in these species. Furthermore, we suggest that all data from the TeGenero and Paraxel research file should be made publicly available for discussion by the international scientific community so that lessons from the TGN1412 trial can be learned and better risk assessment can be developed that will protect future healthy volunteers in clinical studies to develop useful new medicines.

On July 3 and 13, 2006, roughly 4 months after the TGN1412 clinical trial and during the process of bankruptcy, TeGenero submitted two identical cynomolgus CD28 nucleotide sequences to the NCBI database (accession numbers ABG77997 and ABG77998) potentially coding for a CD28 molecule with an extracellular domain identical to the human counterpart. Further analysis is needed to show whether this sequence encodes the target of antibody TGN1412 and the functional CD28 molecule in this species. Even if further work should indicate that the CD28 molecules of man and cynomolgus are identical in structure and function, we believe that in-vitro tests and a comparison of human, rhesus monkey, and cynomolgus monkey CD28 sequences should have been included in the research file and, if not, should have led to questions from all involved in the clinical trial (investigators, clinical research organisation, and assessors).

The complexity of current documentation about new compounds has the inherent risk that important findings and a scarcity of data can be hidden. Our approach to risk assessment should be seen as an initiative for an internationally accepted format.

Conflict of interest statement

MJHK is executive director and AFC is vice-chairman of the Central Committee on Research Involving Human Subjects, The Hague, Netherlands, which is also the competent authority for drug trials in Netherlands. AFC is director of the Centre for Human Drug Research, which is involved in phase I trials with healthy volunteers.

References

1 Bresalier RS, Sandler RS, Quan H, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med* 2005; **352:** 1092–102.

2 Council for International Organizations of Medical Sciences. Safety requirements for the first use of new drugs and diagnostic agents in man: a review of safety issues in early clinical trials of drugs. Geneva: Council for International Organizations of Medical Sciences, 1983.

3 Orme M, Harry J, Routledge P, Hobson S. Healthy volunteer studies in Great Britain: the results of a survey into 12 months activity in this field. *Br J Clin Pharmacol* 1989; **27:** 125–33.

4 Zarafonetis CJ, Riley PA Jr, Willis PW 3rd, et al. Clinically significant adverse effects in a phase 1 testing program. *Clin Pharmacol Ther* 1978; **24:** 127–32.

5 Bouchie A. Gene-therapy death prompts suit. *Nat Biotechnol* 2002; **20:** 647.

6 Carmen IH. A death in the laboratory: the politics of the Gelsinger aftermath. *Mol Ther* 2001; **3:** 425–28.

7 Steinbrook R. Protecting research subjects: the crisis at Johns Hopkins. *N Engl J Med* 2002; **346:** 716–20.

8 Schneider CK, Kalinke U, Lower J. TGN1412: a regulator's perspective. *Nat Biotechnol* 2006; **24:** 493–96.

9 Bhogal N, Combes R. TGN1412: time to change the paradigm for the testing of new pharmaceuticals. *Altern Lab Anim* 2006; **34:** 225–39.

10 Wadman M. London's disastrous drug trial has serious side effects for research. *Nature* 2006; **440:** 388–89.

11 Jiang H, Chess L. Regulation of immune responses by T cells. *N Engl J Med* 2006; **354:** 1166–76.

12 Barr TA, Carlring J, Heath AW. Co-stimulatory agonists as immunological adjuvants. *Vaccine* 2006; **24:** 3399–407.

13 Evans EJ, Esnouf RM, Manso-Sancho R, et al. Crystal structure of a soluble CD28-Fab complex. *Nat Immunol* 2005; **6:** 271–79.

14 Phan GQ, Yang JC, Sherry RM, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc Natl Acad Sci USA* 2003; **100:** 8372–77.

15 Beck KE, Blansfield JA, Tran KQ, et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. *J Clin Oncol* 2006; **24:** 2283–89.

16 Blansfield JA, Beck KE, Tran K, et al. Cytotoxic T-lymphocyte-associated antigen-4 blockage can induce autoimmune hypophysitis in patients with metastatic melanoma and renal cancer. *J Immunother* 2005; **28:** 593–98.

17 Smith JA, Bluestone JA. T cell inactivation and cytokine deviation promoted by anti-CD3 mAbs. *Curr Opin Immunol* 1997; **9:** 648–54.

18 Carpenter PA, Pavlovic S, Tso JY, et al. Non-Fc receptor-binding humanized anti-CD3 antibodies induce apoptosis of activated human T cells. *J Immunol* 2000; **165:** 6205–13.

19 Carpenter PA, Tso JY, Press OW, Yu X, Anasetti C. Non-FcR-binding, humanized anti-CD3 antibody Hu291 induces apoptosis of human T cells more effectively than OKT3 and is immunosuppressive in vivo. *Transplant Proc* 2000; **32:** 1545–46.

20 Parexel. Parexel clinical trial protocol: a phase-I single-centre, double-blind, randomised, placebo controlled, single escalating-dose study to assess the safety, pharmacokinetics, pharmacodynamics and immunogenicity of TGN1412 administered intravenously to healthy volunteers. London: Parexel International, 2006. Available at: http://www.circare.org/foia5/tgn1412protocol.pdf (accessed Aug 23, 2006).

21 TeGenero Immunotherapeutics. Investigator's brochure: TGN1412—humanized agonistic anti-CD28 monoclonal antibody, edn 1.1 2005-12-19. Würzburg: TeGenero AG, 2005. Available at: http://www.circare.org/foia5/tgn1412investigatorbrochure.pdf (accessed Aug 23, 2006).

22 Lühder F, Huang Y, Dennehy KM, et al. Topological requirements and signaling properties of T cell-activating, anti-CD28 antibody superagonists. *J Exp Med* 2003; **197:** 955–66.

23 Altschul SF, Madden TL, Schaffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997; **25:** 3389–402.

24 Legrand N, Cupedo T, van Lent AU, et al. Transient accumulation of human mature thymocytes and regulatory T cells with CD28 superagonist in "human immune system" Rag2–/–γc–/–mice. *Blood* 2006; **108:** 238–45.

25 Venuprasad K, Chattopadhyay S, Saha B. CD28 signaling in neutrophil induces T-cell chemotactic factor(s) modulating T-cell response. *Hum Immunol* 2003; **64:** 38–43.

26 Venuprasad K, Parab P, Prasad DV, et al. Immunobiology of CD28 expression on human neutrophils: I—CD28 regulates neutrophil migration by modulating CXCR-1 expression. *Eur J Immunol* 2001; **31:** 1536–43.

27 Lewis CE, McCarthy SP, Lorenzen J, McGee JO. Differential effects of LPS, IFN-gamma and TNF alpha on the secretion of lysozyme by individual human mononuclear phagocytes: relationship to cell maturity. *Immunology* 1990; **69:** 402–08.

28 Medicines and Health Care Products Regulatory Agency. Pharmaco-toxicological (safety) assessment of TGN1412. London: Medicines and Health Care Products Regulatory Agency, 2005.

29 Department of Health. Expert scientific group on phase one clinical trials: a consultation. http://www.dh.gov.uk/Consultations/LiveConsultations/LiveConsultationsArticle/fs/en?CONTENT_ID=4137501&chk=x%2BoJ/%2B (accessed Aug 23, 2006).