

EXPERT SCIENTIFIC GROUP ON
PHASE ONE CLINICAL TRIALS

FINAL REPORT

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1. Executive Summary

Background

The Expert Scientific Group (ESG) was set up following the very serious adverse reactions that occurred in the first-in-man clinical trial of TGN412 in March 2006. The trial was performed in a private clinical research unit at Northwick Park Hospital in London. TGN1412 is a monoclonal antibody that was being developed as a medicine to treat leukaemia and autoimmune diseases such as rheumatoid arthritis.

In the clinical trial, six healthy male volunteers experienced severe systemic adverse reactions soon after receiving TGN1412 intravenously. All six volunteers developed a cytokine release syndrome with multi-organ failure and required intensive treatment and supportive measures that were provided by the Intensive Therapy Unit (ITU) at Northwick Park Hospital.

Previously, first-in-man clinical trials had a very good safety record and, as far as we can determine, the TGN1412 trial outcome, where all recipients experienced such a severe and similar adverse reaction, is unprecedented.

Following these events at Northwick Park Hospital, the ESG was set up by the Secretary of State for Health, with the following terms of reference:

Terms of Reference

1. To consider what may be necessary in the transition from pre-clinical to first-in-man phase one studies, and in the design of these trials, with specific reference to:
 - Biological molecules with novel mechanisms of action;
 - New agents with a highly species-specific action;
 - New drugs directed towards immune system targets.
2. To provide advice in the form of a report to the Secretary of State for Health for the future authorisation of such trials with an interim report to be provided within three months

The ESG was asked to seek the views of major stakeholders before compiling its report.

Stakeholders raised several areas of concern that were not within our terms of reference. These included topics such as the process of informed consent and clarity of information, communication between clinical investigators and clinical trial subjects before and during a trial, insurance cover, the role of Research Ethics Committees, and clinical follow-up of trial subjects who had experienced an adverse

reaction. Although beyond our remit, we considered these wider concerns to be extremely important, and recommend that they should be taken up as a high priority.

Aims of the ESG

The aims of the ESG were to review what could be learned from the TGN1412 trial, and to make recommendations to increase the safety of future first-in-man trials of the types of medicines described in our terms of reference.

We were asked to make an interim report in three months and to seek stakeholder opinions in formulating our recommendations. Following the publication of our interim report on July 26th 2006, we received some further 38 written and verbal submissions from stakeholders, including four of the trial volunteers and their representatives, the Northwick Park physicians, patient representatives, individuals, national and international public sector institutions, the biotechnology and pharmaceutical industries and contract research organisations. The ESG has considered the stakeholder written submissions and verbal presentations, and has taken them into account in developing the final recommendations.

At the time of our interim report, some results were awaited from independent laboratory tests carried out by the National Institute for Biological Standards and Control (NIBSC) to clarify the toxicity seen in the TGN1412 trial. These results were received in November 2006 and were studied closely. The new experimental results may provide potential answers to scientific questions surrounding the adverse reactions to TGN1412 in the clinical trial, and why similar reactions were not detected in pre-clinical testing in animals. These results have been incorporated as a summary section in our report.

Approach to the Problem

The need for better and safer medicines is clear, as is the fact that the first human exposure to a new medicine will always carry some risk, even if extremely small. Our aim has been to optimise the safety of future first-in-man trials of the types of medicines within our remit without stifling innovation or raising unnecessary barriers to the development of useful new medicines.

The ESG has met 10 times, has held 5 stakeholder meetings and has considered a total of 52 written and 25 verbal submissions from stakeholders.

We reviewed the pre-clinical development of TGN1412, the results from MHRA investigations and the likely causes of the unpredicted severe toxicity at the dose given in the trial. Our conclusion is that the pre-clinical development studies that were performed with TGN1412 did not predict a safe dose for use in humans, even though current regulatory requirements were met.

The dose of TGN1412 given to the human volunteers induced a rapid and large release of cytokines, which are small protein molecules that transmit signals between

immune cells and tissue cells. Cytokines are biologically potent, and their production is normally highly controlled. A rapid and large release of cytokines causing fever, low blood pressure and organ failure has been called a ‘cytokine release syndrome’, or ‘cytokine storm’. This occurred in the trial volunteers but it did not occur in the cynomolgus monkey, the animal model chosen for studies to calculate the dose for the first human exposure to TGN1412. At a dose that was numerically 500 times larger than that given to human volunteers, cynomolgus monkeys did not develop a cytokine release syndrome.

We went on to identify factors relevant to risk assessment and risk management in first-in-man trials of new medicines as described in our remit. We use the word ‘agent’ to describe a chemical or biological compound being developed as a potential medicine for human use.

Scope of the Recommendations

What kind of clinical trial?

The recommendations are intended to apply to “first-in-man” clinical trials, and not to phase one trials in general (which might include trials of agents with an established record of safety in humans). Special caution is needed during first human exposures to higher risk agents at doses with the potential to cause a pharmacological effect.

However, added caution should also be taken when administering a medicine with the potential for high risk to a distinct new population, be they healthy volunteers or patients. Preparation of guidance on first-in-man studies should give consideration to the applicability of the concepts developed to transitions from one population group to another.

The ICH E8 guideline ‘General Considerations for Clinical Trials (CPMP/ICH/291/95) (<http://www.emea.europa.eu/pdfs/human/ich/029195en.pdf>) provides general guidance on clinical trials and contains, in section 3, an internationally agreed description of phase one trials and a good overview of the development phases of medicines from discovery to market.

What kind of agent?

Our remit covers three categories of medicines that may have a higher potential for risk of harm to volunteers during the first human exposures, or where risk may be more difficult to evaluate in pre-clinical development. The categories are:

- Biological molecules with novel mechanisms of action;
- New agents with a high degree of species-specificity;
- New agents with immune system targets.

We intend our recommendations to apply to agents in any one of these three categories unless a careful assessment of the nature of the agent, the physiological role of the target molecules, and the intended recipients, supports a low risk of harm in first human exposures.

We do not suggest that all agents that fall into one of the three categories listed above necessarily pose a high risk on first human exposures, but that a thorough assessment of risk should be made and a clear scientific case provided when risk of harm is assessed as being low.

For example a conventional vaccine, although aimed at stimulating an immune response, may not pose a high risk, or a new agent similar to one with an established safety record in humans, and aimed at a known target where the pharmacology can be predicted with confidence, may not pose a high risk.

We have discussed in this report factors that should raise the level of caution for first human exposures to new agents. These include:

- any agent whose effects might cause severe physiological disturbance to vital body systems;
- agonistic or stimulatory actions;
- novel agents and novel mechanisms of action where there is no prior experience;
- species-specificity of an agent making pre-clinical risk-assessment in animal models difficult or impossible;
- the potency of an agent, eg compared with a natural ligand;
- multifunctional agents, eg bivalent antibodies, FcR binding domains;
- cell-associated targets;
- targets that by-pass normal control mechanisms;
- immune system targets;
- targets in systems with the potential for large biological amplification *in vivo*.

A thorough assessment of risk should always be carried out before first-in-man trials. The risk assessment should be clearly described in the trial documents and be fully examined by the regulator. When there is significant doubt, higher risk should always be assumed. (See Section 7 of the Report.)

The Recommendations

The safety, rights and well-being of subjects, both patients and healthy volunteers, must always be the primary concerns in clinical trials. We have made 22 recommendations that we believe will increase the safety of volunteers in future clinical trials involving the first human exposures to agents with higher potential risks, as categorised in our terms of reference and discussed in Section 8 of our report.

They cover:

- Pre-clinical and early clinical development;
- The process of preparation and review of clinical trial applications, and early access to advice for both regulators and sponsors;
- Determining and administering the initial doses in man;
- The clinical environment for first-in-man studies;
- Developing the skills and training to meet future needs.

There is focus on sharing of information relevant to safety, the calculation and administration of first doses, and regulatory access to independent specialist opinion in the appraisal of trial applications for the first human exposures to new medicines of the types described in our remit where there is higher potential risk.

The rationale for each recommendation, and explanations of our intentions, are summarised in *Section 7, "Predicting Hazards in Pre-clinical to Clinical Transition"*, and *Section 8, "Risk Reduction and Risk Management"*. The recommendations should be considered in the context of these Sections.

Our recommendations are offered to the UK authorities and sponsors of first-in-man trials in the UK, but we believe it is important that agreement is sought at EU and international level, to ensure that equal protection is afforded to clinical trial participants worldwide.

Acknowledgement

We are especially grateful to the four trial volunteers who visited the ESG and gave us valuable personal accounts of their experiences.

We would also like to thank all of the stakeholders for the many thoughtful and constructive comments that they provided in written and verbal submissions to the ESG.

We also thank the secretariat and technical support teams whose hard work in preparing background papers, arranging meetings, taking minutes and liaising with national and international stakeholders has been greatly appreciated.

Summary of Recommendations

Pre-clinical and early clinical development

1. Decisions on the strategy for pre-clinical development of a new medicine and the experimental approaches used to assemble information relevant to the safety of first-in-man clinical trials must be science-based, made and justified case-by-case by individuals with appropriate training.

The pre-clinical development of new medicines is addressed by internationally agreed guidelines, in particular ICH S6 Pre-clinical Safety Evaluation of Biotechnology-Derived Products, and ICH M3 (R1) Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (see further reading below).

Regulators should consider the applicability of this guidance to high-risk medicines and in particular their first use in man. The development of more specific guidance in the present context should be proposed. This might best be achieved by initial guidance at the EU level, and using that as a platform for proposals at international level.

2. The regulatory process for first-in-man trials of higher risk agents and advanced medicinal products based on innovative technologies should be subject to regular review.

With present advances in biology, biological chemistry, informatics and biotechnology, we can expect the identification of many new therapeutic targets and the development of innovative medicines to fill unmet clinical needs. It is important that the regulation of medicines is science-based and that the regulatory process keeps pace with, and is appropriate for, the science and technology that will give rise to tomorrow's medicines.

3. Developers of medicines, research funding bodies and regulatory authorities should expedite the collection of information from unpublished pre-clinical studies relevant to the safety of human exposure. As a first step this should focus on pre-clinical reports of results that signal potential danger to humans with the use of a higher risk medicine or group of medicines. This should provide a platform for information sharing between regulators at EU and international level, e.g. in the form of a confidential database. The submission of such data to a database by the investigators is strongly encouraged.

In the interests of safety, we believe that the ultimate goal should be an open access database, and the feasibility of this needs to be explored. However, to avoid the risk of delay in sharing safety-critical information, reports from studies that would otherwise not be in the public domain could initially be stored in a secure database accessible only to regulators worldwide, with frequent review of the reason for delaying open access.

4. Regulatory authorities should consider ways to expedite the sharing of safety information on phase one clinical trials between regulators within the EU and worldwide. This should certainly include information on first-in-man experience with higher risk medicines. Trials with negative safety outcomes should be included. This database might be widened to include products that may not currently be perceived as high risk, or trials conducted later in development, that suggest a strong warning for first-in-man use of similar products.

In the EU, this collection and sharing of information could be based on the model of the existing clinical trial database EudraCT (for first-in-man trials since 2004) and the EudraVigilance database for ‘Suspected Unexpected Serious Adverse Reactions (SUSARs)’ – (see below). Relevant information from first-in-man trials prior to 2004 could be submitted on a voluntary basis. This would ensure access to relevant safety information by national regulators.

This will require dialogue with the pharmaceutical industry on which study reports should be made available and when they should be posted, but should be achievable since industry is already committed to disclosure of clinical trial results on medicines already on the market, and encourages sponsors to post results of development failures where there may be important safety implications. The regulators should explore the feasibility of open access to this data.

SUSARs are Suspected Unexpected Serious Adverse Reactions. They are unexpected because their nature or severity is not consistent with the applicable product information (e.g. investigator's brochure for an unauthorised investigational product or summary of product characteristics for an authorized product), they are reactions because there is a causal relationship with the product, and they are serious, as described below

Article 2(o) of Directive 2001/20/EC gives the definition of serious adverse reaction: “any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect.”

For all other important medical outcomes not on this list, professional medical and scientific judgement should be exercised when deciding the need to report.

The process of preparation and review of clinical trial applications, and early access to advice for both regulators and sponsors

5. More communication is strongly recommended between developers and the regulator at an earlier stage before an application is filed, especially for higher risk agents, to ensure that there is time for an appropriate consideration of any safety concerns without introducing undue delay to product development. Ways to increase communication between the regulator and research ethics committees should also be considered.

For first-in-man clinical trials of new agents that fall into the higher risk categories described in our remit, pre-submission meetings between sponsors and regulators, to

identify potential concerns, would be useful to both parties, and are strongly recommended. If developers elect not to have pre-submission discussion with the regulator, they should be asked to inform the regulator six weeks in advance of an intended application for a first-in-man clinical trial of a higher risk medicine. This would give the regulator adequate time to consider the need for external expert advice and to identify appropriate experts (see next recommendation). Advance warning would reduce the possibility of prolonging the assessment process.

6. For appraisal of applications for trials of higher risk agents, as defined by the nature of the agent, its degree of novelty, its intended pharmacological target, and its intended recipient, the regulator should have access to additional opinion from independent, specialist experts with research knowledge of their fields.
7. An Expert Advisory Group (EAG) of the Commission on Human Medicines, or a similar body, might undertake this role with a core membership of appropriate experts and the ability to co-opt additional expertise as the need dictates.
8. Consideration should be given to introducing some flexibility in the time-scale of clinical trial appraisal in exceptional cases of unusual complexity.

We emphasise “exceptional cases of unusual complexity”; there is no intention to introduce unjustified delay in the regulatory process in the development of new medicines. The European Clinical Trials Directive (article 6, paragraph 7) (Directive 2001/20/EC, below) already allows for time extension in the appraisal of gene therapy, somatic cell therapy and products containing genetically modified organisms. This might be a model for other highly innovative medicines that bring new science into therapeutics.

Determining and administering the initial doses in man

9. Special consideration should be given to new agents for which the primary pharmacological action, for the proposed therapeutic effect, cannot be demonstrated in an animal model. The underpinning case for dose selection in any trial of this kind should include a clear rationale for the proposed mechanism of action and also for the safety and efficacy of the substance in its intended clinical use.
10. A broader approach to dose calculation, beyond reliance on ‘No Observable Effect Level’ or ‘No Observable Adverse Effect Level’ in animal studies, should be taken. The calculation of starting dose should utilise all relevant information. Factors to be taken into account include the novelty of the agent, its biological potency and its mechanism of action, the degree of species-specificity of the agent, the dose-response curves of biological effects in human and animal cells, dose-response data from *in vivo* animal studies, pharmacokinetic and

pharmacodynamic modelling, the calculation of target occupancy versus concentration and the calculated exposure of targets or target cells in humans *in vivo*.

The 'Minimal Anticipated Biological Effect Level' (MABEL) approach is one good model for achieving this. (See BIA/ABPI report and stakeholder submission.)

11. If different methods give different estimates of a safe dose in humans, the lowest value should be taken as the starting point in first-in-man trials and a margin of safety introduced in calculation of the actual starting doses in man.
12. When it is likely that pre-clinical information, for any reason, may be a poor guide to human responses *in vivo*, the starting doses in first-in-man trials should be calculated to err on the side of caution. Further dose increases should proceed with caution since the initial dose may have been particularly low and there may be a steep dose-response curve.
13. Careful consideration should be given to the route and the rate of administration of the first doses in first-in-man trials, with careful monitoring for an adverse or exaggerated response.

For example, in the case of first human exposure to a higher risk agent given intravenously, a slow infusion over several hours may be more appropriate than a slow bolus over several minutes. This would allow monitoring for an adverse response and stopping the infusion if clinically indicated.

14. The trial design, including number of subjects, decisions on starting doses and the dose escalation regime, should be made on a case-by-case basis, and should be scientifically and statistically justifiable, taking account of all relevant information.
(See stakeholder submission from The Royal Statistical Society)

15. New agents in first-in-man trials should be administered sequentially to subjects with an appropriate period of observation between dosing of individual subjects. The interval of observation between sequential dosing of subjects should be related to the kind of adverse reactions that might be anticipated based on the nature of the agent, its target and the recipient, as well as the potential pharmacokinetics and pharmacodynamics of the agent.

There should be a suitable interval between doses to different individuals as well as between doses to the same individual, and both should be justified on the basis of available evidence.

16. A similar period of monitoring should occur between sequential dosing of the subjects during dose escalation. (*as above*)
17. The decision whether to conduct a first-in-man trial in healthy volunteers or in volunteer patients should be carefully considered and fully justified, taking into account all factors relevant to the safety of the subjects and the value of the scientific information that is likely to be obtained.

In general, there is no anticipated benefit to a patient in a first-in-man trial of a new medicine. Therefore, risk to benefit assessment is not usually a major factor in deciding whether such trials should be performed in volunteer patients or in healthy subjects.

In the field of cancer medicine there may be exceptions since patients who experience a beneficial response to a trial agent are often able to continue treatment with it. In this case the balance of risk and benefit may become a factor in deciding that patients are more appropriate subjects of a clinical trial, especially patients that have not responded to available therapies and when the trial medicine has predictable cellular toxicity (which may, in fact, be its intended pharmacological effect).

However, the paramount factors should always be the rights, safety, and well-being of the volunteers, whether patients or healthy individuals, and the value of what can be learned from the clinical trial.

The clinical environment for first-in-man studies

18. Principal Investigators who are responsible for the care of subjects in first-in-man trials should always be appropriately qualified, and satisfy themselves that they know enough about the agent, its target and mechanism of action to be in a position to make informed clinical judgements.

The development of a national professional accreditation system for Principal Investigators conducting first-in-man clinical trials should be strongly encouraged.

19. In first-in-man studies where there is a predictable risk of certain types of severe adverse reaction, a treatment strategy should be considered beforehand. This should include the availability of specific antidotes where they exist and a clear plan of supportive treatment, including the pre-arranged contingency availability of ITU facilities.
20. First-in-man studies of higher risk medicines should always be conducted in an appropriate clinical environment supervised by staff with appropriate levels of training and expertise, with immediate access to facilities for the treatment and stabilisation of individuals in an acute emergency, and with pre-arranged contingency availability of ITU facilities in reasonable proximity.

There should always be an adequate staffing level in first-in-man studies and adequate 24-hour cover when volunteers are kept in overnight.

All clinical sites conducting such trials should have standard operating procedures for emergency situations, and staff should maintain expertise in implementing these procedures through regular drills. Trial subjects should always be clearly informed about what to do if they experience symptoms of an adverse reaction during or after a clinical trial.

Developing expertise

21. The availability of ‘hands-on’ experience in the planning and conduct of clinical trials should be widened. For example postgraduate training programmes could have within them a secondment period to commercial organisations or a training period in specialist centres within the NHS and Universities (see next recommendation).

The need to train a new generation of doctors in the skills needed for the assessment of safety and efficacy of medicines was highlighted in the UK’s Academy of Medical Sciences 2005 report “Safer Medicines”. Similar needs were identified in “Sustaining the skills pipeline in the pharmaceutical and biopharmaceutical industries”, a recent report of a study by the ABPI.

This will need co-operation between higher education funding bodies and institutions, the NHS and industry. Given that major stakeholders agree on the need to fill this skills gap with a new generation of Clinical Pharmacologists, Toxicologists and related professionals, it should be possible to address it, and we encourage this in the interests of the safety of future first-in-man clinical trials.

22. The feasibility of developing specialist centres for phase one clinical trials of higher risk agents and advanced medicinal products should be explored.

The development of a national inspection and accreditation system for clinical centres that undertake first-in-man studies of higher risk agents should be encouraged. The accreditation should be open to all centres that fulfil defined criteria, in both the public and private sectors.

Further reading

‘Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412’
G. Suntharalingam, M. Perry, S. Ward, S. Brett, A. Castello-Cortes, M. Brunner, and N. Panoskaltzis.

N Engl J Med. 2006, 355:1018-1028.

The European Clinical Trials Directive 2001/20/EC
(http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/vol-1/dir_2001_20/dir_2001_20_en.pdf)

EudraLex Volume 10 –Clinical Trials
(<http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/homev10.htm>)

European Union Guidelines -
<http://www.emea.europa.eu/htms/human/humanguidelines/background.htm>

ICH E8 - General Considerations for Clinical Trials (CPMP/ICH/291/95)
(<http://www.emea.europa.eu/pdfs/human/ich/029195en.pdf>)

ICH S6 Pre-clinical Safety Evaluation of Biotechnology-Derived Products
(CPMP/ICH/302/95)
(<http://www.emea.europa.eu/pdfs/human/ich/030295en.pdf>)

ICH M3 (R1) Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for
Pharmaceuticals (CPMP/ICH/286/95).
(<http://www.emea.europa.eu/pdfs/human/ich/028695en.pdf>).

ICH International Conference on Harmonisation of Technical Requirements for Registration
of Pharmaceuticals for Human Use (ICH) <http://www.ich.org/cache/compo/276-254-1.html>

Academy of Medical Sciences 2005 report “Safer Medicines”
(<http://www.acmedsci.ac.uk/p102.html>).

“Sustaining the skills pipeline in the pharmaceutical and biopharmaceutical industries”,
ABPI. (<http://www.abpi.org.uk/Details.asp?ProductID=285>).

2. Introduction

Adverse events in Phase One Clinical Trial at Northwick Park Hospital

On March 13, 2006, six healthy male volunteers received TeGenero's TGN1412 drug and two received a placebo in a phase one, first-in-man, clinical trial run by Parexel, a contract research organisation. The Parexel unit was in rented space on the Northwick Park Hospital site in London. TGN1412 is a monoclonal antibody that was being developed as a new medicine for the treatment of B cell leukaemia and autoimmune diseases. Within hours of receiving TGN1412, all six volunteers were admitted to the intensive care unit at Northwick Park Hospital with a very severe systemic inflammatory reaction that progressed to multi-organ failure.

Formation of Expert Scientific Group (ESG)

The ESG was established in April 2006 by the Secretary of State for Health following the tragic events at Northwick Park Hospital during the clinical trial of TGN1412.

Terms of Reference of ESG

Following the clinical trial of TGN1412 the Secretary of State for Health appointed the ESG with the following remit:

1. To consider what may be necessary in the transition from pre-clinical to first-in-man phase one studies, and in the design of these trials, with specific reference to:
 - Biological molecules with novel mechanisms of action;
 - New agents with a highly species-specific action;
 - New drugs directed towards immune system targets.
2. To provide advice in the form of a report to the Secretary of State for Health for the future authorisation of such trials with an interim report to be provided within three months

The ESG was asked to seek the opinions of major stakeholders before formulating its recommendations.

The ESG comprised 17 individuals with backgrounds in science, clinical medicine, pharmacology, toxicology, immunology, bio-ethics, clinical trials, medicines development and medicines regulation. There were also two lay members making 19 in all. The ESG had observers in attendance from the Medicines Regulatory Authorities of Japan, USA and the EU, and from the Department of Health, the Department of Trade & Industry and the Gene Therapy Advisory Committee.

Aims of ESG, ways of working and meetings schedule

With the types of agent described in the terms of reference, the aim was to optimise the safety of first-in-man clinical trials without creating unnecessary barriers to the development of useful new medicines.

After an assessment of the events in the TGN1412 trial, the ESG liaised with the National Institute for Biological Standards and Control (NIBSC) to perform independently some of the key experiments that had been undertaken by TeGenero in the pre-clinical development of TGN1412, and to perform further investigations of the biological properties of TGN1412.

With the outcome of the TGN1412 clinical trial in mind, and what was known about the TGN1412 monoclonal antibody, the ESG considered factors that may be generally important for the recognition of *a priori* risk in phase one trials.

The ESG identified and discussed properties of TGN14 that may confer risk, and that could be generalised to other agents in first-in-man clinical trials, such as:

- Species-specificity of action creating challenges for pre-clinical development;
- Agonistic activity on targets within a biological amplification cascade;
- Immune system targets;
- Cell surface targets that activated signalling pathways
- Multifunctional molecules, eg antibodies with potential to activate FcR-bearing cells as well as primary target cells;
- Potency:
 - Increased affinity, occupation or signalling effect on target receptor compared with a natural ligand (e.g. “superagonist antibody”);
 - Prolonged exposure to target via normal (e.g. antibodies) or altered pharmacokinetics (e.g. pegylation);
 - Multivalency, allowing cross-linking of target molecules.

These and other factors related to the nature of the agent and the physiological function of its targets were considered in detail with an attempt to understand what could be generalised to allow recognition of higher risk agents in the categories described in our terms of reference.

Risk reduction and risk management questions related to different aspects of the transition from pre-clinical to clinical development of a new medicine, with a focus on the first human exposures, were then explored in detail. Examples of the questions discussed included:

Pre-clinical

- How to validate the predictive value of animal models?
- Are there methods to optimise the predictive value of *in vitro* experiments on human cells / tissues?
- What is the feasibility and value of developing shared databases on experience with animal model responses to new investigational agents, or classes of agents? Could such a database be contributed to, and accessible by, international regulatory agencies, pharmaceutical and biotechnology industries, academic researchers, research ethics committees?
- Development of *in silico* modelling; how useful is it now, or when might it be in the future?

Clinical

- Adequate safety margin of dose; is NOAEL or NOEL in an animal model a valid starting point for newer types of medicines and is the safety margin in humans sufficiently large taking into account all relevant information?
- Is sequential administration of high-risk agents essential in first-in-man trials? How long to wait before administration to second and subsequent recipients?
- How important is the route and rate of administration with a new agent, especially one that has a novel mechanism of action?
- How to choose the first recipient? How to ensure informed consent?
- When and how to escalate the dose?
- Are there differences in risk/benefit ratio between healthy volunteers and patients, even in phase one trials? Is there a clear rationale for first-in-man trials to involve healthy volunteers or volunteer patients? Is this a case-by-case question?
- What should be the clinical environment of phase one trials?
- Importance of adequate staff, expertise and training?
- Should there be more training opportunities to acquire relevant skills?
- Do first-in-man trials of higher risk agents need to be performed on hospital sites?
- The ready availability of resuscitation and ITU facilities?
- The anticipation of major adverse reactions, and type of reaction?
- Availability of specific therapies for potential ADRs where a risk is identifiable?
- Is there enough disclosure of data to the principal investigator to allow informed clinical judgements?
- Should there be specialist units for first-in-man trials of higher risk or very innovative agents?
- Is there enough sharing of information from previous clinical trial experiences?
- Could clinical trial safety databases be open access?

Regulatory

- Should there be a review of higher risk 'first-in-man' CTA applications by independent experts; eg an Expert Advisory Group of CHM and/or co-option of highly specialised experts when needed?
- Can higher risk agents be defined in a pragmatic way?
- How could this be done within the time scales that apply (during the processing of the application or before the application is submitted)?

- Should the time scales be extended, or the clock able to be stopped?

Meetings and key dates (2006)

23 rd May	ESG meeting. Background papers from secretariat on development of TGN1412 , and adverse reactions in the clinical trial. MHRA Investigation Reports Clinical Reports from Northwick Park ITU physicians
31 st May	ESG meeting. Mechanism(s) of the adverse reactions Identification of risk factors Identification of high-risk agents
2 nd June	ESG meeting. Risk reduction strategies
14 th June	ESG meeting. Risk reduction strategies (continued) Views of stakeholders
15 th June	Sub-group meeting with stakeholders
16 th June	Sub-group meeting with stakeholders
19 th June	Sub-group meeting with stakeholders
20 th June	Sub-group meeting with stakeholders
27 th June	ESG meeting. Assessment of stakeholder views on TGN 1412 trial
11 th July	ESG meeting with stakeholders. Stakeholders views, interim recommendations
20 th July	Interim Report to web as consultation document. Following publication of the interim report as a consultation document, further stakeholder presentations and written submissions were invited in September, October and November 2006. Further meetings were held as follows:
12 th Sept	ESG meeting with stakeholders
24 th Oct	ESG meeting with stakeholders
13 th Nov	ESG meeting with stakeholders

- 14th Nov Sub-group meeting with stakeholders, including four of the clinical trial volunteers and their representatives.
- 15th Nov ESG meeting
NIBSC results
Discussion on stakeholders' views
Final recommendations
- 30th Nov Final Report to Ministers

All stakeholders' comments were considered in detail by the ESG before submission of its final report and recommendations.

3. Background to the TGN1412 incident as at July 2006. Prepared by the Secretariat to the ESG

A Pre-clinical Development of TGN1412

Role of CD28 in Immune System

CD28 is a co-stimulatory receptor expressed on the cell surface of CD4 T lymphocytes (T cells) and on a large fraction of CD8 T cells. It efficiently co-stimulates resting T cells in combination with a signal from the T cell antigen receptor (TCR)¹. The CD28 receptor promotes the development of T_H1 and T_H2 cells. T_H1 and T_H2 cells are helper T cells that aid activation of the responses of white blood cells. T_H1 cells promote 'cellular immunity' in host defence and are also involved in reactions such as transplant rejection. T_H2 cells promote 'humoural immunity' by helping B lymphocytes (B cells) to proliferate and secrete antibodies.

Activation of the CD28 signalling pathway in nature requires simultaneous triggering of the TCR by antigen and of CD28 by its physiological membrane-bound ligands B7-1 (CD80) or B7-2 (CD86). *In vitro*, this process can be mimicked by using a combination of antibodies with specificity for the TCR and CD28. The agonistic anti-CD28 monoclonal antibody TGN1412 bypasses the requirement for TCR signalling and activates human T cells irrespective of their TCR specificity. This property led to the use of the term 'superagonist'.

The finding that early TCR signals are not required for T-cell expansion mediated by agonistic anti-CD28 antibodies was shown for human and rat T cells^{2,3} – see Figure 1.

It was further shown that agonistic anti-CD28 monoclonal antibodies such as TGN1412, bind exclusively to the laterally exposed C'D loop of the immunoglobulin-like extracellular domain of CD28 whereas conventional, co-stimulatory antibodies recognise an epitope close to the binding site for the natural ligands, and that this specificity closely correlates with the agonistic activity of anti-CD28 antibodies³. Most recently, the critical involvement of the C'D loop for binding of agonistic anti-CD28 monoclonal antibodies has also been confirmed by X-ray crystallographic analyses⁴.

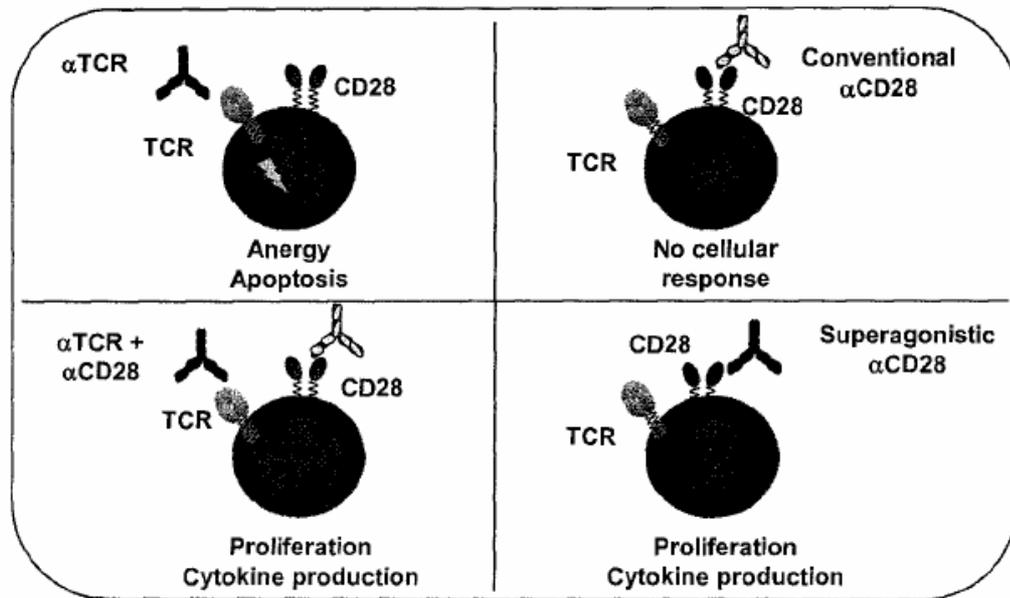


Figure 1: Activation of human T cells in the absence of TCR stimulation TGN1412 bypasses the requirement for TCR signalling triggering and activates human T cells in the absence of TCR stimulation. In T cells, TCR triggering alone leads to anergy and apoptosis. Conventional anti-CD28 antibodies are not capable of inducing cellular T cell response. Concomitant triggering *via* anti-TCR and anti-CD28 antibodies leads to proliferation and secretion of pro-inflammatory cytokines *in-vitro*, but not *in vivo*. In contrast, TGN1412 induces profound *in vitro* T cell proliferation and well-tolerated *in vivo* expansion of T cells.

The TGN1412 humanised monoclonal antibody

TGN1412 is an agonistic anti-CD28 monoclonal antibody, developed as a therapeutic agent for various diseases in which T cells are involved in the pathogenesis of chronic inflammation or haematological malignancies such as leukaemia. The antibody is a recombinant humanised monoclonal antibody that specifically binds CD28 present on T cells. TGN1412 was genetically engineered by transfer of the complementarity determining regions (CDRs) from heavy and light chain variable region sequences of a monoclonal mouse anti-humanCD28 antibody into human heavy and light chain variable region frameworks. Humanised variable regions were subsequently recombined with a human gene coding for the IgG4 γ chain and with a human gene coding for a κ chain, respectively. The human constant domain and variable domain framework structures were expected to confer decreased immunogenicity and an optimum of antibody effector functioning within the human immune system.

The TGN1412 molecule consists of two light chains of ~24 kDa (214 amino acids) and two heavy chains of ~51 kDa (447 amino acids). The protein is expressed in CHO cells and has a molecular mass of ~148 kDa. The TGN1412 drug product is a buffered, isotonic, non-preserved concentrate for solution for infusion. In the clinical material the concentration of the drug substance was 10 mg·ml⁻¹, filled in 40 ml vials. The container was a 50 ml injection vial.

The rationale for development of TGN1412 for haematological malignancies was based on its capability to reconstitute a collapsed T cell compartment (*e.g.* in B-CLL). In *ex vivo* experiments conducted with primary blood samples from a broad spectrum of B-CLL patients, it could be demonstrated that TGN1412 induced both polyclonal T-cell

expansion and activation. TGN1412 bypasses the requirement for TCR triggering and activates T cells irrespective of their TCR specificity. This novel mode of T cell activation has been termed “agonistic” or “superagonistic”. Both terms are used synonymously throughout the literature cited.

Furthermore, TGN1412 was shown indirectly to improve deficient antigen presentation by BCLL cells. The postulated immunomodulatory (anti-inflammatory) properties of TGN1412 were related to its feature of activating and expanding regulatory T lymphocytes and inducing anti-inflammatory cytokines. Agonistic anti-CD28 treatment was demonstrated to be effective in animal models of autoimmune diseases including animal models of rheumatoid arthritis, rat experimental autoimmune neuritis (EAN)⁵ and rat experimental autoimmune encephalomyelitis (EAE)⁶.

Toxicology Report

***In vitro* development using animal and human cells**

Specificity of TGN1412 for human CD28 was shown in various assay systems, including flow cytometry and Biacore analyses. TGN1412 did not cross-react with the closely related receptors Cytotoxic T-Lymphocyte- Antigen-4 (CTLA-4) and Inducible Costimulator (ICOS).

The topological requirements of agonistic anti-CD28 antibodies have been investigated in detail by epitope mapping using chimeric CD28 molecules³. It has been shown that agonistic antibodies specific for rat or human CD28 bind exclusively to the laterally exposed C'D loop of the immunoglobulin-like domain of CD28 whereas conventional, co-stimulatory antibodies recognise an epitope close to the binding site for the natural CD80/CD86 ligands. Moreover, mouse CD28 molecules engineered to express human C'D loop sequences activated T cells without TCR ligation when cross-linked by agonistic anti-human CD28 antibodies, showing that agonistic function is causally related to the C'D loop. More recently, the critical involvement of the C'D loop for binding of agonistic anti-CD28 monoclonal antibodies has also been confirmed by X-ray crystallographic analyses. Therefore, based on shared specificity-function relations, the agonistic anti-rat CD28 monoclonal antibody JJ316 and the anti-human CD28 agonists 5.11.A1, TGN1112 and TGN1412 were considered as true orthologues.

Studies were conducted with TGN1412 and TGN1112 to demonstrate cross-reactions with CD28 expressed on T cells from rodents and non-human primates such as *Macaca mulatta* (rhesus monkey), *Macaca fascicularis* (cynomolgus monkey) and *Callithrix jacchus* (marmoset monkey) in order to support the rationale for the selection of an appropriate species for safety and toxicology studies.

TGN1412 was reactive with human, cynomolgus and rhesus monkey T cells in a pattern characteristic for CD28. Binding to T cells from marmosets could not be demonstrated.

In addition, an amino acid sequence homology analysis of the C'D loop of CD28 from different species was performed. Whereas the C'D loop sequences of human and cynomolgus monkey are identical and differ in one amino acid from rhesus CD28, the

marmoset C'D differs in 2 out of 6 amino acids. The rodent C'D loop has very low homology with the human CD28 C'D loop.

The T cell activating capacity of TGN1412 was established in *in vitro* proliferation assays using total peripheral blood mononuclear cells (PBMC) as well as purified T lymphocytes and highly purified T cell subsets such as CD4+ and CD8+ T cells, naïve CD4+CD45RA+ and memory CD4+CD45RO+ cells or conventional CD4+CD25- as well as regulatory CD4+CD25 high T cells from healthy donors. Co-incubation of PBMC with soluble TGN1412 resulted in polyclonal T cell proliferation and secretion of T cell specific cytokines. The degree of TGN1412-induced proliferation varied among different blood donors, while conventional, co-stimulatory human-specific anti-CD28 antibodies were generally unable to induce substantial cellular proliferation. TGN1412 was, therefore, considered to be unique in its ability to deliver mitogenic signals via CD28 without co-engagement of the TCR.

***In vivo* development in animal models**

.A longitudinal study to assess the tolerability and T cell activating efficacy of TGN1412 and the IgG1 variant TGN1112 was conducted in rhesus monkeys. TGN1112 was able to activate and expand T cells *in vivo*. Importantly, administration of agonistic anti-CD28 monoclonal antibodies to *Macaca mulatta* did not mediate a substantial change in systemic cytokine (IL-5, IL-6, IL-10, IFN γ) serum concentrations and had no long-term (up to day 155) side-effects.

It was observed that TGN1412 had a significantly weaker pharmacological activity in rhesus monkeys than TGN1112. This was explained by the different affinities and/or FcR-binding properties of the two antibody formats.

The T cell activating properties of TGN1412 were determined in studies conducted in cynomolgus monkeys. Flow cytometry results revealed a substantial expansion of CD4+ and CD8+ T cells that showed a clear peak at around day 15 post infusion. The transient T cell expansion was paralleled by cellular activation as measured by CD69 and CD25.

Pharmacokinetics and Toxicokinetics of TGN1412

Due to the well-known pathways of protein degradation, conventional absorption, distribution, metabolism and excretion studies were not performed with TGN1412.

Differences in the kinetics of surrogate agonistic anti-CD28 antibodies (JJ316 and TGN1112) were found when comparing the results in the rat model of adjuvant arthritis and in the rhesus monkey. They appeared to be at least partly reconciled in the kinetics of *in vivo* T cell expansion induced by JJ316 and TGN1112. This difference was expected, as it mirrors the general diversity of rodents and non-human primates.

Therefore, toxicokinetic characteristics of TGN1412, as determined in cynomolgus monkeys were assumed to be most predictive for human pharmacokinetics.

Toxicokinetic evaluation during the repeat-dose toxicity study in cynomolgus monkeys showed that TGN1412 serum concentration *versus* time profiles were generally consistent with intravenous (*iv*) injection of the drug. A terminal elimination half life of 8 days after the first injection of 5 mg·kg⁻¹ was estimated for TGN1412, consistent with the relatively slow elimination of a large biological molecule such as an antibody.

In one animal, relatively low serum concentrations of TGN1412 were observed, which was attributed to the presence of anti-TGN1412 antibodies. No consistent sex-related differences were apparent in any of the toxicokinetic parameters reported for TGN1412.

Systemic exposure to TGN1412 increased by up to around 20-fold as dose increased from 5 to 50 mg·kg⁻¹. In addition, there was evidence for increased mean terminal half-life of TGN1412 as dose increased.

Since TGN1412 does not act directly on malignant tissues, pharmacodynamic effects were not expected to be solely correlated to plasma/ serum concentrations of the active substance, but rather to be dependent on the effect on T cell subsets in the peripheral blood and lymphoid tissues. In this respect, it is important to note that although four peak serum levels were observed following four weekly doses of TGN1412 only one peak of T cell expansion (around day 15) was observed. The lack of repetitive T cell expansion may be due to modulation availability of the CD28 antigen, availability of the TGN1412 target structure, functional unresponsiveness of T cells and/or limitation of T cell expansion by homeostatic mechanisms.

Nonclinical Safety Studies

Since the TGN1412 epitope on the CD28 extracellular domain is restricted to humans and non-human primates, cynomolgus and rhesus monkeys were considered to be the most relevant species for safety and toxicology studies to assess any potential toxicity of TGN1412 administration to humans.

The nonclinical toxicology program included studies of repeat-dose toxicity, local tolerance and immuno-histochemical investigation of cross reactivity with human and cynomolgus monkey tissues. These studies were conducted in compliance with Good Laboratory Practices (GLP). The *IV* route of administration was selected to accord with the intended application in clinical trials.

The results of these studies showed that TGN1412 was well tolerated in cynomolgus monkeys at doses up to 50 mg·kg⁻¹·week⁻¹ for four consecutive weeks. No TGN1412-related signs of toxicity, hypersensitivity or systemic immune system deviation were observed in these studies. No adverse effects on the major physiological systems (cardiovascular system, respiratory system and central nervous system) were observed.

Therefore, 50 mg·kg⁻¹ was considered to be the no-observed-adverse-effect level (NOAEL).

Reproduction and developmental toxicity studies were not performed. Histopathology of reproductive tract tissues was, however, performed as part of the 28-day toxicity study in cynomolgus monkeys. No treatment-related changes were observed.

Due to the biological nature of TGN1412, the standard battery of genotoxicity testing was considered to be inappropriate and was therefore not performed.

Local reactions at the injection sites of treated cynomolgus monkeys were considered not to be related to treatment with TGN1412 but to the administration procedure. In a local tolerance study conducted in rabbits, *IV*, perivenous, or intra-arterial routes of TGN1412 administration were well tolerated and did not produce clinically significant irritation

The immunotoxicity of TGN1412 was assessed as part of the standard toxicology studies and in non-GLP pharmacology studies. For additional studies, an agonistic antibody with specificity for rat CD28 (JJ316) or an IgG1 variant of TGN1412 (TGN1112) was used. Administration of TGN1412 or TGN1112 to non-human primates led to a transient increase in CD4+ and CD8+ T cell numbers between day 13 and 17 after dosing. This was an expected pharmacodynamic effect of TGN1412. Moderate elevations of IL-2, IL-5 and IL-6 serum levels were observed upon TGN1412 treatment in individual animals, however, no clinical signs of a first-dose cytokine release syndrome (CRS) were observed. Moreover, there was no evidence for an anaphylactic reaction, induction of autoimmune disease and/or unintended systemic immuno-suppression in animals treated with any dose of agonistic anti-CD28 monoclonal antibodies.

In the tissue cross reactivity study, the distribution of lymphocyte staining was consistent with the expected distribution of T cells within lymphoid tissue (target antigen specificity). Additional specific staining considered to represent astrocyte staining was seen in central nervous tissues of both human and cynomolgus monkey donors. However, CNS tissue cross reactivity was not associated with CNS related adverse clinical symptoms/toxicology findings in cynomolgus monkeys. Intracytoplasmic staining was recorded in the cervix of cynomolgus donors and in cytotrophoblast cells in the placenta of humans. This intracytoplasmic staining was not regarded as being of clinical importance as exposure of cytoplasmic antigens appeared to be a result of tissue sectioning. The fact that no treatment-related histopathological findings were reported for the reproductive tract of cynomolgus monkeys from the 28-day toxicology study underlined this assumption.

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4. Evans E., Esnouf R., Manso-Sancho R., Gilbert R., *et al.* Crystal structure of a soluble CD28-Fab complex. *Nature Immunol.*, 2005; 6: 271 to 279.

5. Schmidt J., Elflein K., Stienekemeier M., Rodriguez-Palmero M., *et al.* Treatment and prevention of experimental autoimmune neuritis with superagonistic CD28-specific monoclonal antibodies. *J. Neuroimmunol.*, 2003; 140: 143 to 152.
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B Transition to Clinical Development of TGN1412

Use of Human Volunteers

Based on the proposed TGN1412 mode-of-action and on its demonstrated pre-clinical safety in non-human primates, the company considered that an analysis of its immunological safety and pharmacodynamic effects in immunocompetent humans justified the selection of healthy subjects as the target population for a TGN1412 first-in-man trial.

In particular, the company believed that a study in healthy subjects yielding valuable information on TGN1412 safety and pharmacology was warranted prior to studies in patients because:

- i. the target antigen CD28 is expressed comparably in healthy subjects and in rheumatoid arthritis (RA) and B-cell chronic lymphocytic leukaemia (B-CLL) patients, respectively, implying that safety, pharmacokinetic (PK) and pharmacodynamic (PD) data could be at least partially transferred to B-CLL and/or RA patients
- ii. immunological safety of TGN1412 was considered most likely in healthy subjects, since no adverse effects had been observed in healthy animals in multiple pre-clinical studies, including non-human primates. Immunological adverse effects could not be completely excluded in subjects with an existing immuno-pathogenic predisposition
- iii. interpretation of safety and pharmacology data in healthy subjects would not be compromised by pre-activation or dysfunction of T cells or by a pre-existing imbalance of effector/memory and regulatory T cells or other components of the haematopoietic compartment.
- iv. healthy subjects, as outlined in the study protocol, would represent a rather homogenous population, avoiding the impact of confounding factors such as pre/co-medication and/or disease activity on the interpretation of TGN1412 safety and pharmacology.

Dose calculation

Since the specificity of TGN1412 was apparently restricted to CD28 expressed on T cells from humans and nonhuman primates, safety and toxicology studies in non-human primates (cynomolgus and rhesus monkeys) were considered as the most relevant studies to assess a potential toxicity of TGN1412 administration to humans. To determine potential adverse effects related to TGN1412 mode-of-action as well as unintended toxicity, a number of safety and efficacy studies with TGN1412 in non-human primates were conducted using single dose and multiple-dose regimens. The results of these studies showed that TGN1412 was well tolerated at doses up to $50 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{week}^{-1}$ for at least 4 consecutive weeks.

Calculation of the phase I trial starting dose was primarily based on the No Observed Adverse Effect Level (NOAEL) in the repeated dose toxicity study in cynomolgus monkeys and the procedure described in the draft USA Food and Drug Administration (FDA) guideline “Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers”¹. The NOAEL was considered $50 \text{ mg}\cdot\text{kg}^{-1}$.

Using the FDA guidance, an allometric correction factor of 3.1 was applied for the cynomolgus monkey NOAEL in order to calculate the “human equivalent dose” (HED) of $16 \text{ mg}\cdot\text{kg}^{-1}$. When the default safety factor of 10 was then applied, the maximum recommended starting dose (MRSD) was estimated to be $1.6 \text{ mg}\cdot\text{kg}^{-1}$. The company then applied an additional safety margin and proposed a starting dose of $0.1 \text{ mg}\cdot\text{kg}^{-1}$.

The dose range in the proposed phase I trial was considered to ensure both maximum patient safety due to the conservative safety factors of 160 and also to enable investigation of basic PD/PK characteristics of TGN1412, relevant for future clinical trials to be conducted in patient populations.

Using a “Minimal Anticipated Biological Effect Level” (MABEL) Approach

Pharmacological activity of TGN1412 and its surrogate had been observed in rhesus and cynomolgus monkeys at between 2.5 and $25 \text{ mg}\cdot\text{kg}^{-1}$. A no observed effect level (NOEL) of $<0.3 \text{ mg}\cdot\text{kg}^{-1}$ was derived from pre-clinical studies conducted in healthy and arthritic (adjuvant arthritis) rats using the rat CD28 specific homologous antibody JJ316. Optimal pharmacological responses were achieved between 1 and $5 \text{ mg}\cdot\text{kg}^{-1}$. Therefore, the Minimal Anticipated Biological Effect Level (MABEL) dose could be considered to be between 0.3 and $1 \text{ mg}\cdot\text{kg}^{-1}$. If the MABEL was considered to be $0.5 \text{ mg}\cdot\text{kg}^{-1}$ and using the Safety Criteria outlined in the CPMP Position Paper on Non-Clinical Safety Studies to Support Clinical Trials with a Single Microdose², the safe starting dose would have been calculated to be $0.005 \text{ mg}\cdot\text{kg}^{-1}$ or $5 \text{ }\mu\text{g}\cdot\text{kg}^{-1}$.

Examples of starting dose calculation for two products which were “first-in-man” in healthy volunteers as a comparison with TGN1412.

Product 1

MABEL

The maximal inhibition determined in vitro in human plasma is ~40% at a concentration of 0.2ug/ml. No change in the degree of inhibition was observed at concentrations of 2ug/ml or 20ug/ml. The concentration resulting in 20% inhibition was calculated to be 0.01ug/ml. Using a plasma volume of 50ml/kg, this translates into a dose of 0.5ug/kg. From toxicokinetic studies in the monkey, a 5mg/kg bolus dose resulted in a peak plasma concentration of 189ug/ml. The maximum concentration anticipated with a 0.5ug/kg dose is 0.0189ug/ml.

Starting dose 0.5ug/kg

Human Equivalent Dose

1/10 of the HED based upon the NOAEL in the most sensitive species is normally accepted as a maximum safe starting dose in healthy volunteers. An additional safety factor up to 10 should be added if NOAEL has not been defined.

The calculation is as follows

Observed adverse effect dose in the monkey: 5 mg/kg

Corresponding Human Equivalent Dose (HED): 1.6 mg/kg

1/10 of the HED: 0.16 mg/kg

Additional safety factor of 10: 0.016 mg/kg

Starting dose 16ug/kg

Microdose

The dose calculated to yield a pharmacological effect is 0.5mg/kg. The term microdose is defined as less than 1/100th the dose calculated to yield a pharmacological effect. This is 5ug/kg

Starting dose 5ug/kg

Product 2

MABEL

The compound showed no differences in affinity or potency when compared to its rodent homologue in vitro. Both molecules were efficacious in a rodent model of disease. The

minimum effective dose of the test compound was 0.3mg/kg. This corresponds to a plasma level of 0.04mg/ml in the rodent. Using a plasma volume of 50ml/kg, this translates into a dose of 2mg/kg.

Starting dose 2mg/kg

Human Equivalent Dose

1/10 of the HED based upon the NOAEL in the most sensitive species is normally accepted as a maximum safe starting dose in healthy volunteers. An additional safety factor up to 10 should be added if NOAEL has not been defined.

The calculation is as follows

NOAEL in both rat and monkey is 500 mg/kg

Corresponding Human Equivalent Dose (HED): 81 mg/kg (rat)

162mg/kg (monkey)

1/10 of the HED: 8.1 mg/kg

Starting dose 8mg/kg

Microdose

The dose calculated to yield a pharmacological effect is 5mg/kg. The term microdose is defined as less than 1/100th the dose calculated to yield a pharmacological effect. This is 50ug/kg

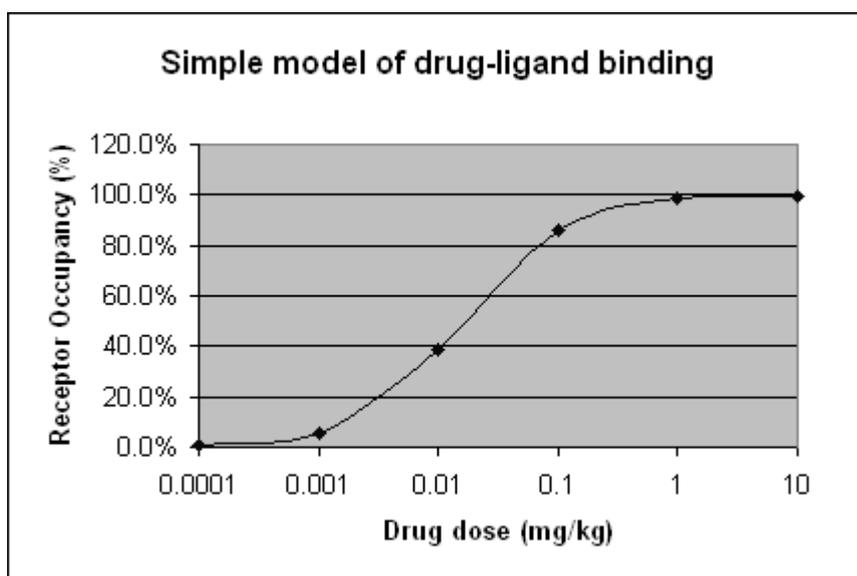
Starting dose 50ug/kg

Calculated receptor occupancy of TGN1412

Example of Receptor Occupancy versus local concentration

Dissociation constant	1.88E-9 (M)	Kd of TGN1412
Molecular weight (Da)	150,000	Molecular weight of TGN1412
Volume of distribution (mL/kg)	57	

Dose (mg/kg)	Plasma conc (M)	Receptor Occupancy (%)
0.0001	1.17E-11	0.6%
0.001	1.17E-10	5.9%
0.01	1.17E-09	38.4%
0.1	1.17E-08	86.2%
1	1.17E-07	98.4%
10	1.17E-06	99.8%



Receptor Occupancy for TGN1412 as calculated by the ABPI/BIA Taskforce

TGN1412: an example

The amount of mAb bound to its target can be estimated from the simple relationship as outlined in the joint ABPI/BIA, where:

mAb (A) + ligand (B) is in equilibrium with mAb-ligand complex (C) The binding affinity (Kd) is represented by $[A][B]/[C]$ and mAbs are selected for high affinity i.e. they favour the binding to target ligand.

In vivo, the concentration of both mAb and the target ligand will change with time. However, as an initial estimate, the amount (or percentage) of mAb bound to the target in circulating blood can be estimated by assuming:

- i) no turnover (elimination) of the target ligand (CD28 receptor)
- ii) no loss of mAb to distribution and clearance
- iii) instantaneous equilibrium in the binding reaction.

Whilst these assumptions are not a true representation of the *in-vivo* situation, they do give an estimate of the likely binding to available target in the first few hours immediately after dosing.

Dose TGN1412 0.1 mg/kg

Body weight 70 kg

Molecular weight TGN1412 150000

Blood volume 5L and plasma volume 2.5

T lymphocyte count at baseline (before dosing) = 1.3×10^9 cells per L blood

CD28 receptors per Tcell 150000 (Bryl et al 2001; 167 (6): 3231-3238)

Kd 1.88 nM (TeGenero, information in public domain)

Total TGN1412 concentration (A + C) in plasma immediately post-dosing 18.7 nM

Total ligand (CD28) concentration (B + C) exposed to plasma at baseline 0.648 nM, assuming $B + C = 1.3 \times 10^9 \times 150,000$ (receptors/cell) / $N_A \times 10^9$

Drug-ligand concentration (C) immediately post-dosing 0.587 nM

Percentage CD28 receptors occupied by TGN1412 90.6%

Clearly, this initial dose is expected to give a very high occupancy of CD28 receptors immediately post-dosing and is likely to achieve the maximum pharmacological effect. It is not uncommon that a very small dose of mAb (below the clinically recommended dose) will give a maximum pharmacological effect, albeit for a short duration of time. The duration of effect being largely governed by the dose of mAb and the rate of turnover of the target ligand. For a receptor agonist, such as TGN1412, this level of receptor occupancy should be viewed in light of that achieved in pre-clinical pharmacology studies and the relative potency between animals and man. This information is not in the public domain.

Some simplifying general principles

A precise estimate of initial ligand binding requires an estimate of the target concentration. In some situations this may prove difficult, for example where the ligand is in tissue or the volume is variable (e.g., synovial fluid). Where the concentration of

ligand is reasonably expected to be much lower than antibody, i.e., $A \gg B$, for monoclonal antibodies, fractional ligand occupancy (R_o) is approximately given by:

$$R_o = 1/(1 + K_d[nM]) / (187[nM/mg/kg] \times \text{Dose}[mg/kg])$$

The relatively low variability in monoclonal antibody distribution means that the factor of 187 is generic to large proteins (MWT in excess of say 100kDA). As a guide, starting doses in mg/kg of $K_d[nM]/200$, will have initial ligand occupancy of about **50%**. For an antibody falling under the terms of reference of the ESG, such as TGN1412, lower initial occupancy is desirable. Initial levels of receptor occupancy should be justified by the applicant in the IMPD application. For example, using this approach, a dose of 0.001 mg/kg TGN1412 would give a projected receptor occupancy in the range 5-10%.

References

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C Phase One Clinical Trial and Adverse Events at Northwick Park Hospital

Introduction

TGN1412, a CD28 superagonist was scheduled to be tested in 'First in man' trial at the Parexel Clinical Pharmacology Research Unit (CPRU) located within leased space at Northwick Park hospital in North London. The Parexel CPRU was the contract Research organisation that undertook responsibility for conduct of the trial of TGN1412. TGN1412 or analogues had been tested in pre-clinical studies in the following species; Rats (JJ316), rabbits, rhesus and cynomolgus monkeys (TGN 1112 and TGN 1412). TGN 1112 is an IgG1 variant of TGN 1412.

5.1 Design of Trial

The proposed trial was a phase-I, single centre, double blind randomised, placebo controlled escalating dose group study to assess the safety, pharmacokinetics, pharmacodynamics and immunogenicity of single doses of TGN1412 administered intravenously to healthy volunteers.

The primary objectives were to assess the safety and tolerability of ascending single doses of TGN1412 in separate cohorts and to assess pharmacokinetics. The

secondary objectives included assessment of effect of TGN1412 on lymphocyte subsets, cytokine profiles and anti-TGN1412 antibody generation after single doses of TGN1412.

The subjects were scheduled to be admitted to the CPRU on day 1 and retained at the unit until day-3. Vital signs were to be measured 0.5, 1, 2, 4, 8 and 12 hours after start of infusion. Further recordings (ambulatory) were planned at specified intervals up to 43 days.

The proposed dosing range was 0.1, 0.5, 2.0, and 5.0mg/kg BW intravenously or placebo.

5.2 Trial Protocol

Inclusion & Exclusion Criteria; (Protocol defined).

A predefined set of criteria were used to select appropriate volunteers;

Inclusion Criteria:

- 18-40 year old healthy males
- Body-mass index 18-28 kg/m²
- In good health based on medical history, examination, ECG and laboratory parameters.
- Normal CRP at screening and day (-1) to exclude inflammatory processes
- Able to give informed consent.

Exclusion Criteria;

A variety of exclusion criteria were applicable, notable of which were;

- Any infection or inflammation (based on CRP)
- Enlarged lymph nodes
- History of major systemic disorder (cardio-respiratory, neurological ...etc)
- History of multiple drug allergies or any clinical allergic disease
- AIDS, hepatitis (B or C),
- Subjects receiving an investigational drug in the previous-4 months, or vaccines
- Subjects who received a previous dose level(TGN 1412) in this study
- Alcohol or substance abuse, mental incapacity, participation in strenuous exercise in the previous 4 days, high BP, low heart rate etc.

Measures provided in the Protocol to minimise risks

The protocol listed the following potential adverse events and the general and specific treatments were planned in the event of such an occurrence;

Event	General measures	Specific measures
Anaphylaxis	Airway/circulation support/ vasoconstrictors	Corticosteroids /Beta- agonists / antihistamines
Immunogenicity /Autoimmunity	Avoid further exposure	Steroids/ antihistamines if required.
General cutaneous reactions	Antihistamines, analgesics,	Corticosteroids if necessary
Cytokine release syndrome	General supportive measures	Corticosteroids and other appropriate clinical measures.
serious adverse events	General supportive measures	System specific measures. Stop trial if a number of serious ADRs were reported.
Immunosuppression (massive induction of anti-inflammatory cytokines & / regulatory T cells)	Appropriate counter measures	Antibiotics for infection etc..

Reasons for stopping subjects in the trial:

Subject withdrawal;

- Withdrawal of consent
- Intolerable adverse event
- An intercurrent illness or procedural complication
- Entry into study in violation of protocol or protocol violation during the study
- Investigator opinion on best medical interests of the subject
- Availability of new data that raised concerns about safety of drug

Premature termination of the trial;

- Number and severity of adverse events justify discontinuation (in the opinion of investigator and sponsor)
- New data becoming available that raise concerns about safety of the drug

5.3 Adverse Events:

Report obtained from SUSARs.

The first screening was 22nd February 2006. The first day of dosing was 13th March 2006.

Eight subjects aged 19-34 (mean 26 years) were recruited. All had normal haematological and biochemical parameters pre-dosing.

Pre-dose parameters

Subjects ⇐	1	2	3	4	5	6
Urea (mmol/L)	6.0	3.6	4.6	4.3	5.8	4.9
Creatinine (umol/L)	89	82	79	74	81	78
Neutrophils [$\times 10^9$ /L]	2.32	1.73	2.54	3.69	5.14	2.16
Lymphocytes [$\times 10^9$ /L]	1.47	1.63	2.28	1.69	2.59	2.03
CRP (units)	<5	<5	<5	<5	<5	<5
ALT	23	22	32	25	36	25

Doses Administered and times

All subjects were dosed between 8-9 AM on the 13th Mar 2006 at ~10minute intervals. Six received active agent and two received placebo.

	Subjects ⇒	1	2	3	4	5	6
Body weight (Kg)		84.3	68.9	88.5	82.4	72.1	81.8
Dosing							
TGN1412		8.4 mg	6.8 mg	8.8 mg	8.24 mg	7.2 mg	8.2mg
Dosing time		08:00	08:20	08:30	08:40	08:50	09:00

Symptoms and timing

A number of symptoms were reported by the subjects fairly soon after administration of the investigational agent (drug/ placebo).

- In 5 of 6 subjects, headache was reported between 50-90 minutes.
- All reported lumbar myalgia.
- Rigors were reported between 58-120minutes in 4 subjects.
- Elevated temperatures $>38^{\circ}\text{C}$ were noted between 2.5 to 6.5 hrs
- Hypotension was noted between 3.5 to 4.6 hrs after administration
- Tachycardia noted at 2.5 to 4.6 hrs

Other symptoms reported include nausea, vomiting, dyspnoea and bowel disturbances. A number of subjects subsequently reported feeling extremely ill and unable to recall events in specific order (reported as amnesia).

Initial treatment

All subjects received intravenous fluids [4 to 8 l overall; (colloids 1.5 to 4.0 l) and crystalloids 1-4 l). All subjects also received vasoconstrictors (metaraminol 1mg), analgesics (including paracetamol and codeine orally).

Corticosteroid (hydrocortisone 100mg) was administered to all subjects between 4 to 6 hours of onset of symptoms.

Transfer to Critical care

All 6 symptomatic subjects were transferred to the Critical Care Unit at Northwick Park hospital between 12 and 16 hours after dosing in view of the continued deterioration in clinical status.

On transfer to critical care unit, the following were the predominant and common features; dyspnoea and tachypnoea in 5 of the 6, respiratory fatigue in 4 of the 6, bilateral radiological pulmonary infiltrates in all, increased blood urea, significant base deficit, and features consistent with disseminated intravascular coagulation [elevated fibrin degradation products (FDP), low fibrinogen and altered prothrombin time]. There was general lymphopenia (values ranging between 0.04 to $0.07 \times 10^9/L$ in the ITU) compared to pre-dose (baseline 1.47 to $2.59 \times 10^9/L$) and this persisted for several days.

Treatment in Critical care

- **Ventilation:**

All subjects (patients) needed assisted ventilation: 4 of the 6 non-intubated patients received continuous positive pressure ventilation (CPAP) for durations of 4 -82 hours. Intermittent positive pressure ventilation (IPPV) was utilised in two subjects (12 -18 hours post dose). These two subjects needed IPPV.

- **Veno-venous filtration:**

Continuous veno-venous filtration (CVVHDF) was utilised in all subjects for varied durations (44 hours to 158 hours) in the ITU setting. Two subjects had oliguria/anuria.

- **Steroids /Immunosuppressants;**

A second dose of Hydrocortisone (100mg each dose, and 200mg total) was administered after the initial dose and this was followed by 3 doses of methylprednisolone 1.0 gramme IV to all subjects, the first at 16 hours with subsequent doses at 40 and 64 hours post TGN412 dosing. Three doses of Dacluzimab (an immunosuppressant monoclonal antibody to prevent T lymphocyte proliferation), 1mg/kg were administered to each subject. Low dose steroids were continued after the above for a range of 21-33 days.

Other clinical events

Atrial fibrillation and peripheral limb ischaemia were seen in two subjects. ;

Generalised desquamation (prolonged in 3 subjects), muscle weakness, myalgia, and hyperalgesia occurred. Other neurological phenomena were also noted including headache, inability to concentrate, impaired ability to recall and peripheral numbness.

Complications/ other events

Three of the six subjects acquired infections while in hospital (Klebsiella, Candida, Staphylococcus aureus infection of foot wounds. Two others had viral reactivation (oronasal herpes and herpetic lesions elsewhere) and these responded to appropriate treatments (antibiotics and high dose acyclovir).

Clinical Follow-up

In all 6 subjects the duration of pyrexia (temp >38 C) varied between 14 hours to 36 hours. At the start of ITU/ICU admission, all subjects needed plasma volume expanders, vasoconstrictors, and veno-venous haemofiltration of duration (44-158 hours). The Intensive therapy unit stay was prolonged in 3 subjects.

Interpretation of clinical events triggered by TGN

The events that followed administration of TGN1412 were similar to those observed with another agent, OKT3 antibody. OKT3 when administered to humans and in some animals caused a cytokine storm, releasing various inflammatory cytokines from lymphocytes/monocytes and other cells.

The events in the 6 subjects who received TGN 1412 followed a similar clinical picture with generalised symptoms (myalgia, headache, hypotension & tachycardia). These progressed as described above with changes in cytokines and lymphocyte subsets.

Cytokine release

Estimation of cytokines was part of the protocol of assessing the response to TGN1412. The cytokine results were obtained from different laboratories. The data from one private organisation is shown summarised here. The data from the immunology and haematology labs of Northwick Park Hospital are provided as non-numerical figures in the appendix. The values from the sponsor company (TeGenero) are not available at this point in time.

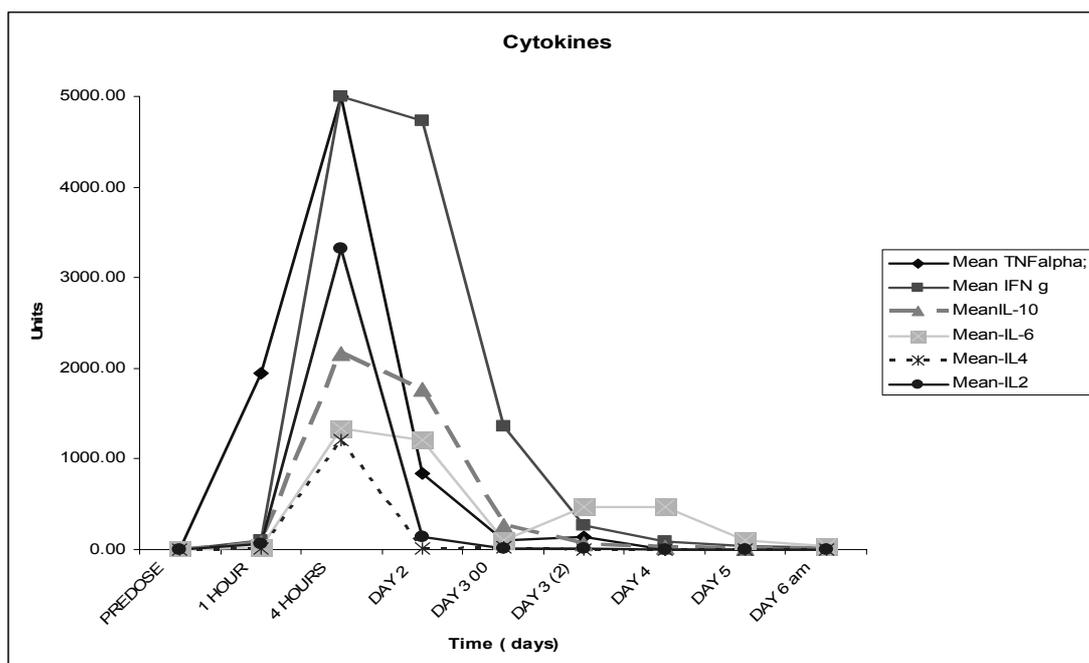
The following cytokines were assayed; TNF α , INF γ , IL-2, IL-4, IL-6 and IL10.

Cytokine table

	PREDOSE	1 HOUR	4 HOURS	DAY 2	DAY 3 00:10	DAY 3 11:50	DAY 4	DAY 5	DAY 6 am
Mean TNF α	<2.8	1943	> 5000	836	107	136	<2.8	<2.8	3.00
Mean IFN γ	<7.1	99	> 5000	4730	1366	270	89	43	27
Mean IL-10	<2.8	76	2158	1771	272	69	19	10	8
Mean-IL-6	< 3.0	29	1330	1204	96	475	466	95	43
Mean-IL4	<2.6	9	1205	13	24	3	3	3	3
Mean-IL2	4.70	57	3317	137	14.	9	4	3.	4

The table only shows mean values rounded to the closest integer. This is only to provide a schematic representation of changes in Cytokine values. The symbols (<) and (>) indicates values below and above the assay limits and hence are not exact values.

All cytokines increased significantly on days 1 and 2. Very large increases (>500-1000 fold) were seen in TNF and Interferon gamma and especially at 4 hours and day 2. Interestingly, TNF rose sharply in all subjects within 1 hour of TGN 1412 administration. A downward trend was seen in all cytokines by day 3 after administration.



The values from the Immunology laboratory of Northwick Park Hospital are not shown here.

Lymphocyte subsets;

All subjects demonstrated lymphopenia after administration of TGN1412. The comparisons of lymphocyte levels throughout the course are shown in the table.

Blood level (units)	Predose (range)*	T=8 hours (range)*	CTU normal range	T=16 hours (range)**	ICU normal range
Lymphocytes (x10 ⁹ /L)	1.86 (1.47 - 2.59)	0.06 (0.05 - 0.09)	1.5 - 4.0	0.04 (0.03 - 0.07)	1.10 - 4.80

Individual T –lymphocyte values varied to a greater extent. In all subjects after TGN 1412 there was a significant drop in CD3+, CD4+ CD8+ cells. The recovery also varied, with CD3+ cells leading the recovery followed by CD4+ and then CD8+. The CD8+ cells showed the slowest and the lower magnitude of recovery.

OUTCOMES;

Sequelae

All subjects have been followed by a clinician for 3 months after the event. All have residual limitations especially of loss of memory, inability to concentrate and headaches. One subject has dry gangrene of the toes and fingers that still require remedial measures. One subject has recurrent aphthous ulcerations.

Further data are awaited on the haemodynamic stability, immunological profile and antibody screen in these individuals.

D MHRA GCP, GMP and GLP Investigations

Introduction

This section covers the investigations carried out by the Medicines and Healthcare products Regulatory Agency (MHRA) regarding the Serious Adverse Events experienced during the ‘first time into man study of the monoclonal antibody TGN1412. It summarises the inspections performed by the MHRA and other European authorities and the testing performed on samples following the incident. The objective of this investigation was to determine whether there were any errors in the conduct of this trial which might have caused the serious adverse events.

Background

Parexel (a contract research organisation) was contracted by the sponsor, TeGenero AG, to conduct an entry into human study of the monoclonal antibody TGN1412. Eight healthy male volunteers were recruited and dosed by Parexel Clinical Pharmacology Research Unit (CPRU) on 13th March 2006. On the same day Serious Adverse Events (SAEs) were reported in 6 of the 8 subjects. According to Parexel CPRU, the subjects experienced “Cytokine Release Syndrome”, which was reported as “Life-Threatening”. The drug codes were broken by Parexel, this confirmed that the 6 subjects who experienced SAEs received active drug and the two subjects who did not experience adverse events received placebo.

Study Design

A Phase I, single-centre, double-blind, randomised, placebo-controlled, single escalating-dose study. Four single doses: 0.1, 0.5, 2.0 and 5.0 mg/kg to be administered in 4 groups of 8. A total of 32 subjects were planned.

First date of screening: 22nd February 2006

First day of dosing: 13th March 2006

Key organisations involved in the trial

Role	Name	Location
Sponsor	TeGenero AG	Wurzburg, Germany
Manufacturer	Boehringer Ingelheim	Germany
GLP Studies	[Redacted under FOIA Section 38]	[Redacted under FOIA Section 38]
Contract Research Organisation / Phase I Clinical Pharmacology Unit	Parexel	Northwick Park Hospital, Harrow, UK

Triggered Inspections of Facilities

Following the incident triggered inspections were performed at the various facilities.

Facility	Inspection Type	Date of Inspection
TeGenero AG, Germany	GCP	17 th of March 2006
Parexel, Northwick Park Hospital, Harrow, UK	GCP	16 th , 17 th and 27 th March 2006
Parexel	GMP	14 th and 16 th March 2006
[Redacted under FOIA Section 38]	GLP	20 th -21 st March 2006
Boehringer Ingelheim, Germany	GMP	22 nd – 24 th of March 2006

Inspection Findings

GCP Inspection of TeGenero AG

The inspection focussed upon the pre-clinical work performed by TeGenero prior to the first in human study. All work performed had been provided to regulatory authorities, as required by the clinical trials application process. No irregularities were found during the inspection.

GCP Inspection of Parexel

During the inspection the following areas were inspected; Parexel Clinical Pharmacology Research Unit (CPRU) Clinic, recruitment offices, reception, areas used to store the infusion pumps, office where blind break envelopes were stored, the sample laboratory and sample storage area. In addition, the Clinbase® electronic clinical trials database was viewed.

The inspectors reported that there were no findings which were believed to be likely to have contributed to the Serious Adverse Events experienced by the trial subjects who received the study drug.

Protocol Adherence

As a result of the GCP inspection a number of discrepancies were identified:

Documentation procedures were not adhered to

It is incumbent upon clinical pharmacology research units to keep appropriate records as laid out in protocol procedures. Parexel failed to complete the full medical background of a trial subject in writing. One Principal Investigator did not update the medical history file in writing following a verbal consultation with one of the volunteers.

Employment procedural errors

There was no contract in existence for the bank screening physician at the time they were employed - one was subsequently issued.

Parexel's Principal Investigator failed to authorise, in their log, the full work remit for the bank screening physician at the start of their employment.

Having interviewed the bank screening physician as part of their inspection, MHRA Inspectors were not satisfied that the individual had adequate training and experience for their role.

Insurance issues

Parexel had a duty to review TeGenero's insurance policy to ensure that one was in place and that there were no exclusion categories within it that might impact upon their volunteers, in this study. They failed to do this although no such exclusions were subsequently found.

The placebo volunteers – non adherence to the unblinding procedure;

The placebo volunteers were permitted to leave the trial before appropriate checks were undertaken to confirm that they were the two subjects that had received the placebo.

Contracts

There was no contract in place between TeGenero and Parexel at the start of the trial – one was subsequently issued and there was only a draft contract in existence between Parexel and private laboratory they had engaged.

Medical Cover

There was no formal system in place to provide 24 hour medical cover.

Parexel GMP Inspection

The scope of the inspection included a review of the facilities, equipment, quality systems, documentation and records associated with the storage, preparation and release of TGN1412 and placebo at the unit.

No deficiencies were raised during the inspection.

[Redacted under FOIA Section 38] GLP Inspection

The purpose of the inspection was to determine that a pivotal toxicology study performed to support the progression of TGN1412-HV into human was conducted in accordance with the principles of GLP.

The study audited was –

[Redacted under FOIA Section 38] Study: “4 week intravenous toxicity study in cynomolgus monkeys with a 6 week observation period”.

Additionally two validation studies and a dose-ranging study were reviewed.

The inspector concluded that the study had been performed in accordance with the principles of GLP and that the results presented in the final report appeared to accurately reflect the raw data. No critical or major deficiencies were identified.

It appears highly unlikely that any of these deficiencies contributed to the condition of the volunteers who participated in the Phase I clinical trial at Parexel CPRU, Northwick Park Hospital.

TGN1412 Product analysis Testing

Following the incident a series of tests were performed by independent laboratories to determine if the products met the batch release specification and additional tests were performed to aid the investigation into the incident. The testing spans the batch used in the toxicology studies (80 litre batch) and the batch used for the subjects (2000 litre batch).

Overview

Following the serious adverse event at the Parexel CPRU samples of the product and the placebo, administered to the trial subjects, had been secured by nursing staff and were seized by the Metropolitan Police investigation team. Staff from the National Institute for Biological Standardisation Control (NIBSC) transported the samples under controlled temperature conditions to NIBSC laboratories at Potters Bar, where they were transferred to temperature monitored refrigerators.

The manufacturer of the product Boehringer-Ingelheim in Germany (BI) provided reference materials and detailed analytical methodology to perform the required analysis. By virtue of their role as the UK Official Medicines Control Laboratory NIBSC were selected by MHRA to perform most of the analyses required on the product. However, tests, which NIBSC could not perform were carried out at three other laboratories. [Redacted under FOIA Section 38] Laboratories performed a rabbit pyrogen test, a sterility test and a total viable count. The Forensic Chemistry Centre (Cincinnati Ohio USA), US Food and Drug Administration (FDA) performed a toxicological screening test. The MHRA laboratory at Teddington developed and validated a suitable method to assay both the protein and acetate in the diluted samples administered to the trial subjects.

MHRA staff produced a testing protocol, based upon the release tests and specifications from the product manufacturer. The test protocol aimed to indicate any differences between the initial 80 Litre batch produced by BI, that was used in the toxicological study, and the full-scale production batch used for the clinical trial.

Samples available for testing

The following test materials were obtained from the CPRU at Northwick Park.:-

Refrigerated (2 - 8°C) Samples of Investigational Medicinal Product (IMP)
TGN1412 Lot No. E5646LO04

Refrigerated (2 - 8°C) Samples of placebo acetate buffer Lot No. 5653LO01

The following reference/retained sample materials were provided by BI Germany:-

Frozen reference samples Ref Std DS/1. These materials were combined from manufacturing runs 90695 and 90710. This was the material produced from the 80L batches used in the toxicity study.

Frozen reference samples Ref Std DS/2. These materials were produced from manufacturing run 100301-01. This was the reference for material Lot 5646LO04

Additionally, NIBSC are storing further samples, as yet un-analysed, of clinical materials and samples seized by the Metropolitan Police from The Doctors' Laboratory.

NIBSC is also storing the actual diluted samples of TGN1412 and placebo as administered to the trial subjects.

Testing Protocol

The following testing protocol was agreed by the MHRA investigation team, in conjunction with the analysts at NIBSC. The protocol was based upon the company batch release specification. However, for the SDS-PAGE and size exclusion chromatography prior to the receipt of the company methods, NIBSC performed an analysis using in-house methods. These tests were designed to confirm that the Investigational Medicinal Product (IMP) Lot No E5646LO04 administered to the trial subjects complied with the manufacturers' release specification and was equivalent to the pilot scale batch (Run 90695/90710) used in the original pre-clinical toxicity study.

Company release specification tests.

Assay by UV spectrophotometry:- To confirm the concentration of total protein present in the IMP batch and that the product was free from insoluble aggregates.

Endotoxins by LAL gel clot:-. To confirm the absence of endotoxins and pyrogenic material.

SDS-PAGE and Isoelectric focussing:-. To confirm that the product was consistent with the proteins expected to be found with a Human monoclonal antibody (MAb) and demonstrate that there was no adverse production of either dimers or half molecules.

Size Exclusion High Performance Liquid Chromatography:- To confirm that the principal peak in the chromatograms obtained was consistent with those produced by a protein of the expected molecular weight and that the content of dimers in the IMP was consistent with both the specification and the chromatographic profile obtained from the pilot batches.

BIAcore (Cell Binding) Assay:- To confirm that the product contains a protein that binds to the CD28 construct used.

Total Viable Count and Sterility:- To confirm that neither the placebo nor the IMP were contaminated with micro-organisms.

Non-Company release specification tests.

Rabbit Pyrogen Test:- To confirm that product did not contain pyrogenic material. Performance of the test also required that the product was injected into another animal species. (Performed by a commercial contract research laboratory).

Abnormal Toxicity Test (British Pharmacopoeia):- . The test was performed to confirm that IMP TGN1412 did not contain unexpected toxic contaminants and the MAb was not toxic to the test species.

Toxicity Screen. The test was performed using various screening techniques by the FDA's Forensic Chemistry Centre (FCC). The tests were based upon those routinely performed by FCC to assure the safety and non-contamination of foodstuffs in the USA. The results of the test would provide the final assurance that the IMP TGN1412 was not contaminated by any unexpected organic or inorganic toxic materials.

Results Obtained

All the results obtained by all methods performed confirmed that TGN1412, as administered to the trial subjects, fully complied with the release specification.

Scientists from NIBSC advised that any minor differences detected between the results obtained with Batch E5646LO04 as administered and those obtained with reference material DS 01 (pilot scale production) or reference material DS 02 (Lot No E5646LO04) were of no significance.

The results of the analysis performed are presented in Table 1 in Appendix K.

Conclusions

The investigation indicated that the adverse incident did not involve errors in the manufacture of TGN1412 or in its formulation, dilution or administration to trial participants. It was therefore concluded that an unpredicted biological action of the drug in humans was the most likely cause of the adverse reactions in the trial participants.

The Expert Scientific Group wishes to emphasize that the residual syringe samples of the material which was administered to the trial subjects have not yet been analysed for content and potential contamination and that, accordingly, the conclusions expressed with respect to the formulation, dilution and administration to trial participants, must be viewed as preliminary, until the results of analyses have been obtained. They also wish to point out that the provisional conclusions above were drawn based on analyses of TGN1412 from the same batch that had been administered to the subjects and handled and diluted according to the trial protocol and in an identical fashion as deduced from the GCP records. It is deemed highly unlikely that only the material given to the six affected trial subjects and not that given to the two subjects who received placebo, would have been subject to any form of contamination. Therefore the provisional conclusion remains reasonable.

**4. CYTOKINE STORM IN A PHASE ONE TRIAL OF
THE ANTI-CD28 MONOCLONAL ANTIBODY
TGN1412**

PERMISSION HAS BEEN GRANTED BY THE AUTHORS, THE
EDITORS OF THE NEW ENGLAND JOURNAL OF MEDICINE
AND THE MASSACHUSETTS MEDICAL SOCIETY FOR
REPRODUCTION OF THIS ARTICLE.

THE ARTICLE WAS WRITTEN BY THE NORTHWICK PARK
HOSPITAL PHYSICIANS WHO TREATED THE CLINICAL
TRIAL VOLUNTEERS AT NORTHWICK PARK HOSPITAL.

BRIEF REPORT

Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412

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SUMMARY

Six healthy young male volunteers at a contract research organization were enrolled in the first phase 1 clinical trial of TGN1412, a novel superagonist anti-CD28 monoclonal antibody that directly stimulates T cells. Within 90 minutes after receiving a single intravenous dose of the drug, all six volunteers had a systemic inflammatory response characterized by a rapid induction of proinflammatory cytokines and accompanied by headache, myalgias, nausea, diarrhea, erythema, vasodilatation, and hypotension. Within 12 to 16 hours after infusion, they became critically ill, with pulmonary infiltrates and lung injury, renal failure, and disseminated intravascular coagulation. Severe and unexpected depletion of lymphocytes and monocytes occurred within 24 hours after infusion. All six patients were transferred to the care of the authors at an intensive care unit at a public hospital, where they received intensive cardiopulmonary support (including dialysis), high-dose methylprednisolone, and an anti-interleukin-2 receptor antagonist antibody. Prolonged cardiovascular shock and acute respiratory distress syndrome developed in two patients, who required intensive organ support for 8 and 16 days. Despite evidence of the multiple cytokine-release syndrome, all six patients survived. Documentation of the clinical course occurring over the 30 days after infusion offers insight into the systemic inflammatory response syndrome in the absence of contaminating pathogens, endotoxin, or underlying disease.

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ON MARCH 13, 2006, EIGHT HEALTHY MALE VOLUNTEERS PARTICIPATED in a double-blind, randomized, placebo-controlled phase 1 study of the safety of TGN1412 (TeGenero), a novel monoclonal antibody. The study drug is a recombinantly expressed, humanized superagonist anti-CD28 monoclonal antibody of the IgG4 κ subclass that stimulates and expands T cells independently of the ligation of the T-cell receptor.¹ In contrast to other antibodies in clinical use or in clinical trials, TGN1412 directly stimulates the immune response *in vivo*. In pre-clinical models, the stimulation of CD28 with TGN1412 (or with murine-antibody counterparts) preferentially activated and expanded type 2 helper T cells² and, in particular, CD4+CD25+ regulatory T cells, resulting in transient lymphocytosis with no detectable toxic or proinflammatory effects.¹⁻⁴

On the day of the trial, six of the eight volunteers received TGN1412 and two received placebo. Subsequently, the six volunteers in the treatment group, who had multiorgan failure with an unknown mechanism and an unpredictable severity, were all admitted to the on-site critical care unit at Northwick Park and

St. Mark's Hospital, a National Health Service (NHS) hospital in London. We detail the clinical and pathological findings during the first 30 days after the infusion.

METHODS

TRIAL CONDUCT

TeGenero sponsored the trial of the monoclonal antibody TGN1412, which was manufactured by Boehringer Ingelheim. The trial was conducted by Parexel International, a contract research organization that operates an independent clinical trials unit in leased space on the premises of Northwick Park and St. Mark's Hospital.

The authors of this report are a group of NHS clinicians who assumed clinical responsibility for the secondary care of these patients after they were transferred to the NHS (between 12 hours [one patient] and 16 hours [five patients] after infusion). The authors have no contractual or operational relationship with either Parexel International or TeGenero.

PATIENTS AND SOURCES OF DATA

All six patients provided written informed consent to the NHS for the publication of data obtained during clinical case management. Clinical data obtained before admission to the NHS, and selected laboratory data obtained before the complications were observed, are reproduced here with permission from TeGenero. The trial was suspended owing to the serious adverse events, and no further tests were performed for research purposes. There was full disclosure of drug information, scientific data, and trial documentation by TeGenero and Parexel International, in order to assist in clinical management decisions at the time of the incident.

CYTOKINE AND CELL SUBGROUP DETERMINATIONS

Data on subgroups of cytokines and lymphocytes were subsequently collected for clinical purposes during the course of the illnesses. For details on the cytokine assays and the cell subgroups, see the Supplementary Appendix (available with the full text of this article at www.nejm.org).

RESULTS

All six patients who received the trial drug were male, with a median age of 29.5 years (range, 19

to 34) (Table 1). None had a notable medical history, and all were clinically well during the 2 weeks before the study; baseline laboratory values were normal (Table 2). Beginning at 8 a.m. on day 1, each volunteer received an intravenous infusion, 10 minutes apart, of either the study drug or placebo. Each infusion lasted 3 to 6 minutes. The six volunteers in the treatment group each received 0.1 mg of TGN1412 per kilogram of body weight, infused at a rate of 2 mg per minute; the remaining two volunteers received a similar volume of saline.

INITIAL RESPONSE AFTER INFUSION OF TGN1412

A series of adverse effects began in the treatment group after infusion, starting with the onset of severe headache in five patients after a median of 60 minutes (range, 50 to 90), accompanied by lumbar myalgia in all six patients after a median of 77 minutes (range, 57 to 95) (Fig. 1). Subsequently, during this early phase, the patients were restless and had varying degrees of nausea, vomiting, bowel urgency, or diarrhea (Table 1). Five subjects had short amnesic episodes associated with severe pyrexia, restlessness, or both. All patients had a systemic inflammatory response that included erythema and peripheral vasodilatation (the timing of which was undocumented), with recorded rigors in four patients at a median of 59 minutes (range, 58 to 120) after infusion. Hypotension (defined by a decline in systolic blood pressure of 20 mm Hg or more) developed in all patients a median of 240 minutes (range, 210 to 280) after infusion, accompanied by tachycardia, with maximal heart rates of 110 to 145 beats per minute. All patients received intravenous lactated Ringer's solution during this time. Body temperatures of 39.5 to 40.0°C were recorded a median of 280 minutes (range, 240 to 390) after infusion. At 300 minutes after infusion, Patient 1 had signs of respiratory failure, with tachypnea and a partial pressure of arterial oxygen (PaO₂) of 52 mm Hg while breathing ambient air; the PaO₂ increased with the addition of supplemental oxygen. Chest radiography revealed pulmonary infiltrates; these findings were not consistent with the expected response of a fit young man to the infusion of less than 4 liters of fluid at this stage. There was no clinical evidence of bronchospasm or laryngeal edema.

All patients were initially empirically treated

Characteristic	Patient No.					
	1	2	3	4	5	6
Age (yr)	24	34	31	19	28	20
Weight (kg)	68.9	84.3	81.8	72.1	88.5	82.4
TGN1412 dose (mg)	6.8	8.4	8.2	7.2	8.8	8.2
Transfer to critical care (hr after dose)	15.5	16.0	16.0	16.0	16.0	12.0
APACHE II score on transfer†	8	10	11	18	20	18
Bilateral pulmonary infiltrates‡	+	++	++	++	++	+++
Duration of abnormalities on chest radiography (days)	7	6	8	>5	6	7
Hemodynamics on transfer						
Blood pressure (mm Hg)	120/50	124/79	107/42	98/40	95/40	80/64
Heart rate (beats/min)	125	103	116	120	105	140
LVEF on echocardiogram (%)	50–55	70	60	50–55	60	55
PaO ₂ :FIO ₂	395.5	195.6	329.5	321.3	201.8§	84.0§
Base deficit (mmol/liter)	-5.1	-6.5	-5.6	-5.8	-10.3	-8.2
Lactate (mmol/liter)¶	3.1	4.5	5.7	6.0	5.9	4.2
Urinary output (ml/hr)	20	30	30	45	30	0
Treatment						
Days spent in ICU	4	7	7	5	11	21
Days receiving corticosteroids (including tapering)	21	21	21	21	24	33
Epihenomena						
Generalized desquamation	+	++	+	+	++++	+++
Muscle weakness‡	+	++	+	+	++	++
Late myalgia	Calf	Calf and hip adductors	Calf	—	Calf	—
Neurologic findings	Headaches and hyperalgesia	Hyperalgesia	Hyperalgesia and numbness	Headaches	Headaches and numbness	—

* LVEF denotes left ventricular ejection fraction, PaO₂ partial pressure of arterial oxygen, and FIO₂ fraction of inspired oxygen.

† Acute Physiology and Chronic Health Evaluation (APACHE) II scores range from 0 to 71, with higher values indicating more severe illness.

‡ Plus signs represent the degree of infiltrates or of muscle weakness.

§ The patient was on assisted mechanical ventilation.

¶ The normal range for lactate is 0.5 to 2.2 mmol per liter.

|| One plus symbol represents one episode of generalized desquamation, two represent two episodes, and three and four represent increasingly prolonged generalized desquamation.

in the independent clinical trials unit. A dose of 200 mg of hydrocortisone was administered intravenously in divided doses (with the initial 100-mg bolus a median of 331 minutes [range, 315 to 346] after infusion), in addition to 10 mg of chlorpheniramine intravenously, 1 g of acetaminophen intravenously, 4 to 8 mg of ondansetron intravenously, and 0.5 to 3.0 mg of metaraminol intravenously (in divided doses, titrated to effect). Blood samples were analyzed 8 hours after infusion at an off-site private laboratory (according to the study protocol) and therefore were not avail-

able as the situation evolved; the results were abnormal (Table 2).

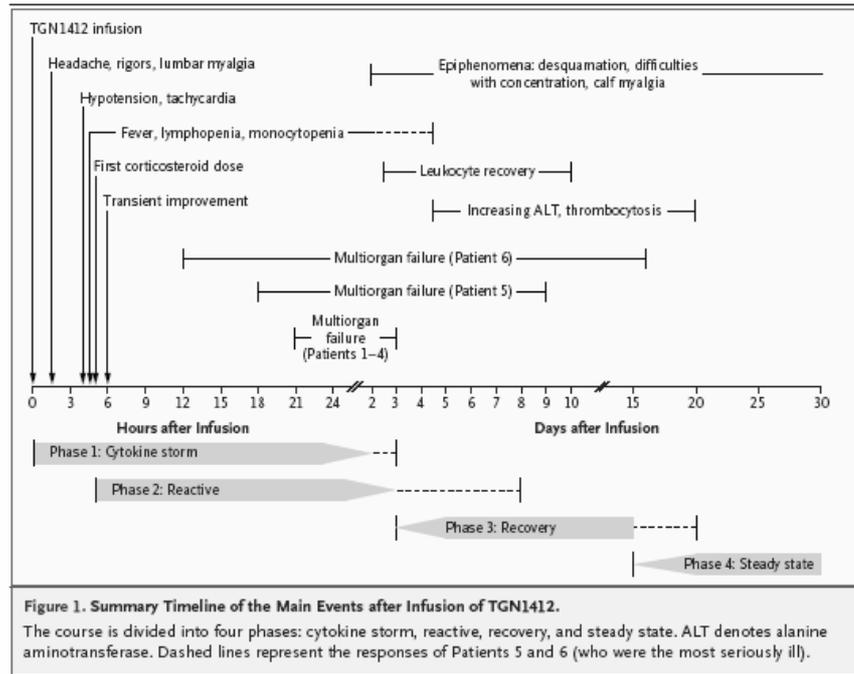
SUBSEQUENT EVENTS

After an initial recovery, Patient 6 became hypotensive (blood pressure, 65/40 mm Hg), and 12 hours after infusion, he had metabolic acidosis and marked respiratory distress with hypoxemia that was refractory to treatment with supplemental oxygen. He underwent intubation and mechanical ventilation, after which he was admitted to the intensive care unit (ICU) at Northwick Park

BRIEF REPORT

Table 2. Median Results of Blood Tests for the Six Patients before Infusion and 8 and 16 Hours after Infusion of TGN1412.*					
Blood Level of Constituent	Independent Clinical Trials Unit			Intensive Care Unit	
	Before Infusion	8 Hours after Infusion	Normal Range	16 Hours after Infusion	Normal Range
Creatinine ($\mu\text{mol/liter}$)					
Median	80	128	—	163	—
Range	74–89	106–195	66–112	125–325	62–115
Urea (mmol/liter)					
Median	4.8	6.4	—	9.3	—
Range	3.6–6.0	6.1–7.6	1.7–8.3	7.3–7.7	3.2–7.4
Uric acid ($\mu\text{mol/liter}$)					
Median	330	418.5	—	404	—
Range	309–426	339–465	266–474	251–590	210–420
Alanine aminotransferase (IU/liter)					
Median	25	21	—	32	—
Range	22–36	15–191	10–50	18–161	0–55
Hemoglobin (g/dl)					
Median	15.4	11.7	—	12.7	—
Range	15.1–15.9	11.0–14.1	13.0–17.0	10.3–15.6	13.0–17.5
Neutrophils ($\times 10^{-3}/\text{mm}^3$)					
Median	2.43	2.31	—	6.50	—
Range	1.73–5.14	1.99–4.95	2.00–7.50	5.09–11.14	1.80–7.70
Monocytes ($\times 10^{-3}/\text{mm}^3$)					
Median	0.26	0.03	—	0.03	—
Range	0.08–0.59	0.01–0.15	0.20–1.00	0.01–0.05	0.20–0.80
Lymphocytes ($\times 10^{-3}/\text{mm}^3$)					
Median	1.86	0.06	—	0.04	—
Range	1.47–2.59	0.05–0.09	1.50–4.00	0.03–0.07	1.10–4.80
Platelets ($\times 10^{-3}/\text{mm}^3$)					
Median	222	98	—	132	—
Range	164–261	51–144	150–400	69–169	140–450
Prothrombin time (sec)					
Median	11.2	14.2	—	26.2	—
Range	10.5–11.7	13.1–19.5	10.0–12.0	19.5–33.2	11.5–16.0
Activated partial-thromboplastin time (sec)					
Median	NA	NA	—	43.5	—
Range				40.1–61.9	26.0–38.0
Fibrinogen (g/liter)					
Median	NA	1.47	—	1.69	—
Range		0.66–1.75	1.50–4.00	0.99–1.98	2.00–4.50
D-dimer (ng/ml)					
Median	NA	NA	—	1784	—
Range				1350–4535	0–250

* To convert values for creatinine to milligrams per deciliter, divide by 88.4. To convert values for urea to milligrams per deciliter, divide by 0.357. NA denotes not available.



and St. Mark's Hospital. He had severely abnormal hemodynamics, coagulation, and pulmonary function, with a PaO₂ of 84 mm Hg while breathing 100% oxygen (ratio of PaO₂ to the fraction of inspired oxygen, 84) (Table 1).⁸ Because there was concern that all patients would follow a similar course of rapid deterioration, all remaining patients were transferred to NHS ICU facilities 16 hours after infusion.

FURTHER TREATMENT

Between 16 and 20 hours after infusion of TGN1412, the patients had further signs of respiratory deterioration: all six had signs of tachypnea, use of accessory muscles, inability to complete spoken sentences, and bilateral pulmonary infiltrates on chest radiography (Fig. 2A and 2B), and two had symptoms of dyspnea. There was also evidence of substantial renal impairment and disseminated intravascular coagulation, as indicated by an elevated prothrombin time, low fibrinogen level, high level of D-dimers, and decreased platelet counts in all six patients (Table 2). All patients had severe lymphopenia and monocytope-

nia, with sparing of neutrophils. Blood smears showed toxic granulation with Döhle's bodies and a dysplastic appearance of the neutrophils, with pseudo-Pelger-Huët anomaly (Fig. 2C and 2D).

There was no clinical evidence of primary cardiogenic shock, nor was there bronchospasm, laryngeal edema, or cutaneous signs indicating anaphylaxis. There were no overt or focal neurologic symptoms or signs that suggested neurogenic vasodilatory shock. All electrocardiograms and echocardiograms were normal (Table 1), and there was no clinical indication for lumbar puncture or electroencephalography.

All patients received empirical treatment with 1 g of methylprednisolone sodium succinate intravenously a median of 16 hours (range, 15.5 to 17) after infusion with TGN1412, with subsequent doses 40 hours and 64 hours after. Because of the expected effects of TGN1412 on T cells, all patients were empirically treated daily for 3 days with an anti-interleukin-2 receptor antagonist antibody, daclizumab (Roche), beginning a median of 25.5 hours (range, 23.5 to 28.0) after infusion. This treatment was stopped after 3 days

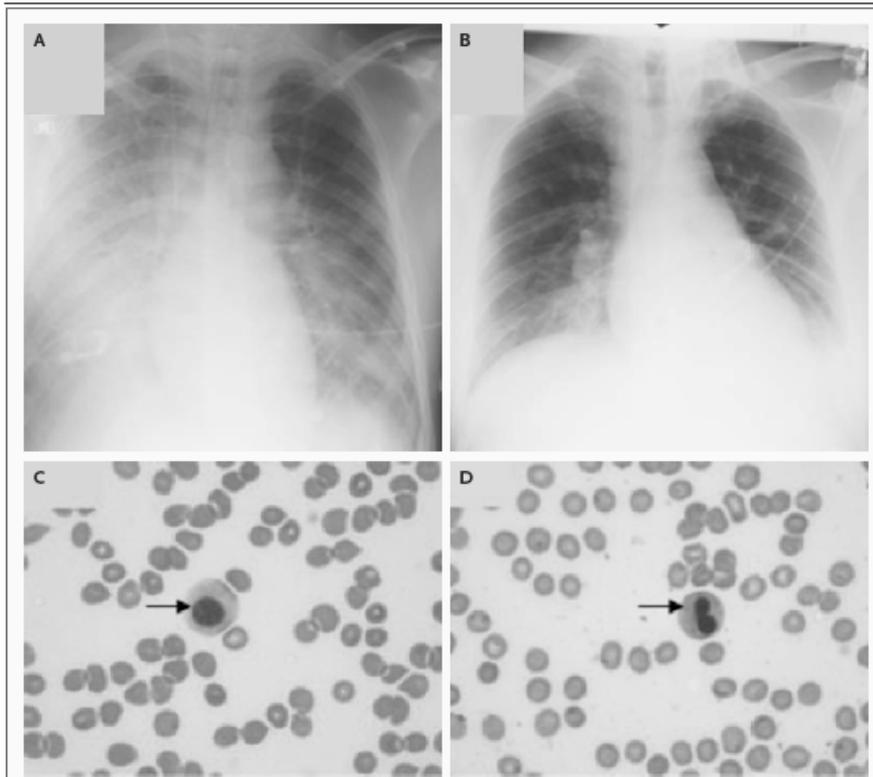


Figure 2. Representative Chest Radiographs (Panels A and B) and Blood Smears (Panels C and D) of the Six Affected Patients.

All anteroposterior chest radiographs were similar in appearance, with interstitial infiltrates first noted 5 to 16 hours after infusion of TGN1412 (Panels A and B). The blood films did not show red-cell fragmentation but did show dysplastic changes in the neutrophils, including pseudo-Pelger-Huët anomaly (arrows, Panels C and D; May-Grünwald-Giemsa stain), which was first noted within 24 hours after TGN1412 infusion. Although toxic granulation, vacuoles, and Döhle's bodies were initially observed, later blood smears showed neutrophils that were hypogranular.

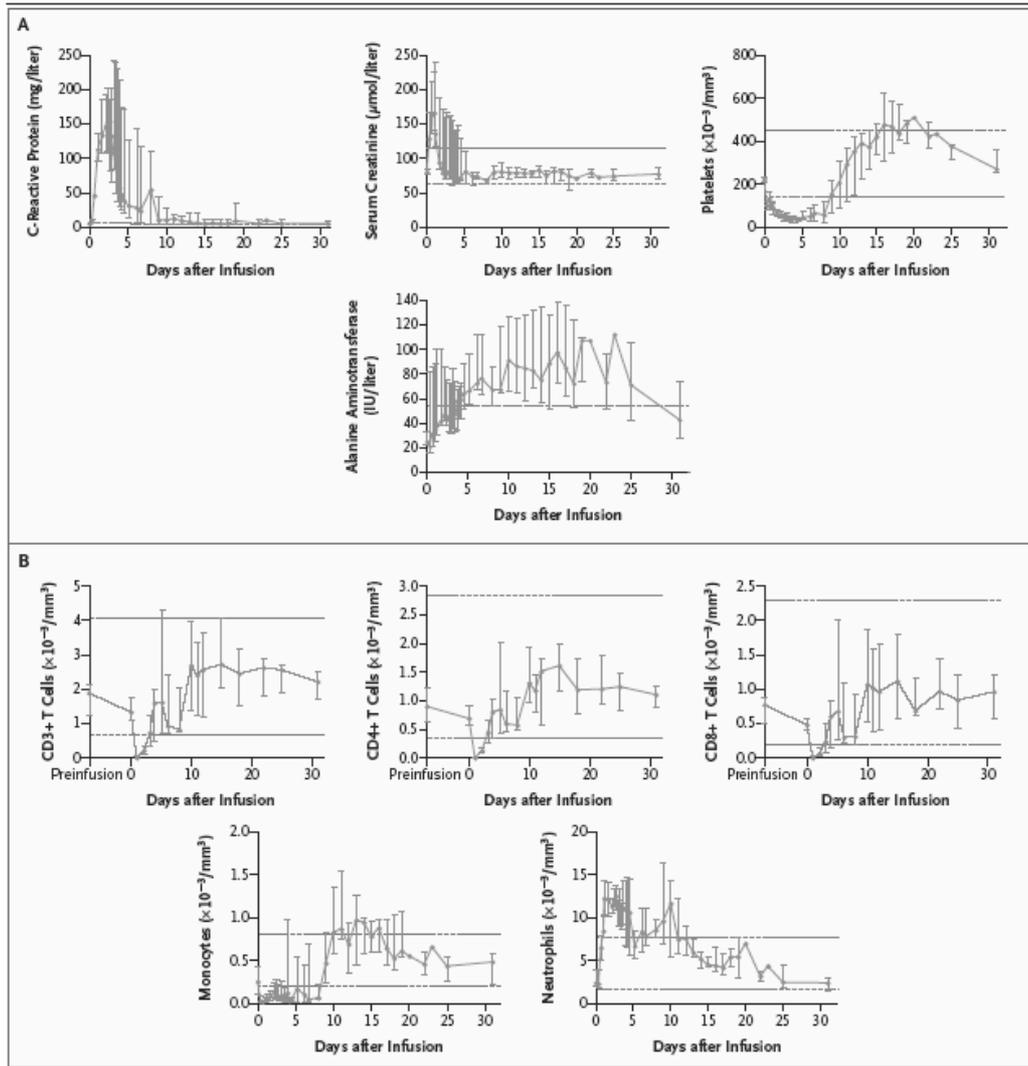
in the absence of TGN1412-induced lymphocytosis. In addition, potential activation of a histaminergic response was treated with 50 mg of intravenous ranitidine every 8 hours and 10 mg of intravenous chlorpheniramine maleate every 8 hours (continued from earlier doses).

SUPPORTIVE MANAGEMENT

Patients 1 through 4 received continuous positive airway pressure of 10 cm H₂O by means of a tight-fitting face mask. Patients 5 and 6 underwent mechanical ventilation, with tidal volumes limited to 6 to 8 ml per kilogram of dry body weight and

positive end-expiratory pressure maintained at 15 to 20 cm H₂O. All six patients had oliguria, metabolic acidosis, and increasing creatinine levels; they therefore received renal support by means of continuous venovenous hemodiafiltration with the use of a standard polyacrylonitrile membrane (Gambro Hospal U.K.) within 36 hours after their exposure to TGN1412. Dialysate rates were set to 1 liter per hour and were subsequently increased to 4 liters per hour.

All patients required the replacement of blood components by means of the infusion of fresh-frozen plasma and cryoprecipitate to correct co-

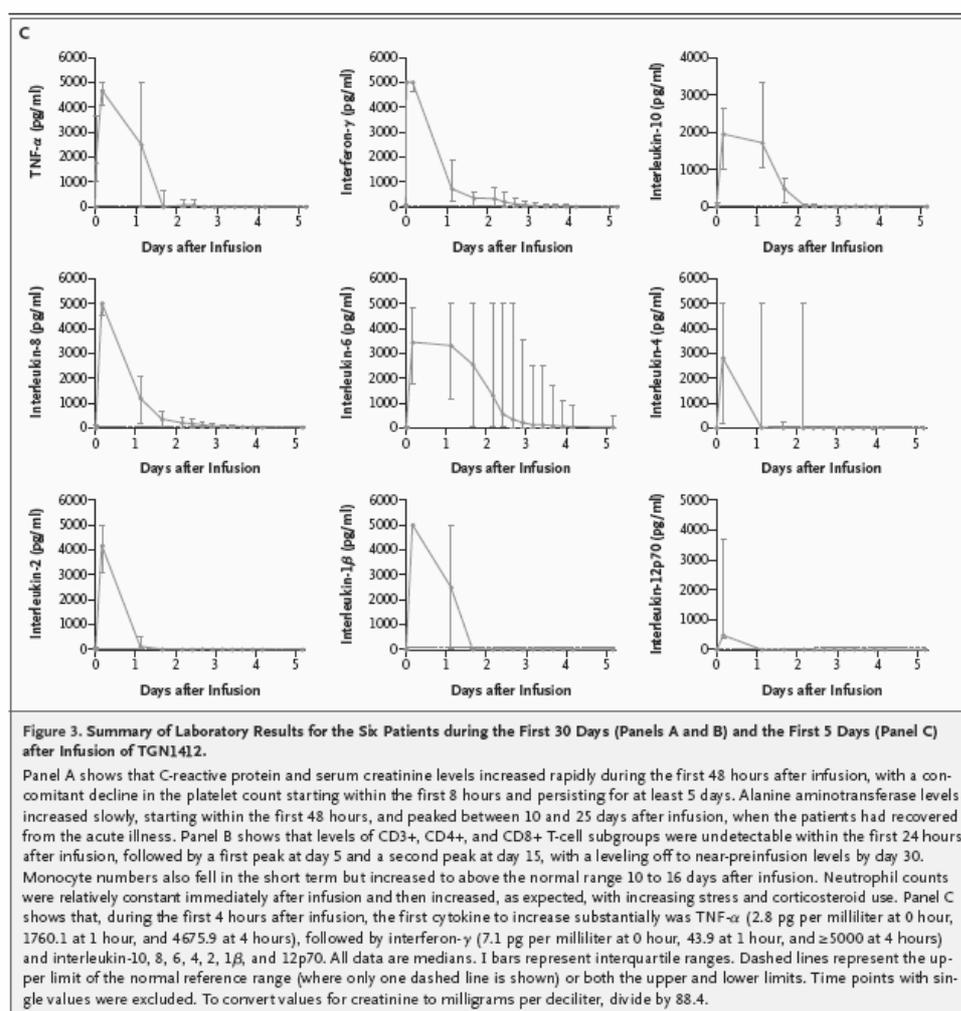


agulopathy. Owing to their severe lymphopenia, the patients were treated according to a protocol of infusions of irradiated red cells and platelets, as required, to prevent possible graft-versus-host disease.

CLINICAL PROGRESSION

Patients 1, 2, 3, and 4 continued to have intermittent fever, myalgia, and diffuse erythematous flushing for 48 hours, at which point their clinical

symptoms and signs diminished markedly. Immunomodulatory treatment in these four patients was reduced to a tapering dose of intravenous hydrocortisone followed by oral prednisolone (total duration of corticosteroid treatment in each case, 21 days). Continuous venovenous hemodiafiltration was stopped after a median of 28 hours (range, 22 to 35), and continuous positive airway pressure was stopped after 4 hours in Patient 1 and after a median of 77 hours (range,



57 to 82) in Patients 2, 3, and 4 (Fig. 4A and 4B through 7A and 7B in the Supplementary Appendix). Patient 2 was also successfully treated for presumed nosocomial *Klebsiella pneumoniae* bacteremia, isolated on day 6 after TGN1412 infusion.

Patients 5 and 6 had a more complex course, as detailed in the Supplementary Appendix. Although both patients initially had diminished erythema and fever 48 hours after infusion, they

subsequently had recurrent fever, increased peripheral vascular permeability, and episodes of diffuse erythematous flushing lasting several days. Both patients required intubation and mechanical ventilation. Peripheral ischemia was observed in a glove-and-stocking distribution in Patient 6. It fluctuated over time, independently of the changing vasopressor dose. Most of the peripheral ischemia slowly resolved, except in patch-

es of necrosis on the fingers of both hands and all the toes.

Over the next 30 days, all patients had generalized desquamation (most marked in Patients 5 and 6) and muscle weakness on discharge from the ICU. Five patients had late myalgia, headache after the discontinuation of corticosteroids, difficulties with concentration, and short-term difficulties in finding words (particularly names). Three patients had delayed hyperalgesia, and two had peripheral numbness. None had documented lymphadenopathy or splenomegaly while in the ICU or after discharge.

HEMATOLOGIC AND IMMUNOLOGIC PROGRESSION

The laboratory values for the six patients are summarized in Figure 3; data on the clinical course of each patient are provided in the Supplementary Appendix. Severe thrombocytopenia was observed, initially accompanying disseminated intravascular coagulation but persisting even after the other clotting values normalized (Fig. 3A, and Fig. 4A and 4B through 9A and 9B in the Supplementary Appendix). All patients had mild normocytic anemia that persisted beyond discharge from the ICU, followed by a slow recovery. Neutrophil numbers initially were preserved and then increased in response to corticosteroids (Fig. 3B), but they were dysplastic in appearance (Fig. 2C and 2D), a feature that eventually resolved. By contrast, marked lymphopenia and monocytopenia were noted in all patients 8 hours after TGN1412 infusion (Table 2).

Lymphocyte numbers were too low to allow for the measurement of cell subgroups 1 day after infusion. Subsequent blood tests showed increasing levels of CD4+ and CD8+ T cells (Fig. 3 through 9 in the Supplementary Appendix), CD19+ B cells, and CD16+ presumed natural killer cells, starting 48 hours after infusion. In Patients 1, 2, 3, and 4, who recovered the most rapidly, T-cell recovery occurred in a CD4+:CD8+ ratio of 1:1, with a temporary rise to levels just above normal in two patients (Fig. 4D and 7D in the Supplementary Appendix). Patients 5 and 6, the two who were most severely ill, had a slower recovery, with lower overall numbers of T cells (Fig. 8 and 9 in the Supplementary Appendix) and a CD4+:CD8+ ratio of 2:1.

The lymphocyte and monocyte nadirs in each patient occurred within 24 hours after TGN1412 infusion, overlapping with the cytokine storm

(Fig. 3B, and Fig. 4C through 9C in the Supplementary Appendix). A dramatic increase in the level of tumor necrosis factor α (TNF- α) was observed in all patients within an hour after TGN1412 infusion, followed by elevations in the level of interleukin-2, 6, and 10 and interferon- γ within the first 4 hours after infusion (Fig. 3C, and Fig. 4C through 9C and 10 in the Supplementary Appendix). This cytokine release resolved after the first doses of hydrocortisone and methylprednisolone, and in Patients 1, 2, 3, and 4 the values normalized within 2 days. By contrast, in Patients 5 and 6, the cytokine storm was prolonged by 1 to 2 days; discrete elevations in the interleukin-6 and interleukin-4 levels, out of proportion to those noted in the other patients, were observed.

DISCUSSION

The intravenous infusion of TGN1412 in healthy persons produced a sudden and rapid release of proinflammatory cytokines. These unexpected clinical data provide insight into the natural course of the cytokine storm and the systemic inflammatory response syndrome (SIRS) in the absence of contaminating organic factors. Regulatory authorities, who tested TGN1412 from the same batch as the infused drug, found no errors in its manufacture, formulation, or administration and found no contamination with endotoxin, pyrogen, or microbiologic or other agents.⁶ This type of cytokine release had not been observed in the preclinical studies of TGN1412, and it is currently unclear whether the severe effects of this type of cytokine release in vivo in humans is caused by the direct ligation of CD28 on T cells or by the ligation and activation of other cell types, leading to the release of preformed TNF- α , which then triggers the remainder of the cascade. The Secretary of State for Health has convened an expert scientific group to study the events of the clinical trial in greater detail.⁶

Clinically, the most striking phenomenon in the cohort was the stereotypical response to the study drug in all six patients and in all organ systems affected (albeit to varying degrees) (Table 3). All six patients initially had clinical signs that fit the criteria for SIRS.⁸ Subsequently, the most prominent clinical feature was the early appearance of respiratory distress and pulmonary infiltrates, accompanied by renal impairment and

profound disseminated intravascular coagulation. This pattern of organ impairment may be consistent with a generalized multiorgan response to inflammation or critical illness.^{9,10} However, the rapid onset and concordance of the lung injury among patients seemed unusual, and in the presence of high cytokine (especially interferon- γ and TNF- α) levels, these features may be consistent with immune-mediated injury that is specific to the lung.^{11,12}

Alveolar macrophages in humans are normally inefficient in the costimulation of T cells through the CD28 pathway¹³; thus, our data suggest that anti-CD28 agonists *in vivo* may be able to potentiate immune activation and therefore lung injury. Neither cytokine storm nor lung injury was observed in the preclinical studies of TGN1412. This probably indicates that the presence of high levels of proinflammatory cytokines is a requirement for the pulmonary compromise, regardless of whether CD28 is ligated in the lung. In contrast to the pulmonary compromise that eventually ensues in SIRS, the more rapid onset of lung injury in our patients may have been due to the combination of the direct effects of the antibody and cytokines on lung tissue.

Equally striking was the consistent pattern of immunologic effects and recovery in all six patients. In particular, the severe lymphopenia observed in these patients was unexpected; a temporary lymphocytosis had been observed in preclinical studies of TGN1412 in animals.^{3,4,14} This unanticipated lymphopenia in humans may have reflected cell death or the migration of cells to other tissues such as lymph nodes, although lymphadenopathy was not detected. Lymphopenia has been observed as part of the cytokine storm induced by other monoclonal antibodies.¹⁵⁻¹⁷ However, the low cell numbers observed in these studies were anticipated, given the mechanism of action and the antilymphocyte specificity of the infused antibodies. Sepsis in humans may also induce lymphopenia that is selective for B cells and CD4+ T cells over the course of several days.¹⁸ In contrast, the onset of lymphopenia within 8 hours after infusion of TGN1412, and the involvement of all mononuclear cells (CD4+ and CD8+ T cells and monocytes), may suggest that the depletion of cells in our patients was a response to the infused T-cell agonist drug rather than to the cytokine storm alone.

The clinical progression after infusion of

Table 3. Common Features after Infusion of TGN1412.

System	Feature
Cardiovascular	Capillary leak Hemodynamic instability Lactic acidemia
Renal	Early acute renal impairment Urinary sediment 10–100 White cells <10 Red cells Granular casts (two patients)
Pulmonary	Acute pulmonary changes (six patients) Met criteria for acute lung injury (two patients)* Met criteria for acute respiratory distress syndrome (one patient)*
Hematologic and immunologic	Cytokine storm (TNF- α ; interferon- γ ; interleukin-10, 6, 2) Increased C-reactive protein level and erythrocyte sedimentation rate Lymphopenia Monocytopenia Thrombocytopenia Disseminated intravascular coagulation Normochromic, normocytic anemia Dysplastic neutrophils but preserved numbers
Hepatic	Increased alanine aminotransferase and alkaline phosphatase levels
Integumentary	Diffuse erythema Late desquamation
Neurologic	Delirium Partial amnesia Paresthesia or localized numbness Difficulty concentrating (late) Headaches (early and late)
Autonomic, gastrointestinal, or both	Bowel urgency or diarrhea Nausea or vomiting
Musculoskeletal	Myalgia in lower back (early) and calves (late)

* Criteria are from the American–European Consensus Conference on ARDS.⁷

TGN1412 can be separated into four phases (Fig. 1). Phase 1 began within an hour after infusion, continued through days 1 and 2 (and day 3, in Patients 5 and 6), and consisted of the cytokine storm, involving the rapid induction of type 1 and type 2 cytokines (to varying degrees) and severe lymphopenia and monocytopenia. Phase 2, the reactive phase, occurred from day 1 through day 3 (or days 1 through 8 in Patients 5 and 6, who were the most seriously ill); it consisted of renal failure, disseminated intravascular coagulation, pulmonary infiltrates, and respiratory failure. Phases 1 and 2 overlapped; phase 2 was not necessarily directly caused by the events in phase 1. The recovery phase, phase 3, occurred between day 3 and day 15 (or between day 5 and day 20, for the patients who were the sickest) and was

characterized by the recovery of renal and pulmonary function. This recovery was reflected in thrombocytosis and increases in alanine aminotransferase and monocyte and lymphocyte levels (mostly in a 1:1 ratio of CD4⁺:CD8⁺ T cells). The last phase, phase 4, can be described as a plateau or steady-state phase. It began 15 days after infusion (or 20 days after in Patients 5 and 6) and consisted of normalization of the measured variables. As compared with reactions to the infusion of other immunomodulatory agents (such as anti-CD20,¹⁵ anti-CD3,¹⁶ and anti-CD52 monoclonal antibodies¹⁷), the response to TGN1412 initially had similar kinetics, including the rapid increase in the levels of first TNF- α and then interferon- γ and interleukin-6, followed by cardiovascular instability, and disseminated intravascular coagulation. However, from phase 2 onward, features unique to the response to TGN1412 were apparent — including early acute lung injury, diffuse

erythema with late desquamation, neurologic sequelae, and post-illness myalgias (Table 3).

These events occurred during the first dosing interval in a phase 1 drug trial of a humanized immunomodulatory monoclonal antibody involving healthy subjects. The events provide insight into an immune-mediated cytokine storm leading to multiorgan failure in the absence of infection, contamination with endotoxin, or underlying disease. The TGN1412 variant of the syndrome had some features that set it apart from a typical cytokine storm, most notably early acute lung injury and marked lymphopenia.

No potential conflict of interest relevant to this article was reported.

We are indebted to the NHS staff members involved in the care of the patients on the day of the event and after, for their skill and dedication and for overcoming the unprecedented clinical and logistic challenges that the event presented; and especially to the six patients for consenting to the publication of their clinical data in order to inform ongoing discussion and debate.

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5. NIBSC Special Cytokine Study and Follow Up Studies

Initial cell-based testing of TGN1412

Background

Following the Serious Adverse Events that occurred in all six recipients of the stimulatory CD28 Monoclonal antibody (Mab) TGN1412 during its clinical trial at Northwick Park on 13 03 06, an initial phase of analysis was carried out which showed that the drug complied with its specification and appeared to be of clinical grade. Much of this evaluation was carried out at NIBSC and is now complete.

Following discussions with the MHRA, the chairman of ESG and in-house consideration, a second phase of the investigation was begun at NIBSC to investigate the mechanisms underlying the pathological responses and to gain additional information about the biological activities of this molecule. Adverse reactions to TGN1412 began soon after its injection and continued for a period of weeks. The initial responses of fever, pain, and organ failure due to hypotension were consistent with responses to bacterial endotoxin even though the drug was not found to be contaminated with endotoxin in the bacterial endotoxins (Limulus) test. Also, the drug passed a “classical” rabbit pyrogen test, which is used to detect both endotoxin and “non-endotoxin” pyrogenic contaminants, but it is relatively insensitive compared with *in vitro* tests (1) and there are examples of the rabbit pyrogen test failing to detect contaminants that subsequently caused adverse reactions in man (2,3).

For some years an alternative “non-Pharmacopoeial” test for pro-inflammatory and pyrogenic contaminants has been applied to certain products associated with adverse reactions in man but which were negative in the bacterial endotoxins test or the rabbit pyrogen test or both (1, 2, 3,4). The alternative test is variously described as an “*in vitro* pyrogen test”, “monocyte activation test” or “cytokine release test”. In this procedure, human peripheral blood monocytes, which respond to contaminants by releasing pro-inflammatory cytokine proteins, are incubated with the drug being tested and one or more cytokines in the cell-conditioned medium are quantified. The monocytes are usually stimulated either as the mononuclear cell fraction or as diluted whole blood and the cytokine readouts are tumour necrosis factor alpha (TNF α), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6) (5,6).

Since the initial phase of the adverse reactions to TGN1412 suggested the release of pro-inflammatory cytokines (e.g. TNF α , IL-1 β and IL-6) it was decided to assess TGN1412 for cytokine releasing activity in two “cytokine release tests”: one utilising peripheral blood mononuclear cells (PBMC) purified from human peripheral blood and one utilising diluted human peripheral blood. In all experiments the blood was freshly drawn from healthy volunteers (NIBSC staff). The procedure using PBMC was chosen because this system (with IL-6 as readout) gained regulatory significance when it was FDA-approved in 2002 for use at a pharmaceutical company as an end product release test; also, the procedure is used (with FDA-approval) for *in process* testing and has been used to good effect in detecting a non-endotoxin pyrogen (bacterial peptidoglycan) that was causing adverse drug reactions to a licensed product (4). The test system using diluted whole

blood was chosen because this test system includes the neutrophils, platelets and red cells that are lost when the mononuclear cells are purified. Unlike the PBMC-based test, tests using whole blood have been validated only for testing for endotoxin, though such tests do have a capability to detect “non-endotoxin” contaminants (8,9)

When testing for contaminants in cytokine-release tests, usually it is a requirement that the drug under test does not have intrinsic cytokine-releasing activity (7) but in the case of TGN1412, the severity and duration of the adverse drug reactions suggested that the drug might have intrinsic pro-inflammatory activity, especially as TGN1412 was negative in the bacterial endotoxins test and the rabbit pyrogen test. So, in the present study, the cytokine release test is being used to detect responses that could be due to either contaminants or an unwanted biological activity or both.

In addition to the cytokine release testing, experiments were begun to investigate effects on lymphocyte proliferation mediated by TGN1412 as this could be implicit in the phenomena observed in the six volunteers at Northwick Park.

Methods

Tests using the peripheral blood mononuclear cell fraction, PBMC

The PBMC cytokine release test applied at NIBSC is a method that has been harmonised with tests in use in the pharmaceutical industry (7). PBMC are isolated, by density-gradient centrifugation, from human heparinised peripheral blood that is not more than four hours old. The test preparation is cultured with 0.5 to 2.0 million PBMC/ml in culture medium and the donor’s own plasma. The culture is carried out, using aseptic technique and reagents and consumables that are sterile and pyrogen-free, at $37\pm 1^\circ\text{C}$, in 5% CO_2 in humidified air for 16 to 24 h. The cytokine responses to the test preparation are compared, in validated ELISAs, with responses to standard endotoxin and appropriate positive controls, in this case the CD3-stimulating monoclonal antibody UCHT-1 and the lectin phytohaemagglutinin (PHA). Readouts were: IL-6, $\text{TNF}\alpha$, IL-8, Tissue Factor (TF), IL-2, interferon gamma ($\text{IFN}\gamma$)

(Not all readouts were measured in all tests). Two experiments were carried out using PBMC: in each experiment PBMC from four independent donors were tested.

Tests using diluted whole blood

Human heparinised peripheral blood (20% final concentration) that is not more than four hours old is cultured with the test preparation. Aseptic technique and reagents and consumables that are sterile and pyrogen-free are used. The blood culture is carried out at $37\pm 1^\circ\text{C}$, in 5% CO_2 in humidified air for 16 to 24 h. The cytokine responses to the test preparation are compared, in validated ELISAs, with responses to standard endotoxin and appropriate positive controls, in this case the CD3-stimulating monoclonal antibody UCHT-1 and the lectin phytohaemagglutinin (PHA). Readouts were: IL-6, $\text{TNF}\alpha$, IL-8, Tissue Factor (TF), IL-2, interferon gamma ($\text{IFN}\gamma$). (Not all readouts were measured in all tests). Four experiments were carried out using diluted whole blood: in each experiment blood from four independent donors was tested.

Rationale for doses of TGN1412 tested in cytokine release tests

TGN 1412 was injected into volunteers at 0.1 mg/kg and into monkeys at the much higher dose of 50 mg/kg, i.e. 500x the human dose.

When given as 0.1 mg/kg. Average male = 70 kg, and has approximately 5 litres of blood. Drug dissolves in plasma which is approximately 60 % of total blood volume and so effective dilution volume is 3 litres.

0.1 mg/kg x 70 kg = 7 mg TGN 1412 per 3 litres plasma
= approx 2 mg/litre plasma
= approx 2 µg/ml plasma

Dose given to monkeys = 50 mg/kg = 500x more than that given in man. The larger dose is equivalent to approx 1000 µg/ml.

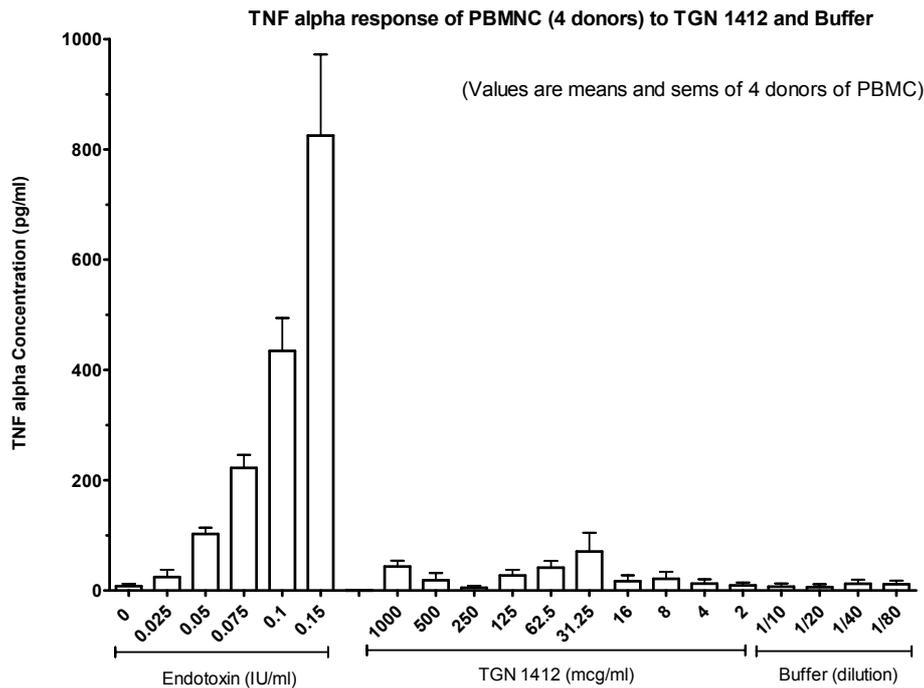
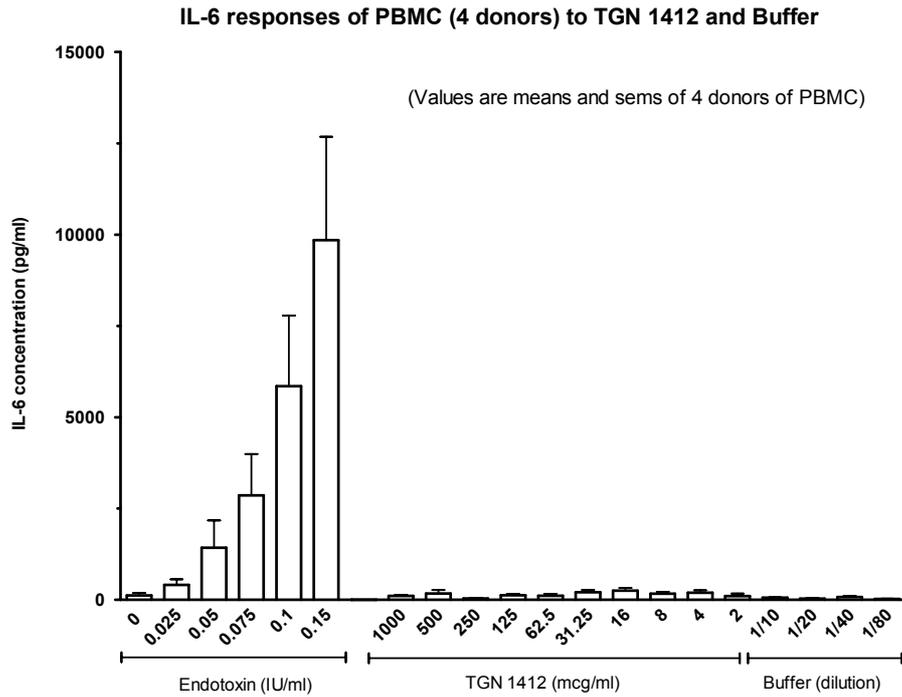
So, TGN 1412 was tested in cytokine release tests at 1000, 500, 250, 125, 62.5, 31.25, 16, 8, 4 and 2 µg/ml final concentration.

Tests for cell proliferation

Interaction of TGN1412 with CD28 is reported to induce a proliferative stimulatory signal. To investigate this, cell proliferation was assessed using a flow cytometry based assay that utilised “wet” and “dry” coating of Abs. Proliferation stimulated by TGN1412 wet coated at 10 µg/ml and in some wells air dried (giving 1 and 10 µg per well) was assessed in 96 well plates. The mitogens PHA (10 µg/ml) and anti-CD3 monoclonal antibody UCHT-1 coated at 10 µg/ml and in some wells air dried (giving 1 µg per well) were employed as positive controls. Briefly, isolated human PBMC were pulsed with the fluorescent membrane dye PKH26, washed and plated in triplicate at 1×10^6 PBMC per ml. After 72 h, PBMC were counterstained with anti-human CD4 FITC and anti-human CD8 APC conjugates, washed and fixed with 2% v/v paraformaldehyde. Triplicates were pooled for acquisition of 30,000 lymphocytes using a Becton Dickinson FACSCalibur flow cytometer. Lymphocytes undergoing proliferation divide their cell membrane between the daughter cells resulting in a halving of fluorescent dye and a reduction in fluorescent intensity compared with parent and resting cells. This appears as bands of cells with reducing PKH26 fluorescence on the flow cytometer. Using cell cycle analysis software the reduction in fluorescent intensity of proliferating cells was used to calculate precursor frequencies. The CD4/CD8 ratio of proliferating cells was analysed using Cell Quest software. The methodology has been described previously (10,11).

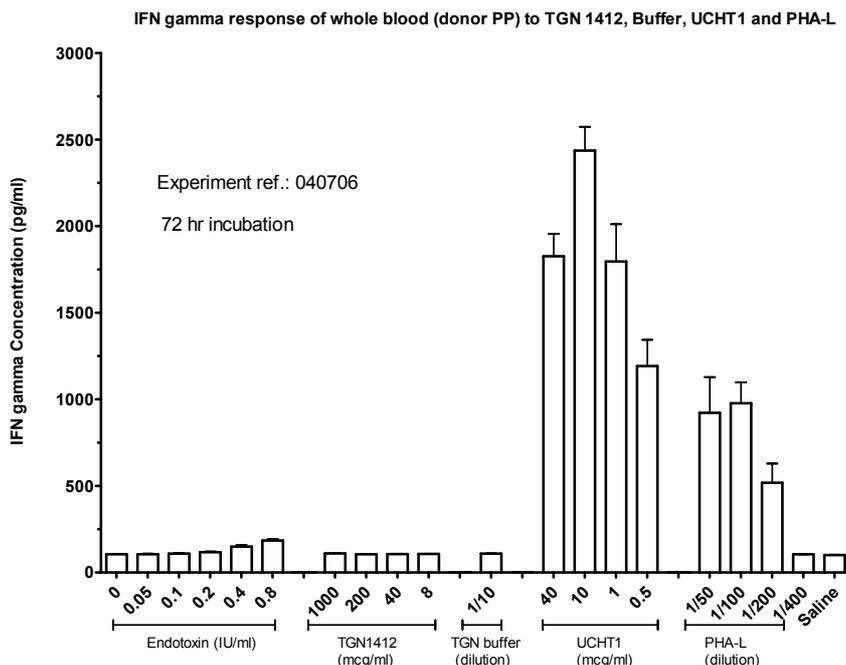
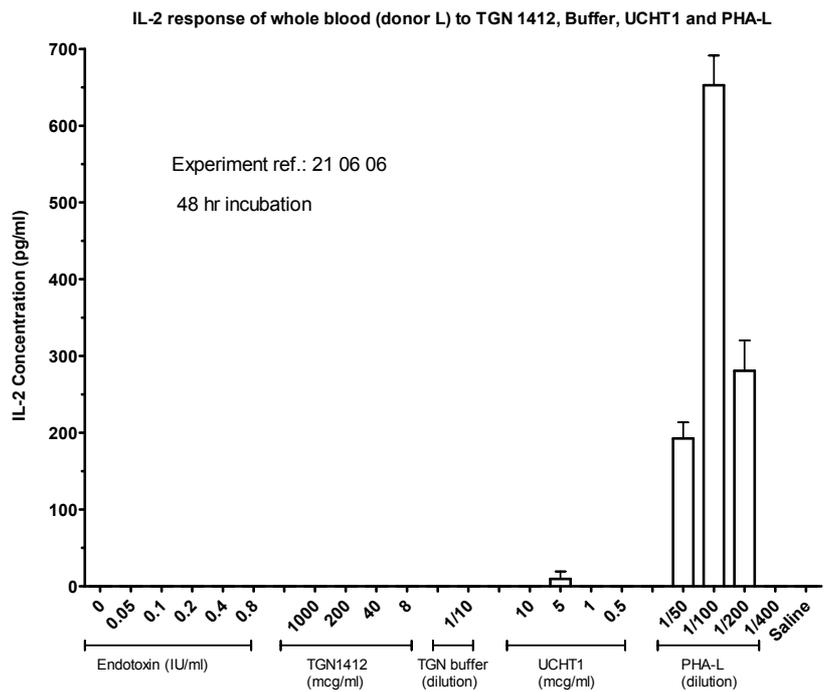
Results

TGN1412 (2 to 1000 µg/ml) incubated with PBMC (1 m cells/ml) for 16-24h did not stimulate the production of IL-6, TNF α , IL-8 or Tissue Factor (TF). Results for IL-6 and TNF α are shown in the two figures immediately below.

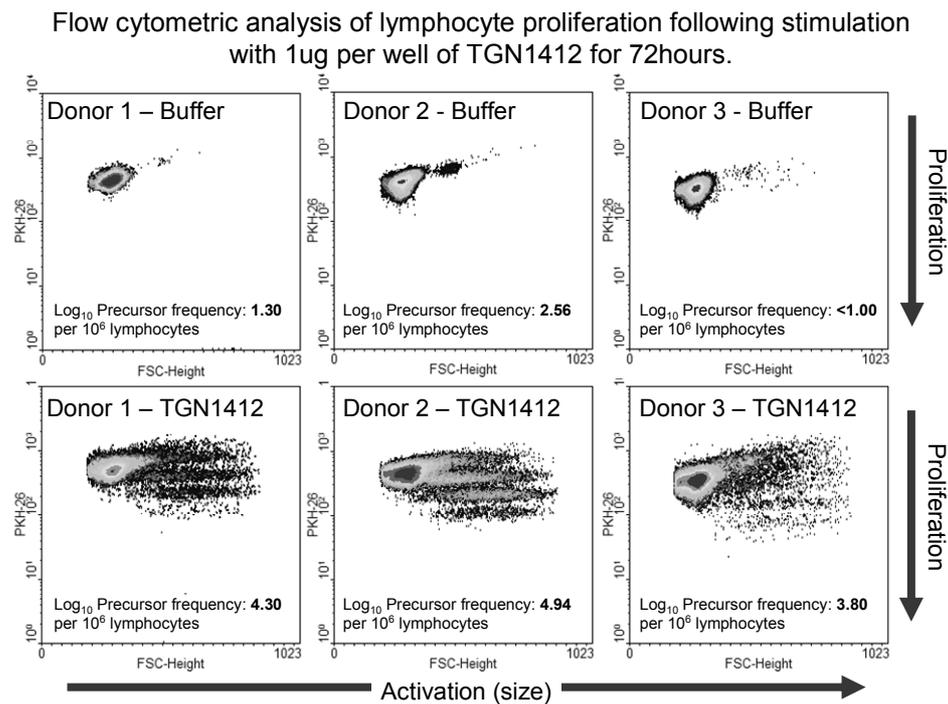


Similarly, TGN1412 (2 to 1000 $\mu\text{g/ml}$) incubated with diluted whole blood (20% v/v) for 16-24h did not stimulate the production of IL-6, TNF α , IL-8 or IFN γ (data not shown). In subsequent experiments with whole blood, the incubation time was increased to 48 or 72 h and IL-2 and IFN γ were the chosen readouts. In some of these experiments the TGN1412 tested was taken from a second vial and the Biacore binding assay was repeated on the TGN from this second vial to confirm that the TGN1412 had not lost any of its capability to bind to CD28. The Biacore results for the TGN1412 from the second vial were very similar to those obtained in the initial evaluation, confirming that the

TGN1412 had retained its biological activity. TGN1412 (8 to 1000 $\mu\text{g/ml}$) incubated with diluted whole blood (20% v/v) for 48 or 72 h did not stimulate the production of IL-2 or $\text{IFN}\gamma$. In contrast, the positive controls of the CD3 MAb UCHT-1 (0.5 to 10 $\mu\text{g/ml}$) and PHA (2.5 to 20 $\mu\text{g/ml}$) stimulated the release of $\text{IFN}\gamma$, and PHA (2.5 to 20 $\mu\text{g/ml}$) stimulated the release of IL-2. The $\text{IFN}\gamma$ responses were somewhat variable, i.e. donor-dependent, but in any given experiment most donors responded, though for some donors, UCHT-1 was a more effective stimulus than PHA and for some donors PHA was a more effective stimulus than UCHT-1. However, the striking observation was that TGN1412 was ineffective when simply incubated with whole blood or PBMC. The two figures immediately below illustrate this finding.



The lack of effect of TGN1412 in the above experiments contrasts with its effectiveness in an initial experiment to investigate its effects on lymphocyte proliferation: see the figure below. In this experiment, TGN1412 was “wet coated” or “dry-coated” onto the walls of the 96-well plate. When “dry-coated”, but not “wet coated”, at 1 $\mu\text{g}/\text{well}$, TGN1412 stimulated cell proliferation, though it was less effective when “dry coated” at 10 $\mu\text{g}/\text{well}$. The level of stimulation with TGN1412 (1 $\mu\text{g}/\text{well}$) was similar to that seen with UCHT-1 and PHA. Although the results of this experiment are still being analyzed, further proliferation experiments are already planned, as are further cytokine release tests in which TGN1412 is “dry-coated” onto the wells of 96-well plates, since this appears to be a more effective way of presenting the drug to cells.



Conclusions from initial experiments and suggestions for further experiments

Simply adding TGN1412 to PBMC or diluted blood does not stimulate the release of a “cytokine storm” or stimulate lymphocyte proliferation. This suggests that the dramatic adverse effects experienced by the volunteers at Northwick Park cannot be explained by a rapid induction of cytokines resulting from simple ligation of CD28. “Dry coating” TGN1412 onto the walls of the wells of 96-well plates would appear to be a more effective methodology, possibly due to cross-linking.

Adding TGN1412 to the wells of plates coated with an antibody against human IgG4 Fc should be investigated. Additionally, experiments that address a possible role for sCD28 in human and cynomolgus macaque (cyno) blood, the effects of TGN1412 on responses to other stimuli, e.g. endotoxin, and the effects of TGN1412 on endothelium and endothelium + PBMC might provide valuable information about TGN1412. Flow cytometry to assess intracellular cytokines and *in vivo* cyno experiments to repeat the toxicology at the dose injected in man, the 500x larger dose tested in cynos and an intermediate dose or doses would also be very informative.

Follow up cell-based testing of TGN1412

Background

Analysis of TGN1412 from unopened bottles and syringes recovered from Northwick Park Hospital was completed in October 2006 (please see report of Robin Thorpe to MHRA) and showed that the drug complied with its specification and appeared to be of clinical grade. Following discussions with the MHRA, the chairman of ESG and in-house consideration, a second phase of the investigation was begun at NIBSC in an attempt to learn something of the mechanisms underlying the pathological responses and to gain additional information about the biological activities of this molecule. An interim report (10 07 06) of the cell-based testing of TGN1412 has been presented to the ESG. Since that time, further cell-based testing of TGN1412 has been carried out at NIBSC and the results of this work are summarised below. While much has been learned, particularly concerning the most effective way to present TGN1412 to cells *in vitro*, it should be noted that the work is still ongoing and some of the results require confirmation.

Methods

In vitro experiments. Cytokine release studies using peripheral blood mononuclear cells (PBMC) and tests for lymphocyte activation and proliferation using flow cytometry were carried out as described in the interim report of 10 07 06 except that TGN1412 was presented to human or Cynomolgus macaque PBMC in a number of different protocols:

- (1) As an aqueous solution
- (2) Wet coated directly onto the walls of the wells of 96-well plates
- (3) Dry coated directly onto the walls of the wells of 96-well plates
- (4) As an aqueous solution, in the presence of a monolayer of cells derived from human endothelium (HUVEC(JR))
- (5) As an aqueous solution, in combination with aqueous anti-human Fc antibody
- (6) Wet coated onto an anti-human Fc antibody that had itself been dry coated onto the walls of the wells of 96-well plates

Cytokine release studies at 16-24 h were confined to protocols (1)–(4) because all anti-human Fc antibodies tested were positive for bacterial endotoxin, which is a potent stimulus for the release of pro-inflammatory cytokines, and tests for lymphocyte activation and proliferation remain to be carried out on lymphocytes taken from the co-cultures with human endothelial cells.

As previously, appropriate positive and negative controls were included: CD3-stimulating monoclonal antibody UCHT-1, the lectin phytohaemagglutinin (PHA) and an isotype matched (IgG4) human monoclonal antibody of irrelevant specificity, i.e. not anti-CD28.

Additional studies to measure the expression of T lymphocyte activation markers after a 3-day stimulation with TGN1412 and intracellular cytokine staining for interleukin-2 (IL-2) and interferon gamma (IFN γ) after a 6-hour stimulation with TGN1412 have now been performed.

In vivo experiments. These were carried out in 12 Cynomolgus macaques, two of which were injected intravenously with TGN1412 buffer (control) and two of which were injected intravenously with each of the following doses of TGN1412: 0.1, 0.5, 5.0 and 50 mg/kg, using TGN1412 from a freshly opened vial of the drug. Additionally, two animals were injected with 0.1 mg/kg of TGN1412 taken from syringe #5, which was recovered from Northwick Park. TGN1412 was administered as a slow intravenous injection that mimicked the rate of administration in the Northwick Park trial as closely as was practicable. The rationale for the choice of these doses of these materials is as follows: 0.1 mg/kg was the dose administered to the six volunteers at Northwick Park and 5.0 and 50 mg/kg were the doses injected in Cynomolgus macaques in the pre-clinical safety testing reported by TeGenero; 0.5 mg/kg was chosen to fill in the dose–response curve between 0.1 and 5.0 mg/kg and syringe #5 contained an aliquot of TGN1412 associated with the most severe adverse events.

TGN1412 was administered to Cynomolgus macaques under anaesthesia. Anaesthesia was maintained for 2 hours after administration of TGN1412 to allow monitoring of blood pressure, heart rate and core body temperature at hourly intervals. Blood samples were taken at these times for haematology, biochemistry and liver function tests, cytokine release measurements and pharmacokinetics. This allowed for detailed monitoring of any immediate adverse reactions. Twenty-four hours after administration of TGN1412, macaques were anaesthetized for a peripheral lymph node biopsy to look for changes in lymphoid tissue lymphocytes and to take blood samples for haematology, biochemistry and liver function tests, cytokine release measurements and pharmacokinetics. A further blood sample was taken at day 3 or 4 for haematology, cytokine release measurements and pharmacokinetics. Macaques were terminated on day 7, blood samples taken for testing and tissues for histopathology.

Results

Cytokine release studies.

TGN1412 (2.0–1000 µg/ml = 0.25–125 µg/well) added in aqueous phase to PBMC (200,000 cells/well) did not stimulate the release of tumour necrosis factor alpha (TNF), interleukin-6 (IL-6) and IL-8. Similarly, TGN1412 wet coated onto the walls of wells (1 µg/well) was not a stimulus for pro-inflammatory cytokine release from PBMC (125,000 cells/well). In contrast, TGN1412 (1.0 and 10 µg/well, but not 0.1 µg/well) dry coated onto the walls of wells in which PBMC (125,000 cells/well) were cultured for 16–24h stimulated the production of TNF, IL-6 and IL-8. The magnitude of the cytokine responses was in the ratio IL-8>TNF>IL-6. TGN1412 (0.1, 1.0 and 10 µg/ml) added in aqueous phase to a co-culture of PBMC (50,000 or 125,000 cells/well) and human endothelial cells (a monolayer of 15,000 or 68,000 cells/well) and cultured for 16–24h stimulated the production of TNF, IL-6 and IL-8. The magnitude of the cytokine responses was in the ratio IL-8>TNF=IL-6.

Tests for lymphocyte activation and proliferation.

Proliferation studies

Human PBMC pulsed with the fluorescent membrane dye PKH26 were assessed by flow cytometry for their proliferative response to TGN1412 at doses ranging from 0.5ug/ml to

1000µg/ml. TGN1412 in aqueous phase, wet coated onto plates or cross-linked in aqueous phase with an anti-human FC antibody did not produce any significant proliferative response. Only TGN1412 immobilised by dry coating onto plates or captured in aqueous phase by anti-human Fc antibody dried onto plates, followed by washing, resulted in significant lymphocyte proliferation in experiments in which PBMC were incubated for 3 days with an immobilised isotype control antibody or TGN1412. Phenotypic analysis of proliferating lymphocytes identified CD4⁺ lymphocytes as the principle responding lymphocyte population. Analysis of the proliferation data showed that all of the CD4⁺ cells were committed to dividing, there eventually being no resting CD4⁺ cells remaining. The level of proliferation induced by immobilised TGN1412 was profound and equivalent to the response observed with mitogens used as positive controls in this assay. Even greater proliferative responses were achieved with anti-human Fc antibody captured TGN1412, suggesting that the responses measured with dried on TGN1412 may be an underestimation of the magnitude of the response. Stimulation of lymphocytes with TGN1412 was accompanied by up regulation of the activation marker CD25 by proliferating cells.

Proliferative responses were quantified as precursor frequencies per 10⁶ lymphocytes. Measurement of proliferative responses to TGN1412 with this assay identified the optimal dose response as between 2 and 10 µg/ml. With higher doses of 100 or 1000µg/ml, equivalent to the doses tested in *Cynomolgus* macaques, either a low or no proliferative response was observed. In contrast, with 2µg/ml, equivalent to the human dose administered at Northwick Park, a strong polyclonal proliferative response was observed.

Following a 3-day stimulation with TGN1412, lymphocytes washed and transferred to fresh plates without TGN1412 continued to proliferate for a further 3 days.

Intracellular cytokine staining

Human PBMC stimulated with immobilised TGN1412 for 6 hours in the presence of a secretion inhibitor were assayed for IL-2 and IFN γ . Stimulation with immobilised TGN1412 for 6 hours resulted in significant production of IL-2 by CD4 positive lymphocytes compared with controls.

The level of IL-2 positive lymphocytes was equivalent to the response seen with mitogens used as strong positive controls in this assay. Staining for intracellular IFN γ on CD4 positive lymphocytes was observed following stimulation with immobilised TGN1412. However, the response was much smaller than that observed with mitogen which activates CD8 positive lymphocytes to produce far more IFN γ than CD4 positive lymphocytes. The dose–response of immobilised TGN1412 for intracellular cytokine staining matched the dose–response of proliferative responses. Even higher levels of IL-2 positive lymphocytes were obtained with anti-Fc antibody captured TGN1412, suggesting that the responses measured with dried on TGN1412 may be an underestimation of the magnitude of the response achievable with TGN1412.

The intracellular cytokine staining responses for IL-2 and IFN γ matched the ELISA data for supernatants from 3 day cultures stimulated with TGN1412. That is high levels of IL-2 and low levels of IFN γ in response to TGN1412 at small but not large doses.

Data from Cynomolgus macaques

At all doses of TGN1412 administered to Cynomolgus macaques no gross adverse reaction was observed in terms of general health, blood pressure, heart rate, temperature, haematology, biochemistry or liver function. Further analysis, tests and histopathology are ongoing. There was no adverse reaction associated with administration of syringe material at the human dose.

In initial *in vitro* assays, in which PBMC from Cynomolgus macaques were stimulated with immobilised TGN1412, cells did not undergo a proliferative response. Early indications are that Cynomolgus macaque PBMC are activated by TGN1412 but do not undergo proliferation. However, when exogenous human IL-2 was added to cultures of Cynomolgus macaque PBMC stimulated with immobilised TGN1412 then a strong proliferative response was observed. No proliferative response was observed following the addition of human IL-2 alone to Cynomolgus macaque PBMC cultures.

Summary of *in vitro* activation and proliferation responses of human and Cynomolgus macaque lymphocytes to immobilised TGN1412

	TGN1412 evoked activation	TGN1412 evoked proliferation	IL-2 evoked activation	IL-2 evoked proliferation	TGN1412+IL-2 evoked activation	TGN1412+IL-2 evoked proliferation
Human PBMC	++++	++++	–	–	Could not be tested*	Could not be tested*
Macaque PBMC	++	–	–	–	+++	+++

*: TGN1412 stimulates activation, IL-2 secretion and proliferation when given alone.

Conclusions

TGN1412 is here shown to have the capability to evoke cytokine release and lymphocyte proliferation *only* when presented to cells in the effective manner identified in these studies. When immobilised by drying onto plates, binding to endothelial cells or captured by immobilised anti-Fc antibody, TGN1412 stimulated the release of cytokines, including TNF, IL-2, IL-6, IL-8, and IFN γ , and profound proliferation of human CD4+ lymphocytes in *in vitro* assays. TGN1412 did not induce cytokine release or proliferative responses when presented in aqueous phase or when cross-linked in aqueous phase. The data from these *in vitro* assays suggests that the dose of TGN1412 given to volunteers in the Northwick Park trial was close to the maximum immunostimulatory dose. In contrast to man, Cynomolgus macaques given TGN1412 at any of the doses tested did not experience any gross adverse reactions and Cynomolgus macaque lymphocytes did not proliferate when stimulated with immobilised TGN1412 unless IL-2 was added to cultures.

Further studies

Experiments are underway to determine whether or not pulsed TGN1412, i.e. added to a monolayer of endothelial cells with unbound TGN1412 then washed away before addition of PBMC, is a more effective stimulus for cytokine release. This could be the

case since unbound TGN1412 would be expected to compete with the bound TGN1412 and so serve as a competitive antagonist of the bound TGN1412 at CD28 receptors. Also, experiments are underway with co-cultures of human PBMC and human MRC5 fibroblasts to determine whether or not a monolayer of cells other than endothelial cells can be used to bind TGN1412 to render it biologically active *in vitro*. These experiments will then be repeated with Cynomolgus macaque PBMC substituted for human PBMC as far as is practicable given the small volumes of Cynomolgus macaque blood that is available for such studies. Additionally, TGN1412-stimulated inflammatory cytokine release from human diluted whole blood and Cynomolgus macaque diluted whole blood will be compared.

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6. Summary of Stakeholder Consultation

Stakeholder Oral Presentations to the Expert Scientific Group

A wide range of stakeholders identified by the Chairman were invited to submit oral or written views concerning the incident.

The following stakeholders made oral presentations to the Chair and representatives of the Expert Scientific Group.

Stakeholders

Dr. Brian Gennery, Director of the Clinical Research Centre, University of Surrey,
Professor Patrick Vallance, Academy of Medical Sciences,
The Association of the British Pharmaceutical Industry (ABPI) and the BioIndustry Association (BIA), represented by Sir Colin Dollery, Dr. David Chiswell, Dr. Richard Peck, Dr. Peter Lloyd, Dr. Richard Barker and Ms Aisling Burnard,
Professor Terry Hamblin, Professor Martin Gore and Dr. Monica Preuss, representing the Gene Therapy Advisory Committee (GTAC),
Dr. Sally Burtles, representing Cancer Research UK,
Dr Malcolm Boyce, Chairman of the Association for Human Pharmacology in the Pharmaceutical Industry (AHPPI),
Dr. J. S. de Bono, Institute of Cancer Research
Professor Peter Weissberg, British Heart Foundation,
Professor Mark Feldmann, Head, Kennedy Institute of Rheumatology Division, Imperial College,
Dr Liz Allen, Chair, Contract Clinical Research Association,
Professor Robert Hawkins, Cancer Research UK professor of Medical Oncology, Christie Hospital, Manchester.

Expert Scientific Group Representatives

Professor Gordon Duff (Chair), Professor Robert Lechler, Sir Leszek Borysiewicz, Dr Stephen Inglis, Ms Vivienne Parry, Professor Mark Walport, Professor Alex Markham., Professor Neva Haites, Professor Andrew McMichael, Sir Bob Hepple, Professor Munir Piromohamed, Professor Johannes Löwer, Professor Lars Klareskog, Professor Kevin Park, Professor Herman Waldmann.

Opening remarks by the Chairman to the stakeholders

The stakeholders were welcomed by the Chair who thanked them for meeting with Members of the Expert Scientific Group. He explained that the ESG was independent of the MHRA and CHM and would seek to identify generally applicable factors that conferred a high degree of risk to new pharmacological agents. It would then attempt to codify these risk factors in order to make recommendations on how to optimise the safety of first-in-man clinical trials without creating unnecessary barriers to the development of useful new medicines. Part of this process would be to take the opinions of a wide range of stakeholders from the academic, industrial and public arenas; hence, the reason for “stakeholder consultation” meetings.

The Chair explained that all papers and proceedings were confidential until the report of the ESG was published when all information, excepting material withheld under Section 38 of the FOIA, would enter the public domain.

Dr. Brian Gennery, Director of the Clinical Research Centre, University of Surrey.

Dr Gennery has a long history of responsibility for Phase I clinical trials and intimated that the Clinical Research Centre at the University of Surrey carried out a lot of studies for industry. He stated that the TGN 1412 incident prompted him to contact the Expert Scientific Group with his views regarding the quality and quantity of data provided by sponsors to clinical units.

The presentation was on the information needs in early stage clinical trials and although the information he presented to the ESG was not directly related to the TGN 1412 incident, it was relevant to the wider safety of subjects in early phase clinical development.

The presentation covered general principles, chemistry and pharmacy of trial products, pre-clinical biology and safety testing, adequacy of clinical data and the importance of the investigator's brochure.

Professor Patrick Vallance, Academy of Medical Sciences.

Professor Vallance chaired the Academy of Medical Sciences Working Group set up following the TGN 1412 incident. The Academy had previously provided the Expert Scientific Group on Phase One Clinical Trials with a position paper on testing antibody therapies which was the subject of his presentation. He recognised that the Academy did not have access to information not in the public domain, but that the conclusions reached by the Working Group were generally valid as best practice principles for this type of therapy.

The presentation covered the predictive value of pre-clinical data, prediction of the starting dose, considerations of the clinical site and identification of risk factors.

The predictive value of pre-clinical testing included the importance of proportionate effects in animal and human *in vitro* and *in vivo* data and the importance of parallel comparisons of drug effects in human and animal cells *in vitro* in judging the predictive value of the animal model.

The Association of the British Pharmaceutical Industry (ABPI) and the BioIndustry Association (BIA), represented by Sir Colin Dollery, Dr. David Chiswell, Dr. Richard Peck, Dr. Peter Lloyd, Dr. Richard Barker and Ms Aisling Burnard.

Dr Chiswell introduced the group and explained that the ABPI/BIA team were representing a single taskforce of approximately 20 experts, co-chaired by Sir Colin Dollery and himself, who were studying the lessons from the TGN1412 incident. He stated that a forensic inquiry into TGN 1412 had not been carried out as not all of the information was in the public domain, rather a "best practice" guide would be presented for future studies.

It was stressed that the current review procedures and regulations relating to early phase clinical development had proved to be most effective and had made such studies very safe. The TGN 1412 incident should be viewed as wholly exceptional and any new recommendations should not lead to a check list approach being adopted.

The APBI/BIA presentation covered the mechanism of action and disposition of biological agents, animal models used and their predictability, the translation of pre-clinical data into the clinical situation, the conduct of the trial itself, manufacturing and control of biologicals, protocol and regulatory review and education and training of scientists and physicians with regard to safety assessment.

Professor Terry Hamblin, Professor Martin Gore and Dr. Monica Preuss, representing the Gene Therapy Advisory Committee (GTAC).

Professor Gore has a long experience of protocol review and has been following development of monoclonal antibodies for several years. Professor Hamblin has a long history of immunotherapy and had been treating patients with antibodies since 1974. He presented unpublished data regarding a study he had carried out in a single patient subject in 1994 using a tri-specific anti-CD3/CD2/CD28 antibody.

The presentation covered two main areas, first dosing in man, healthy volunteers versus patients and the first in man study of a tri-specific anti-CD3/CD2/CD28 antibody which was performed in 1994. The effects of this antibody had parallels with the effects of TGN1412.

Dr. Sally Burtles, representing Cancer Research UK,

Dr Sally Burtles has responsibility for early phase clinical trials and pre-clinical development at Cancer Research UK and informed the ESG that Cancer Research UK was very active in bringing new cancer treatments into man. Dr Burtles described the CRUK portfolio of projects and it was noted that 9 were biological products, some of which fell into the Expert Scientific Group's terms of reference

Her presentation was on the translation from pre-clinical studies to first in man trials and the design of these trials. The areas covered were general experience, expertise and experience, minimising the use of animals, independent peer review of studies and data, oncology drug development, risk-benefit analysis, transition into man and safety considerations.

Dr Malcolm Boyce, Chairman of the Association for Human Pharmacology in the Pharmaceutical Industry (AHPPI),

Dr Boyce is the Clinical Director of a phase I trials unit with an interest in clinical pharmacology. He is also Chairman of the Association for Human Pharmacology in the Pharmaceutical Industry (AHPPI). AHPPI was founded in 1988 and has 162 members with representation from most of the big pharmaceutical companies and contract research organisations (including Parexel). The main purpose of AHPPI was to provide continuing education in clinical pharmacology to its members.

Dr Boyce delivered a presentation entitled "First administrations of a new drug substance to humans".

The presentation covered Phase I units in the UK, trial sponsor, types of trial, biological versus small molecules, pre-trial considerations, staff training, dialogue amongst concerned parties, past safety in phase I trials, healthy subjects versus patients, choice of dose and study design, review and assessment.

Dr. J. S. de Bono, Institute of Cancer Research

His presentation was entitled “Phase one trials and cancer” and covered what were considered key issues, a review of cancer phase I trials, antibody therapeutics, antibody phase I trials, minimising risk – maximising benefit and volunteer studies for cancer drugs.

He stressed that, for cancer studies, that different reasons existed for using healthy volunteer or cancer patients and that the chance of benefit with novel therapeutics had increased in recent years. Antibody therapeutics has been deemed a major success in cancer therapy which had led to significant therapeutic advances.

Professor Peter Weissberg, British Heart Foundation

Professor Weissberg informed the ESG that the British Heart Foundation (BHF) supported a wide range of research, including clinical trials; they did not, however, commission research. He said that the BHF position was that patient safety was the first priority and it supported decreasing potential risks to subjects in phase I clinical trials. He considered that it was important to learn as quickly as possible from any adverse events that occurred during a clinical trial and suggested that trial data on adverse events should be submitted to a central point (e.g. the regulatory authority) that would then promulgate the information to relevant parties.

Professor Mark Feldman FRS (Head, Kennedy Institute of Rheumatology, Imperial College London)

Professor Feldman delivered a presentation entitled “Trial Design for Species Specific Molecules”. The Kennedy Institute’s experience of developing and using an anti-TNF α monoclonal antibody in rheumatoid arthritis and their approach to risk reduction were described. Professor Feldman completed his presentation by discussing methods to improve the testing of new species specific therapeutics including extensive *in vitro* analysis and the use of transgenic mice.

Dr Liz Allen (Chair, Contract Clinical Research Association)

Dr Allen was joined by Dr B Holt and delivered a presentation entitled “Considerations of Phase I Studies with Biologics and NCEs with Novel Targets”.

Members were informed that the Contract Clinical Research Association (CCRA) was a trade organisation with 28 members, 9 of which were involved in phase I studies. The CCRA considered that in the pre-clinical review of biologics and NCEs with specific targets, safety considerations were paramount and special consideration should be given to the mechanism of action and potential consequences of target effects and to the predictability and homology of animal models.

It was stated that safety of phase I clinical trials in the UK was very good and that the TGN 1412 experience was unprecedented. Data was provided showing that from 1992 to 2000 a serious adverse event had occurred in 171 subjects out of 81,471 (0.21%).

Professor Robert Hawkins (Cancer Research UK Professor of Medical Oncology, Christie Hospital, Manchester)

Professor Hawkins delivered a presentation entitled “Clinical Development of Cancer Immunotherapies” in which he related his experiences working with cancer vaccines, targeted super-antigens and gene modified T-cells.

Full details of the oral presentations and associated discussions are presented in the complete minute of the meetings in Appendix C.

Stakeholder Written Submissions to the Expert Scientific Group

The following stakeholders provided written submissions.

ABPI / BIA, Joint Report of the Early Stage Clinical Trial Taskforce,
Faculty of Pharmaceutical Medicine of the Royal Colleges of Physicians
Nuffield Council on Bioethics (Professor Sandy Thomas, Director),
Professor Sir Ravinder Maini, Kennedy Institute of Rheumatology, ICL
Dr Camilo Colaco, ImmunoBiology Ltd, Cambridge
National Cancer Research Institute, Consumer Liaison Group (Mr Roger Wilson (Chair)
The Royal Society (Professor David Read FRS , Biological Secretary and Vice President)
Dr Lynda Wright, Executive Director, The Organisation for Professionals in Regulatory
Affairs
Kate Webb, Senior Policy Adviser, Which.
The Royal Statistical Society, Professor Tim Holt,
Focus on Alternatives, Dr Gill Langley,
J Robinson Associates,
Datamonitor, Press release.

ABPI / BIA, Joint Report of the Early Stage Clinical Trial Taskforce

A full report was submitted which covered the background to the incident, mechanisms of action and biological activity, selection of species/animal models for safety evaluation, translation of pre-clinical data to the clinic, clinical trials conduct, manufacturing, protocol and regulatory review and education and training. Findings and recommendations on these areas were discussed and points to consider for first time in man studies with novel biopharmaceuticals were put forward.

Faculty of Pharmaceutical Medicine of the Royal Colleges of Physicians

This paper focussed on the areas of review of information prior to clinical trials, the clinical procedures adopted in trials, the responsibilities of sponsors and reviewers and the training and competence of staff involved in clinical trials.

Nuffield Council on Bioethics (Professor Sandy Thomas, Director,)

This paper investigated the issues of informed consent and the inducements offered to trial participants and also covered the validity of research involving animals and replacements for their use in research.

Professor Sir Ravinder Maini, Kennedy Institute of Rheumatology, ICL

The comments provided in this document include the ability of animal models to predict toxic effects, the capacity to recognise toxic effects and the need to react swiftly and appropriately. It also states that first time in man studies should be undertaken by clinicians who understand the biology of the target and the expected response.

Dr Camilo Colaco, ImmunoBiology Ltd, Cambridge

This was represented as an article published in the May 5th issue of The Scientist and the recommendation of the author is to develop a mechanism whereby external expert review panels can liaise with the MHRA to provide timely relevant and confidential expert input.

National Cancer Research Institute, Consumer Liaison Group (Mr Roger Wilson (Chair))

The issues addressed in this submission covered the consent process, animal research, Standard Operating Procedures, participant selection, simultaneous dosing and the analysis of data generated in previous studies.

The Royal Society (Professor David Read FRS, Biological Secretary and Vice President)

This paper focussed on three specific areas, biological molecules with novel mechanisms of action and new agents with a highly species specific action, new drugs directed towards immune targets and the validity of pre-clinical testing using animals.

Dr Lynda Wright, Executive Director, The Organisation for Professionals in Regulatory Affairs (TOPRA)

TOPRA's response included a background to the organisation and an outline of what it considered it can contribute to clinical trials and the wider area of education.

Kate Webb, Senior Policy Adviser, Which.

The Which response document was presented under the headings of general comments, clinical trial protocol, management of the trial, regulating the trial and conclusions and recommendations.

The Royal Statistical Society, Professor Tim Holt,

The Society established a working party to review statistical design considerations for 'first in man' studies with particular reference to monoclonal antibodies and the wider class of new biologicals and biotechnologies.

Focus on Alternatives, Dr Gill Langley,

The written submission included sections on the perceived systemic weakness of animal tests, an approach to safer clinical studies and the need for a new paradigm and associated conclusions.

J Robinson Associates,

Did not give permission for publication in the report.

Datamonitor, Press release.

Independent commentary on Biologics driving growth in the pharmaceutical sector.

Full details of the written submissions and associated documents are presented in Appendices C and D

7. Predicting Hazards in Pre-clinical to Clinical Transition

Background

The TGN1412 trial had been approved by two European Regulatory Agencies and a local research ethics committee, yet all of the recipients of the antibody experienced unpredicted and very severe adverse reactions shortly after dosing. The results of further laboratory tests are awaited, but so far there is no evidence that the serious adverse effects seen were caused by anything other than an ‘on-target’ effect of the antibody triggering a cytokine release syndrome in the trial volunteers.

As far as we can determine, such a serious adverse reaction in all recipients of a trial agent, as occurred with TGN1412, was unprecedented. It highlighted an urgent need to review the safety of first-in-man trials of novel agents, and to examine how risks in medicines development are currently assessed and minimised by sponsors, investigators and regulators.

TGN1412 had undergone pre-clinical testing according to current regulatory requirements, but a safe starting dose for the first trial in humans had not been predicted from the pre-clinical studies. Several of the laboratory tests that were performed in the pre-clinical development phase of TGN1412 were repeated by the National Institute for Biological Standards and Control (NIBSC), and similar results were obtained.

It is important to note, however, that further tests performed at NIBSC showed that it was possible to create conditions *in vitro* that resulted in TGN1412-induced release of cytokines and T cell proliferation in human blood cells. Immobilisation of the antibody by dry-coating it onto a plastic surface, or using an immobilised second antibody, or exposing human blood cells to TGN1412 in the presence of an endothelial cell monolayer resulted in cytokine release and/or T cell proliferation.

Notably, similar stimulatory effects on the proliferation of T cells from cynomolgus monkeys have not, so far, been reproduced *in vitro*, and cynomolgus monkeys showed no adverse reaction when given TGN1412 over a wide dose range *in vivo*.

These studies add information on the potential mechanism of the adverse reaction, and why pre-clinical tests performed in human and animal cells, and in animal models, failed to predict the human response to the starting dose of TGN1412 given in the trial. The more recent results from work done at NIBSC, if confirmed and developed, may well point the way to different methods that should be included in pre-clinical testing in the future, particularly for agonistic (stimulatory) antibodies with cell surface targets.

The aim of the ESG has been to define what can be learned from the TGN1412 experience and to consider how far the findings can be generalised to increase the safety of human volunteers in future first-in-man trials of higher risk new agents. Within our terms of reference, we examined whether and how hazards might be recognised in the nature of the agent, its pharmacological target and its intended recipients.

We concluded that there are identifiable hazards inherent in the nature of some new agents and in the molecular targets at which they are aimed. The recognition of these hazards is especially important for the safety of first-in-man clinical trials. The conclusions below summarise ESG discussions and take account of both written and verbal submissions received from stakeholders.

Toxicity of a new agent in a first-in-man trial may be seen early, occurring soon after administration, or late, occurring some time afterwards. The ESG focused on serious acute toxicity that occurs early, or immediately, and may often be life-threatening. The need to monitor for evidence of late toxicity, and the duration of monitoring, are questions best addressed case-by-case, taking into account the type of agent, its pharmacological targets and its half-life *in vivo*.

We have not attempted to quantify or rank risk in any formal way, we use the term “higher risk agents” to mean those that fall within our terms of reference where risk factors can be recognised.

Many of our recommendations refer to decisions affecting the safety and value of first-in-man trials that we believe must be made on a case-by-case basis and be scientifically justifiable. By this we mean that developers should provide justifications for case-by-case decisions and that the regulator should assess the justifications, taking into account the safety of trial volunteers and the information that will be gained.

The recommendations are intended to apply to first human exposures of higher risk agents within the terms of reference but some recommendations may apply to phase one trials in general.

Inherent Hazards of Certain Types of New Medicines

Species-specificity of action

Some medicines, especially biological medicines such as recombinant cytokines or polypeptide hormones designed to have affinity for human cellular receptors, and monoclonal antibodies selected against a human target antigen, may show a high level of species-specificity.

In general, agents aimed very specifically at human molecular targets may show much reduced activity in other species such as in mice, rats and rabbits, but have some activity in non-human primates where molecular structures are closer to those in humans.

As well as differences in molecular affinity for the target molecule, other factors can affect the biological response in a test animal, both quantitatively and qualitatively, compared with the response in the human. For example, there may be a different tissue distribution of the molecular target, different cellular consequences of target binding, different cellular regulatory mechanisms, different metabolic pathways, or different compensatory responses to an initial physiological perturbation.

With new agents where there is evidence of species-specificity of action from *in vitro* studies with human cells compared with cells from a test species, the value of the *in vivo* response of the test species will be significantly reduced in terms of predicting the *in vivo* human response.

This problem may be more likely to arise with biological medicines, although an agent of any type, including small chemical molecules, if highly specific for a human target, may show species-specificity of action.

In practice this means that pre-clinical animal studies with relatively species-specific agents:

- may not reproduce the intended pharmacological effect in humans;
- may give misleading pharmacokinetic and pharmacodynamic results;
- may not reflect 'on-target' toxicity.

In addition, large human-compatible biological molecules are likely to cause immune responses in other species, and animal studies may not predict the effects of repeat dosing of such agents because of their immunogenicity.

Species-specificity of an agent does not imply that there is always an increased risk in first-in-man trials, but it makes pre-clinical evaluation of the risk in animal experiments much more difficult, and sometimes perhaps impossible. Therefore, a highly cautious approach is needed.

It should also be noted that similar responses in human and animal cells *in vitro* is not necessarily a guarantee that the *in vivo* responses will be similar.

Animal studies taking due regard of the three 'Rs', (refinement, reduction and replacement of animals in testing) remain necessary for many aspects of pre-clinical development of novel agents including testing of 'off-target' and 'on-target' toxicity and understanding the fundamental biology relevant to a new medicine and its target molecules in the human. Most, if not all, new medicines arise from biological insights gained from well-designed animal studies. The key point we want to make is the importance of deciding what can be learned from animal studies in the pre-clinical development of a new medicine, and what limitations there might be when it comes to predicting the response, and dose-response relationship, in humans.

Novel agents and mechanisms of action

The risks of unpredictable adverse reactions in first-in-man clinical trials are, in general, greater with agents that have very novel mechanisms of action since there will be no prior experience to guide pre-clinical development and the transition to clinical development.

If there is also species-specificity, the prediction of *in vivo* responses in the human, from *in vitro* studies with human cells or tissues, assumes a central importance.

Novel agents and novel mechanisms of action may encompass the physical nature of the agent, the target at which it is aimed, and the anticipated biological response(s). All of these factors may be affected by the *in vivo* environment, for example, enzymatic and non-enzymatic modification of the agent, the *in vivo* distribution of the agent and its duration at sites of action, the extent and cellular distribution of the target, unpredicted downstream events including amplification by cellular or biochemical pathways that were not present in the *in vitro* models studied, and the effects of compensatory homeostatic mechanisms.

Agonistic mechanism of action

Many new agents are designed to act through cellular receptors that have not been used previously as therapeutic targets. Receptors on the surface of cells specifically recognise extracellular molecules generally known as ligands. Ligand-receptor interaction leads to a cellular response, often transmitted to the cell nucleus by a biochemical signalling pathway. In many cases the response in the nucleus is a change in the regulation of gene activity. Some receptors, eg those for steroid hormones, are located within the cell itself and therefore have no signalling pathway from the cell surface.

New agents that target cellular receptors may either mimic the effect of the natural ligand, or may occupy the receptor without causing a cellular response but preventing the natural ligand from interacting with the receptor. At receptor level, the former is described as a receptor agonist, the latter as a receptor antagonist.

Medicines can have agonistic or antagonistic actions on receptors that suppress cellular activity as well as receptors that stimulate cell activity. Thus receptor agonists and antagonists can either stimulate or suppress cells depending upon the physiological role of the receptor with which they interact.

TGN1412 was designed as a super-agonist for the CD28 receptor on T lymphocytes, which is a co-stimulatory receptor in normal physiology, leading to activation of the T cell with both an increased production of cytokines and an increased rate of cellular proliferation.

In general, agents that have agonistic actions leading to cellular activation and proliferation may be associated with more risk, especially if their target is within a biological amplification cascade (see below).

Potency

New agents may be engineered to have a more potent effect than the natural ligands they are designed to simulate.

For example, this might be achieved by:

- higher affinity for a receptor, or greater receptor occupancy compared with the natural ligand;

- greater signalling effect on the target receptor compared with the natural ligand;
- multiple valency that allows cross-linking of more than one target receptor.

The effects of some agents may also be exaggerated by pharmacokinetic properties that result in an increased exposure of target cells to the agent compared with the natural ligand.

TGN1412 was designed to interact with a different part of the CD28 molecule than the natural ligands for this receptor, resulting in direct stimulation of cellular signalling without requiring a co-stimulus from the T cell antigen receptor, as occurs in nature. It therefore has a novel mode of action.

TGN1412 by-passed the normal control mechanism that requires antigen stimulation to activate an immune response by expanding only those clones of T cells that carry an appropriate antigen receptor and, instead, stimulated many T cell clones independently of their antigen specificity.

TGN1412 is a high molecular weight monoclonal antibody that does not pass through the kidney, giving it a long half-life in the body that is measured in weeks. Being an antibody, TGN1412 was difficult to remove from the body once injected. It would have entered the immunoglobulin pool containing many antibodies necessary for defence against infections.

Multifunctional molecules

Antibodies are a good example of multifunctional molecules. They are complex proteins with a high degree of specificity to recognise three-dimensional molecular structures. While their natural function is to recognise microbial pathogens and contribute to their elimination, the molecular recognition properties of antibodies can be exploited in the development of new drugs that will recognise specific molecular targets.

If the target is a soluble mediator, the antibody may bind to it and neutralise its effects, as is the case with antibodies to TNF used in the treatment of rheumatoid arthritis. If the target is a cell surface receptor, an antibody can either block it as an antagonist, or stimulate it as an agonist. The binding of certain types of antibody to a cell can also lead to cell death through activation of cytotoxic proteins (complement) and other mechanisms.

Since antibodies have two antigen-recognising arms, it is theoretically possible that one antibody could bind to two cell surface receptors and greatly increase the signalling response in the cell. There is also the possibility that an antibody to a cellular receptor could bind to receptors that had been cleaved or released from the cell membrane, forming a soluble immune complex. The functional consequence of this cannot be predicted without experimental studies. However, where differences in receptor shedding between the human and a test species occurs, the results from animal testing may not be a

good guide to the responses in human, even when the binding affinity of the antibody for the target is similar.

A major part of an antibody, distinct from the antigen recognition regions, can also have important biological effects by engaging cellular receptors on a range of different cell types. This part of an antibody is called the Fc portion and its cell surface receptors are called Fc receptors. By engaging a cell surface receptor via its antigen recognising part, and engaging Fc receptors on different cells via its Fc part, an antibody can act as a bridge, bringing different cells into close contact with the potential for additional cellular activation of both cross-linked cell types. Although Fc receptors for IgG4, the particular antibody type of TGN1412, are thought to be rare, such cross-linking activity of TGN1412 *in vivo* cannot be excluded on current evidence.

Inherent Hazards of Certain Types of Pharmacological Target

In general, a high level of caution is needed in the first human exposures to any agent whose effects might cause severe physiological disturbance to vital body systems.

Immune system targets

The immune system recognises microbes and abnormal cells and generates clones of lymphocytes to remove them. Initial activation of the immune system triggers a self-amplifying cascade of lymphocyte proliferation and release of soluble mediators to produce an immune response.

Normally the response is limited to a small number of lymphocyte clones determined by the specificity of their antigen receptor through which the initial activation is signalled.

The effects of pharmacological agents that act on the earlier phases of the immune response may be greatly amplified *in vivo* by activation of the immune cascade.

The potential for over-stimulation of the immune system is increased when the agent is an agonist targeted at activating receptors on lymphocytes, and when the antigen receptor is by-passed allowing polyclonal lymphocyte activation to occur. Similar considerations may apply to antagonists that act within regulatory pathways that turn off an immune response.

Other targets with potential for biological amplification

Factors were identified above as hazards with agents aimed at the immune system, but there may be inherent risk in pharmacological stimulation of other self-amplifying cascade systems such as the inflammatory response and the thrombosis-haemostasis pathways.

Summary of risk factors

The ESG concluded that it is not possible to make a fully comprehensive list of all possible risk factors related to investigational agents and their targets, because of the

inherent scientific uncertainties associated with innovative medicines. The assessment of risk or hazard must therefore be a science-based exercise performed case-by-case.

There are, however, certain factors that clearly may confer higher risk in pre-clinical to clinical transition. These include:

- any agent whose effects might cause severe physiological disturbance to vital body systems;
- agonistic or stimulatory actions;
- novel agents and novel mechanisms of action where there is no prior experience;
- species-specificity of an agent making pre-clinical risk-assessment difficult or impossible;
- the potency of an agent, eg compared with a natural ligand;
- multifunctional agents, eg bivalent antibodies, FcR binding domains;
- cell-associated targets;
- targets that by-pass normal control mechanisms;
- immune system targets;
- targets in systems with the potential for large biological amplification *in vivo*.

In addition to these identifiable factors that should be taken into account in the risk-assessment of an agent in a first-in-man clinical trial, other factors, some of which may become more significant in the future, need to be considered. Examples include:

- Agents with a target that is not present in animal models;
- Combination products e.g retroviral vectors in gene therapy (even if the components have been used in humans before, the new combination may generate new risks);
- ‘Bio-similars’ that are significantly different from the innovator product, for example, in post-translational modifications;
- Vaccines with novel adjuvants, especially those that direct antigen to signalling molecules on the surface of immunocytes, or are designed to stimulate pro-inflammatory cytokine production from lymphocytes or antigen presenting cells;
- Major changes to the route of administration, posology, or formulation of an agent.

Our terms of reference list three categories of agent that may be considered *a priori* to pose increased risk of harm in first human exposures, or where pre-clinical assessment of risk may be more difficult. The categories are:

- Biological molecules with novel mechanisms of action.
- New agents with a high degree of species-specificity.
- New agents with immune system targets.

While our recommendations for caution are aimed at medicines in any one of these three categories, we recognise that exceptions may be made when the potential for harm in first human exposures can be assessed as low, based on a careful scientific evaluation of risk.

Although some of the examples of risk factors mentioned above may be more likely with biological agents, they may also apply to small molecule agents. The descriptions given of factors that may increase risk in first-in-man trials, and where increased caution is indicated, are not intended to form a complete or comprehensive list, and are merely given as examples.

Clearly the more potential risk factors that are applicable to an agent and its pharmacological targets, the higher the risk in transition from pre-clinical to clinical development and the more caution is required, especially when species-specificity is a factor.

8. Risk Reduction and Risk Management

Overall Aim of the ESG

The aim has been to review and generalise what can be learned from the TGN 1412 trial, when a safe starting dose in humans was not predicted from the pre-clinical studies that were done. We considered how to optimise the safety of future ‘first-in-man’ trials without inhibiting innovation or creating unnecessary barriers to the development of useful new medicines.

The recommendations were formulated bearing in mind the ESG’s terms of reference and the general features identified as potentially increasing risk. Although we have not attempted to quantify or rank risk in any formal way, we use the term “higher risk agents” to mean those that fall within our terms of reference where there are factors that may indicate a higher risk of harm to volunteers in first-in-man clinical trials.

Many of our recommendations refer to decisions affecting the safety and value of first-in-man trials that we believe should be made on a case-by-case basis and be scientifically justified. By this we mean that developers should provide clear justifications for such decisions and the regulator should assess the justifications, taking into account the safety of trial volunteers and the information that will be gained from the trial.

The views outlined on increasing the safety of first-in-man trials with higher risk agents are summary conclusions from ESG discussions taking account of all written and verbal comments received from stakeholders before and after the publication of our interim report on 26th July 2006.

Background

The current guidelines for pre-clinical development and first-in-man studies evolved from decades of experience with low molecular weight chemicals, and have been adapted to apply to previous types of biological medicines, mainly altered or natural biological products such as vaccines and products purified from human and animal sources. They served well with previous types of medicines, and phase one trials, before the TGN1412 trial, had a good safety record. However, many of the newer medicines, like TGN1412, are innovative products of advanced biotechnology that may require a different approach.

The first generation of biotechnology agents, including therapeutic monoclonal antibodies, recombinant cytokines, interferons and hormones achieved real clinical advances and large benefits to patients in the therapeutic areas of cancer, autoimmunity, inflammatory diseases and many other debilitating non-malignant diseases. We may expect, and hope, to see a large increase in these new medicines in the future and other novel biological medicines, such as those based on nucleic acids, for the treatment and prevention of disease.

The degrees of specificity and potency that can be engineered into such agents compared with simpler chemical entities create difficult challenges in pre-clinical and clinical

development. It is now apparent that the pre-clinical development of such agents cannot rely on methods that served well with smaller chemical medicines or previous generations of biological medicines.

There are many potential classes of new agents, and the inherent risks may be quite different in each class although some generalisations are possible. With heterogeneity of risk, there is unlikely to be any single, major risk reduction technique or approach that will be sufficient to optimise the safety of first-in-man trials of all types of higher risk agent.

The ESG concluded that the integration of information from many sources, in an iterative process, offers the best approach to optimising the safety of volunteers in first-in-man trials of certain types of new biological medicines. Similar considerations will apply to species-specific small molecule agents when the detection of ‘on-target’ toxicity in animal studies may be unreliable. This may also apply to unanticipated “off-target” toxicity, which may reveal new biology.

A multi-level approach to risk reduction and risk management applied to all aspects of medicines development should be the norm, and although operational distinctions are made between different stages of development, such as pre-clinical and clinical phases, an integrated overview of the entire process should be maintained, and refined as new information accumulates.

Pre-clinical

The decision to begin development of a new medicine should be based on the identification of an unmet clinical need and a sound scientific rationale for therapeutic benefit. In general, the higher the potential risk associated with the type of agent and its pharmacological target, the greater the importance of the underpinning scientific and clinical rationale for its development.

Important information can be learned about the intended pharmacological response of a new medicine, and its potential for adverse reactions, from studies in animal models. However, the predictive value of the animal model for responses in humans needs to be understood, especially when there is known or suspected species-specificity of effect arising from the nature of the medicine or its target molecule(s) *in vivo*.

For highly novel agents, it is essential that there is a discussion between regulatory scientists and independent scientists in the relevant research field, to evaluate the full spectrum of biological effects of the new class of agent. Decisions on what can usefully be learned from whole animal studies and from *in vitro* experimentation using human and animal tissues must be science-based in the light of the value of the information obtained with different technologies and its relevance to human subjects *in vivo*.

For example:

- Using cells and tissues *in vitro* to assess comparability in man and in animal model species of the binding affinity for target molecule(s).

- Assessing the comparability of measurable cellular or tissue responses to receptor occupancy in terms of morphological or biochemical changes, including pre-specified responses as well as cellular changes that may be detected by screening procedures such as RNA or protein profiles. The aim is to test similarity of biological responses in man and model species. Both qualitative and quantitative comparisons should be made.
- In some cases where a new agent is highly species-specific, particularly with biological medicines such as antibodies or cytokines, it may be valuable to test biological responses in appropriate animal models using species-homologous counterparts of the human agent. This can increase the understanding of the physiological role of the target but cannot necessarily predict the response in humans.
- There may also be value in using genetically modified animal cells, tissues or whole animal models, or cellular chimeras, to evaluate responses to human-specific agents.
- The correlation between responses to a new agent in animal and human cells or tissues, and the relation between *in vitro* and *in vivo* animal responses may allow, by iteration, a progressive refinement of the ability to predict the human response *in vivo*.
- The design of the trial should be guided by appropriate statistical methods that maximise the information value of the trial and minimise the exposure to risk of the trial subjects. It may be possible to develop new statistical methods or computer modelling to optimise the predictive value of pre-clinical information for first-in-man trials in humans. Statisticians involved in the design of first-in-man clinical trials should be fully informed of all relevant factors from the pre-clinical development phase that may affect the safety of trial subjects, and the aims to be achieved in terms of new information gained.

Ultimately, the strategy for pre-clinical development of a new medicine, and the experimental approaches used to assemble information that optimises the safety of first-in-man trials are science-based decisions that must be made case-by-case by competent and appropriately trained investigators. .

Rigid adherence to guidelines developed from experience with existing medicines may carry the risk of creating a false sense of security when dealing with new classes of medicines and there may be over-reliance on methods that are not appropriate in specific cases.

- Decisions on the strategy for pre-clinical development of a new medicine and the experimental approaches used to assemble information relevant to the safety of first-in-man clinical trials must be science-based, made and justified case-by-case by individuals with appropriate training.

The pre-clinical development of new medicines is addressed by internationally agreed guidelines, in particular ICH S6 and M3 (M 3 (R1) Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals CPMP/ICH/286/95, S 6 Pre-clinical Safety Evaluation of Biotechnology-Derived Products CPMP/ICH/302/95).

Regulators should consider the applicability of this guidance to high-risk medicines and in particular their first use in man. Developing more explicit guidance on these should be considered. This might best be achieved by initial guidance at the EU level, and using that as a platform for proposals at ICH level.

The recent results from experiments performed at NIBSC suggest that different and informative approaches can be taken in *in vitro* studies that might be more predictive of *in vivo* responses. With higher risk agents, the pre-clinical development programme should ensure a sound understanding of the biological activity, and potency, of the agent.

- The regulatory process for first-in-man trials of higher risk agents and advanced medicinal products based on innovative technologies should be subject to regular review.

With present advances in biology, biological chemistry, informatics and biotechnology, we can expect the identification of many new therapeutic targets and the development of innovative medicines to fill unmet clinical needs. It is important that the regulation of medicines is science-based and that the regulatory process keeps pace with, and is appropriate for, the science and technology that will give rise to tomorrow's medicines.

Sharing of information

Pre-clinical studies

It would seem essential to attempt the collection of unpublished pre-clinical information relevant to the safety of human subjects in first-in-man trials to inform the planning of future trials with similar agents. A UK or European database could be linked with others in different countries to form a global database. There is some indication that the pharmaceutical and biotechnology industries and research funding bodies would be willing to explore the sharing of information in this way, and researchers from other sectors, such as academic centres and research institutions might also contribute.

- Developers of medicines, research funding bodies and regulatory authorities should expedite the collection of information from unpublished pre-clinical studies relevant to the safety of human exposure. As a first step this should focus on pre-clinical reports of results that signal potential danger to humans with the use of a higher risk medicine or group of medicines. This could provide a platform for information sharing between regulators at EU and international level, e.g. in the form of a confidential database. The submission of such data to a database by the investigators is strongly encouraged.

We believe that the ultimate goal should be an open access database, and the feasibility of this needs to be explored. To avoid the risk of delay in sharing safety-critical information, reports from studies that would otherwise not be in the public domain could initially be stored in a secure database accessible only to regulators worldwide, with frequent review of the reason for delaying open access.

Phase one trials

Before the implementation in 2004 of the European Clinical Trials Directive, there was no necessity in the UK for investigators to apply to the regulator for authorisation to conduct a phase one trial in healthy volunteers where there was no potential for therapeutic benefit to the volunteer. A similar situation may have existed in some other European countries and elsewhere.

It is not known how many first-in-man trials were conducted in the UK before 2004, or what the outcomes were. Useful information gained during previous studies of an agent is not always in the public domain and accessible to others who are undertaking a trial of the same, or a similar agent.

The ESG heard a specific example during a stakeholder consultation where information from a phase one trial of an anti-lymphocyte antibody with reactivity against three targets, including the target of TGN1412, and relevant to safety in humans, was never published.

There may be several reasons for non-publication of the outcome of clinical trials, including negative results that are less likely to be accepted for publication in a scientific journal, issues of commercial confidentiality, or the abandonment of the project in the early stages of development.

Both the developers of new medicines and the regulatory authorities should consider ways of encouraging and expediting the collection of past information from first-in-man clinical trials, and future information as it becomes available, onto a database. The feasibility of open access to this information should be explored. Access to the database would be helpful to Research Ethics Committees, for example.

- Regulatory authorities should consider ways to expedite the sharing of safety information on first-in-man clinical trials between regulators within the EU and worldwide. This should certainly include information on first-in-man experience with higher risk medicines. Trials with negative safety outcomes should be included. This database might be widened to include products that may not currently be perceived as high risk, or trials conducted later in development, that suggest a strong warning for first-in-man use of similar products.

In the EU, this collection and sharing of information could be based on the model of the existing clinical trial database EudraCT (for first-in-man trials since 2004) and the EudraVigilance database for ‘Suspected Unexpected Serious Adverse Reactions

(SUSARs)'. Relevant information from first-in-man trials prior to 2004 could be submitted on a voluntary basis. This would ensure access to relevant safety information by national regulators.

Since 2004 in the UK there has been a requirement for Suspected Unexpected Serious Adverse Reactions (SUSARs) within phase one trials to be reported to the regulator. The same applies to all countries of the EU and similar arrangements exist in other countries. It is the intention within Europe to share this information between regulators but we suggest that this should include the sharing of information between regulators worldwide. The feasibility of open access to this data should also be explored.

Transition from pre-clinical to clinical development

Calculation of the starting dose in a first-in-man trial is a central factor affecting the safety of the subjects in the trial. Pre-clinical studies with TGN1412 did not predict a safe starting dose for human trials, and all the evidence available so far indicates that too high a dose was given. The guidelines for dose calculation that have worked well in the past for small molecule drugs may not be universally applicable to large molecule biological medicines, especially when species-specificity of action makes it difficult, or impossible, to predict a safe human dose from studies in animals.

Conventionally, with small molecule drugs, a low dose that has no effect in animals, (No Observable Effect Level, NOEL) or no adverse effects (No Observable Adverse Effect Level, NOAEL) could be established as a starting point, and then a dose reduction with a high margin of safety calculated as the starting dose in first-in-man trials. In general, the more species-specific an agent is, the less reliable will be information from animal studies as a guide to the starting dose in humans.

To overcome this, a wider approach to dose calculation, based on all relevant information should be undertaken. For example, this might include taking account of the novelty of the agent and its mechanism of action, the degree of species-specificity of the agent, the dose-response curves of biological effects in human and animal cells and dose-response data from relevant *in vivo* animal studies.

With receptor ligands it is also important to measure or calculate receptor occupancy versus concentration and the extent of exposure of targets and target cells *in vivo*. In general, doses for first human exposure, even when the relationship between receptor occupancy and biological response is not known, should be calculated to provide concentrations at targets that would give rise to low receptor occupancy.

It was suggested in a stakeholder submission that the starting dose in first-in-man trials of biological medicines should be the dose required to reach the bottom end of a dose response curve in man. This could be estimated from human receptor occupancy and cellular dose-response studies, combined with information from qualified animal models and experience with similar agents. This would give a 'Minimum Anticipated Biological Effect Level' (MABEL) below which the starting dose should be set. The ESG thought that this was a very useful approach to calculating a safe dose in first-in-man trials.

This use of information from both physico-chemical and biological studies combined with prior knowledge of similar agents is likely to increase the safety of first-in-man trials with higher risk agents, and could be enhanced with better access to results from previous pre-clinical and phase one studies with similar agents.

The overall opinion of the ESG was that when it is likely that pre-clinical information, for any reason, may be a poor guide to human responses *in vivo*, the starting dose should be calculated to err on the side of caution.

There is a point of view that giving a dose that is much too low to provide any useful information would call into question the ethics of a trial. However, the opinion of the ESG was that since it may be difficult *a priori* to predict a dose that is too low to be useful, a very low starting dose in the context of an appropriate dose escalation regime would increase the safety of first-in-man trials without creating a serious logistical barrier.

The view that higher risk agents should be given at specified low dose levels, for example in the microgram or nanogram range ('microdosing', 'nanodosing') may have value as a general guideline. But there may be instances of highly potent agents where such specified levels could still be too high for safety in first-in-man trials and the ESG felt that, although these guides were useful to focus care on dose calculation, a 'rule of thumb' was less valuable than a thorough case-by-case evaluation of all relevant information.

It has also been pointed out in stakeholder comments that a very small sub-pharmacological dose, while decreasing risk to the recipient, does not necessarily reduce the risk when pharmacological dose levels are reached during dose escalation. This may be especially true with agents where there is a very steep dose-response curve in humans *in vivo*.

- Special consideration should be given to new agents for which the primary pharmacological action for the proposed therapeutic effect cannot be demonstrated in an animal model. The underpinning case for dose selection in any trial of this kind should include a clear rationale for the proposed mechanism of action and also for the safety and efficacy of the substance in its intended clinical use.
- A broader approach to dose calculation, beyond reliance on 'No Observable Effect Level' or 'No Observable Adverse Effect Level' in animal studies, should be taken. The calculation of starting dose should utilise all relevant information. Factors to be taken into account include the novelty of the agent, its biological potency and its mechanism of action, the degree of species-specificity of the agent, the dose-response curves of biological effects in human and animal cells, dose-response data from *in vivo* animal studies, pharmacokinetic and pharmacodynamic modelling, the calculation of target occupancy versus concentration and the calculated exposure of targets or target cells in humans *in vivo*.

The 'Minimal Anticipated Biological Effect Level' (MABEL) approach is one good model for achieving this.
(See ABPI/BIA report and stakeholder submission.)

- If different methods give different estimates of a safe dose in humans, the lowest value should be taken as the starting point in first-in-man trials and a margin of safety introduced in calculation of the actual starting doses in man.
- When it is likely that pre-clinical information, for any reason, may be a poor guide to human responses *in vivo*, the starting doses in first-in-man trials should be calculated to err on the side of caution. Further dose increases should proceed with caution since the initial dose will have been particularly low and there may be a steep dose-response curve.
- Careful consideration should be given to the route and the rate of administration of the first doses in first-in-man trials, with careful monitoring for an exaggerated response.

For example, in the case of first human exposure to a higher risk agent given intravenously, a slow infusion over several hours may be more appropriate than a slow bolus over several minutes. This would allow monitoring for an adverse response and stopping the infusion if clinically indicated.

The trial design, and decisions on the number of subjects, starting doses and the dose escalation regime should be based on detailed evaluation of predicted and possible risks related to the nature of the agent, its target *in vivo* and the intended recipients. As with many of the questions surrounding pre-clinical to clinical transition of new, higher risk agents, ultimately these are science-based decisions that must be made carefully case-by-case, and should be justifiable, based on all relevant information.

- The trial design, including decisions on the number of subjects, starting doses and the dose escalation regime, should be made on a case-by-case basis, and should be scientifically and statistically justifiable, taking account of all relevant information.

(See stakeholder submission from The Royal Statistical Society).

Sequential dosing of trial subjects

In general, new agents in first-in-man trials should be administered sequentially to human subjects with an appropriate interval between dosing of subjects to limit the number of people that may be affected by a severe adverse reaction. The intervals of observation between administration to the first, second and subsequent subjects should be determined by the kind of adverse reaction that might be anticipated based on the nature of the agent, its target, and the intended recipient.

Although there will be no available pharmacokinetic data in the human, some prediction of pharmacokinetics may be possible from animal studies, for example with large biological molecules that will not be filtered in the kidneys.

For first-in-man trials of a higher risk agent, a guideline minimum observation period after administration to the first subject was discussed by the ESG, but it was acknowledged that the period of observation between dosing the first and subsequent subjects must take into account factors related to the agent used, its target, the recipients, and the range of possible adverse reactions.

The monitoring period between dosing of different subjects should be decided case-by-case. Protocols exist for the progressive escalation of dosing in phase one trials related to periods of monitoring for adverse reactions. A longer monitoring period of the first recipient of an escalated dose would also increase safety.

- New agents in first-in-man trials should be administered sequentially to subjects with an appropriate period of observation between dosing of individual subjects. The interval of observation between sequential dosing of subjects should be related to the kind of adverse reactions that might be anticipated based on the nature of the agent, its target and the recipient, as well as the potential pharmacokinetic and pharmaco/toxico-dynamics of the agent. There should be a suitable interval between doses to different individuals as well as between doses to the same individual, and both should be justified on the basis of available evidence.
- A similar period of monitoring should occur between sequential dosing of the subjects during dose escalation. (*as above*)

Choice of subjects for first-in-man phase one trials

The aim of a phase one trial is not to assess the therapeutic benefit of a new agent and subjects are not expected to derive any benefit. In general, the decision to perform phase one trials in either volunteer healthy subjects or volunteer patients should be made on a case-by-case basis.

Several factors must be considered, such as the risks inherent in the type of agent under test and its molecular target, the presence of the target in healthy subjects and patients, the value of the information that may be obtained in healthy subjects or in patients, and how far the information may be generalised.

In the cancer field there is a history of conducting clinical trials with cytotoxic agents with high potential for toxic effects. The practice has usually been to perform first-in-man trials in volunteer patients, which ensures that the intended drug target is present, and toxicity arising from both ‘on-target’ and ‘off-target’ effects would be detectable.

Although there is no anticipated therapeutic benefit to the subjects in a phase one trial, patient volunteers may be more appropriate subjects than healthy volunteers on the basis of a ‘risk to benefit’ assessment in the case of higher risk agents targeted at serious diseases where all existing therapeutic options for the patient have been exhausted. This assumes that should a patient derive any benefit in a first-in-man clinical trial, the trial medicine could be made available for continued treatment after the trial.

There may be circumstances, however, where healthy volunteers are more appropriate subjects in a phase one clinical trial. For example where concurrent medication in patients would cause difficulties in the interpretation of results.

- The decision whether to conduct a first-in-man trial in healthy volunteers or in volunteer patients should be carefully considered and fully justified, taking into account all factors relevant to the safety of the subjects and the value of the scientific information that is likely to be obtained.

The paramount factors should always be the safety, rights and well-being of the volunteers, whether patients or healthy individuals, and the value of what can be learned from the clinical trial.

Clinical aspects and environment of first-in-man trials

The Principal Investigator (PI) must be appropriately qualified to undertake and supervise the trial. The PI should be satisfied that full information has been made available about the nature of the agent being tested, the details of pre-clinical development relevant to safety in humans and the rationale of the trial design and protocol. If there is doubt, the PI should seek further information. The methods used to calculate the starting dose and the scientific rationale behind the dose escalation strategy should also be made available to the PI. Principal Investigators should always satisfy themselves that they are in a position to make informed clinical judgements.

In cases where there is a predictable risk of a certain type of severe adverse reaction occurring in humans, a treatment strategy should be considered beforehand. This should include the availability of specific antidotes where they exist and a clear plan of supportive treatment including the availability of ITU facilities.

First-in-man trials should be conducted in appropriate clinical environments supervised by staff with appropriate levels of training and expertise and an understanding of the trial agent, its target and mechanism of action. There must be immediate access to facilities for the treatment of medical emergencies (such as cardiac emergencies, anaphylaxis, cytokine release syndrome, convulsions, hypotension), facilities for stabilising individuals in an acute emergency and ready availability of ITU facilities.

- Principal Investigators who are responsible for the care of subjects in first-in-man trials should always be appropriately qualified and satisfy themselves that they know enough about the agent, its target and mechanism of action to be in a position to make informed clinical judgements.

The development of a national professional accreditation system for Principal Investigators conducting first-in-man clinical trials should be encouraged.

- In first-in-man studies where there is a predictable risk of certain types of severe adverse reaction, a treatment strategy should be considered beforehand. This should include the availability of specific antidotes where they exist and a clear plan of supportive treatment including pre-arranged contingency availability of ITU facilities.
- First-in-man studies of higher risk medicines should always be conducted in an appropriate clinical environment supervised by staff with appropriate levels of training and expertise with immediate access to facilities for the treatment and stabilisation of individuals in an acute emergency and with pre-arranged contingency availability of ITU facilities.

There should always be an adequate staffing level in first-in-man studies and adequate 24-hour cover when volunteers are kept in overnight. All clinical sites conducting such trials should have standard operating procedures for emergency situations, and staff should maintain expertise in implementing these procedures through regular drills. Trial subjects should always be clearly informed about what to do if they experience symptoms of an adverse reaction during or after a clinical trial.

Regulatory considerations

Regulation of medicines, including the conduct and safety of clinical trials, should be based on the science underpinning medicines development. In recent years, advances in molecular biology and biotechnology have produced new classes of potent and clinically valuable medicines. As the pace of development in biosciences increases, major advances can take place over comparatively short time periods and this is likely to continue well into the future. It is therefore essential that a wide range of expertise in emerging areas of science should be available to the regulator.

Regulatory access to independent advice

Professional scientific and medical assessors should be supported by external experts who are in touch with the research front in their fields to ensure that regulatory decisions are based on state-of-the-art science.

The regulator should be able to call upon such support for assessing first-in-man phase one clinical trial applications when the requirement is recognised, eg with applications for trials of higher risk agents as defined by factors discussed previously such as the nature of the agent, its degree of novelty, its intended pharmacological target and the intended recipients.

It would be possible to develop the existing scientific advisory structure to make highly specialised expertise available to regulators. For example, an Expert Advisory Group (EAG) of the Commission on Human Medicines might undertake this role, with the appointment of a core membership of relevant experts and the ability to co-opt additional experts as the need dictates.

At present, the regulations for appraisal of applications for clinical trial authorisations create a potential logistical difficulty in seeking outside opinion because a strict time limit is imposed on the process. The advantage of a standing EAG is that good communications with the regulator can be established and responses could be provided on a short timescale.

The logistical problem should be significantly reduced if more communication can be encouraged between developers and the regulator at an earlier stage, before an application is filed, especially for a higher risk agent. This would also enable the regulator to access external expert opinion at an earlier time, if necessary. Increased opportunity for communication between the regulator and research ethics committees on applications for trials of higher risk medicines would also contribute to the overall safety environment.

While it is against the interests of patients to introduce unnecessary delays in medicines development through a slower regulatory process, consideration should be given to introducing some flexibility in the time-scale of clinical trial appraisal in exceptional cases of unusual complexity.

- More communication is strongly recommended between medicines developers and the regulator at an earlier stage before an application is filed, especially for higher risk agents, to ensure that there is time for an appropriate consideration of any safety concerns without introducing undue delay in product development. Ways to increase communication between the regulator and research ethics committees should also be considered.
- For appraisal of applications for first-in-man trials of higher risk agents, as defined by the nature of the agent, its degree of novelty, its intended pharmacological target, and its intended recipient, the regulator should have access to additional opinion from independent, specialist experts with research knowledge of their fields.
- An Expert Advisory Group (EAG) of the Commission on Human Medicines, or a similar body, might undertake this role with a core membership of appropriate experts and the ability to co-opt additional experts as the need dictates.
- Consideration should be given to introducing some flexibility in the time-scale of clinical trial appraisal in exceptional cases of unusual complexity.

Future needs

Skills and training

Many first-in-man phase one trials in the UK, possibly the majority, are currently performed in commercial Clinical Research Organisations (CROs) unrelated to NHS hospitals and universities.

This means that UK Schools of Medicine, Nursing, Dentistry and Pharmacy, as well as the NHS, have decreasing opportunities to develop and teach the skills needed by the next generation of phase one clinical trial specialists. This has negative implications for the future of phase one clinical trials in the UK, and for clinical trials in general. The situation may be similar in other countries.

Provision for ‘hands-on’ experience in the planning and conduct of clinical trials could be increased if academic and NHS institutions created links with commercial CROs. University or NHS postgraduate training programmes could incorporate placement periods within CROs. Another way to retain and develop clinical trial skills within the NHS and universities might be to encourage the formation within them of specialist centres for phase one trials (see below).

The need to train a new generation of doctors in the skills needed for the assessment of safety and efficacy of medicines was highlighted in the UK’s Academy of Medical Sciences 2005 report “Safer Medicines” (<http://www.acmedsci.ac.uk/p102.html>). Similar needs were identified in “Sustaining the skills pipeline in the pharmaceutical and biopharmaceutical industries”, a recent report of a study by the ABPI. (<http://www.abpi.org.uk/Details.asp?ProductID=285>).

This will need co-operation between higher education funding bodies and institutions, the NHS and industry. Given that major stakeholders agree on the need to fill this skills gap with a new generation of Clinical Pharmacologists and related professionals, it should be possible to address it, and we encourage this in the interests of the safety of future first-in-man clinical trials.

- The availability of ‘hands-on’ experience in the planning and conduct of clinical trials should be widened, for example by secondment periods to commercial organisations within postgraduate training programmes, or the development of specialist centres within the NHS and Universities (see next recommendation).

As the science underpinning innovative medicines becomes increasingly sophisticated we may anticipate ever more advanced medicinal products in the future. For example, such products might be aimed very specifically at signalling pathways or sequences of nucleic acids, and may incorporate genetic elements, cellular elements and advanced physical or biological delivery systems.

Such innovative products, known as Advanced Therapy Medicinal Products in the EU, are considered to have great potential for therapeutic benefit in diseases where there is current clinical need. But the promise of more effective medicines carries the possibility, or likelihood, of unpredictable risk. When innovative products enter first-in-man trials, it is important that the skills needed to anticipate and recognise these risks are developed in parallel. The feasibility of developing specialist centres within universities and the NHS for phase one clinical trials of higher risk and advanced medicinal products should be explored.

- The feasibility of developing specialist centres for Phase One clinical trials of higher risk agents and advanced medicinal products should be explored.

The development of a national inspection and accreditation system for clinical centres conducting first-in-man studies of higher risk agents should be encouraged. Although we have highlighted universities and the NHS, the accreditation should be open to all centres in both the public and private sectors that fulfil defined criteria. This would enhance the training opportunities and the skill base.

9. Scope and Summary of the Recommendations

The aim of the ESG has been to define what can be learned from the TGN1412 trial and to consider how far the findings can be generalised to optimise the safety of volunteers in future trials of higher risk new medicines within the ESG's terms of reference. In this report we have made 22 recommendations that we believe will optimise the safety of future first-in-man phase one clinical trials without creating unjustifiable barriers to the development of useful new medicines.

We have considered pre-clinical, clinical and regulatory aspects of phase one trials where safety may be increased, both now and with a view to the future. The recommendations are made with first-in-man trials of higher risk agents in mind, as defined by the nature of the agent, its mode of action, its pharmacological target and its intended recipients, but some of the recommendations may have applicability to phase one trials in general.

Our recommendations are intended to supplement current regulatory guidelines and refine their applicability to newer types of agents where a higher risk of toxicity in first-in-man trials may be recognised.

Several of our recommendations relate to decisions that we believe are more safely made on a case-by-case basis during pre-clinical development and clinical transition. We recommend that such decisions should be justified, and by that we mean that the medicines developer should provide the justification which should be carefully assessed by the regulator, calling on independent expert advice when it is needed.

Scope of the Recommendations

What kind of clinical trial?

The recommendations are intended to apply to “first-in-man” clinical trials, and not to phase one trials in general (which might include trials of agents with an established record of safety in humans). Special caution is needed during first human exposures to higher risk agents at doses with the potential to cause a pharmacological effect.

However, added caution should also be taken when administering a medicine with the potential for high risk to a distinct new population, be they healthy volunteers or patients. Preparation of guidance on first-in-man studies should give consideration to the applicability of the concepts developed to transitions from one population group to another.

The ICH E8 guideline ‘General Considerations for Clinical Trials (CPMP/ICH/291/95) (<http://www.emea.europa.eu/pdfs/human/ich/029195en.pdf>) provides general guidance on clinical trials and contains, in section 3, an internationally agreed description of phase one trials and a good overview of the development phases of medicines from discovery to market.

What kind of agent?

Our remit covers three categories of medicines that may have a higher potential for risk of harm to volunteers during the first human exposures, or where risk may be more difficult to evaluate in pre-clinical development. The categories are:

- Biological molecules with novel mechanisms of action;
- New agents with a high degree of species-specificity;
- New agents with immune system targets.

We intend our recommendations to apply to agents in any one of these three categories unless a careful assessment of the nature of the agent, the physiological role of the target molecules, and the intended recipients, supports a low risk of harm in first human exposures.

We do not suggest that all agents that fall into one of the three categories listed above necessarily pose a high risk on first human exposures, but that a thorough assessment of risk should be made and a clear scientific case provided when risk of harm is assessed as being low.

For example a conventional vaccine, although aimed at stimulating an immune response, may not pose a high risk, or a new agent similar to one with an established safety record in humans, and aimed at a known target where the pharmacology can be predicted with confidence, may not pose a high risk.

We have discussed in this report factors that should raise the level of caution for first human exposures to new agents. These include:

- any agent whose effects might cause severe physiological disturbance to vital body systems;
- agonistic or stimulatory actions;
- novel agents and novel mechanisms of action where there is no prior experience;
- species-specificity of an agent making pre-clinical risk-assessment in animal models difficult or impossible;
- the potency of an agent, eg compared with a natural ligand;
- multifunctional agents, eg bivalent antibodies, FcR binding domains;
- cell-associated targets;
- targets that by-pass normal control mechanisms;
- immune system targets;
- targets in systems with the potential for large biological amplification *in vivo*.

A thorough assessment of risk should always be carried out before first-in-man trials. The risk assessment should be clearly described in the trial documents and be fully examined by the regulator. When there is significant doubt, higher risk should always be assumed. (See Section 7 of the Report.).

Summary of the Recommendations

The safety, rights and well-being of subjects, both patients and healthy volunteers, must always be the primary concerns in clinical trials. We have made 22 recommendations that we believe will increase the safety of volunteers in future clinical trials involving the first human exposures to agents with higher potential risks, as categorised in our terms of reference and discussed in Section 8 of our report. They cover:

- Pre-clinical and early clinical development;
- The process of preparation and review of clinical trial applications, and early access to advice for both regulators and sponsors;
- Determining and administering the initial doses in man;
- The clinical environment for first-in-man studies;
- Developing the skills and training to meet future needs.

There is focus on sharing of information relevant to safety, the calculation and administration of first doses, and regulatory access to independent specialist opinion in the appraisal of trial applications for the first human exposures to new medicines of the types described in our remit where there is higher potential risk.

The rationale for each recommendation, and explanations of our intentions, are summarised in *Section 8, "Predicting Hazards in Pre-clinical to Clinical Transition", and Section 9, "Risk Reduction and Risk Management"*. The recommendations should be considered in the context of these Sections.

Stakeholders raised several areas of concern that were not within our remit. These included topics such as the process of informed consent and clarity of information, communication between clinical investigators and clinical trial subjects before and during a trial, insurance cover, the role of Research Ethics Committees, and clinical follow-up of trial subjects who had experienced an adverse reaction. Although beyond our remit, we believe these wider concerns are all extremely important, and recommend that they are taken up as a high priority and considered in detail.

Our recommendations are offered to the UK authorities and sponsors of first-in-man trials in the UK, but we believe it is important that agreement is sought at EU and international level, to ensure that equal protection is afforded to clinical trial participants worldwide.

Final Recommendations

Pre-clinical and early clinical development:

1. Decisions on the strategy for pre-clinical development of a new medicine and the experimental approaches used to assemble information relevant to the safety of first-in-man clinical trials must be science-based, made and justified case-by-case by individuals with appropriate training.

The pre-clinical development of new medicines is addressed by internationally agreed guidelines, in particular ICH S6 Pre-clinical Safety Evaluation of Biotechnology-Derived Products, and ICH M3 (R1) Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (see further reading below).

Regulators should consider the applicability of this guidance to high-risk medicines and in particular their first use in man. The development of more specific guidance in the present context should be proposed. This might best be achieved by initial guidance at the EU level, and using that as a platform for proposals at international level.

2. The regulatory process for first-in-man trials of higher risk agents and advanced medicinal products based on innovative technologies should be subject to regular review.

With present advances in biology, biological chemistry, informatics and biotechnology, we can expect the identification of many new therapeutic targets and the development of innovative medicines to fill unmet clinical needs. It is important that the regulation of medicines is science-based and that the regulatory process keeps pace with, and is appropriate for, the science and technology that will give rise to tomorrow's medicines.

3. Developers of medicines, research funding bodies and regulatory authorities should expedite the collection of information from unpublished pre-clinical studies relevant to the safety of human exposure. As a first step this should focus on pre-clinical reports of results that signal potential danger to humans with the use of a higher risk medicine or group of medicines. This should provide a platform for information sharing between regulators at EU and international level, e.g. in the form of a confidential database. The submission of such data to a database by the investigators is strongly encouraged.

In the interests of safety, we believe that the ultimate goal should be an open access database, and the feasibility of this needs to be explored. However, to avoid the risk of delay in sharing safety-critical information, reports from studies that would otherwise not be in the public domain could initially be stored in a secure database accessible only to regulators worldwide, with frequent review of the reason for delaying open access.

4. Regulatory authorities should consider ways to expedite the sharing of safety information on phase one clinical trials between regulators within the EU and worldwide. This should certainly include information on first-in-man experience with higher risk medicines. Trials with negative safety outcomes should be included. This database might be widened to include products that may not currently be perceived as high risk, or trials conducted later in development, that suggest a strong warning for first-in-man use of similar products.

In the EU, this collection and sharing of information could be based on the model of the existing clinical trial database EudraCT (for first-in-man trials since 2004) and the EudraVigilance database for ‘Suspected Unexpected Serious Adverse Reactions (SUSARs)’ – (see below). Relevant information from first-in-man trials prior to 2004 could be submitted on a voluntary basis. This would ensure access to relevant safety information by national regulators.

This will require dialogue with the pharmaceutical industry on which study reports should be made available and when they should be posted, but should be achievable since industry is already committed to disclosure of clinical trial results on medicines already on the market, and encourages sponsors to post results of development failures where there may be important safety implications. The regulators should explore the feasibility of open access to this data.

SUSARs are Suspected Unexpected Serious Adverse Reactions. They are unexpected because their nature or severity is not consistent with the applicable product information (e.g. investigator's brochure for an unauthorised investigational product or summary of product characteristics for an authorized product), they are reactions because there is a causal relationship with the product, and they are serious, as described below:

Article 2(o) of Directive 2001/20/EC gives the definition of serious adverse reaction: “any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect.”

For all other important medical outcomes not on this list, professional medical and scientific judgement should be exercised when deciding the need to report.

The process of preparation and review of clinical trial applications, and early access to advice for both regulators and sponsors

5. More communication is strongly recommended between developers and the regulator at an earlier stage before an application is filed, especially for higher risk agents, to ensure that there is time for an appropriate consideration of any safety concerns without introducing undue delay to product development. Ways to increase communication between the regulator and research ethics committees should also be considered.

For first-in-man clinical trials of new agents that fall into the higher risk categories described in our remit, pre-submission meetings between sponsors and regulators, to identify potential concerns, would be useful to both parties, and are strongly recommended. If developers elect not to have pre-submission discussion with the regulator, they should be asked to inform the regulator six weeks in advance of an intended application for a first-in-man clinical trial of a higher risk medicine. This would give the regulator adequate time to consider the need for external expert advice and to identify appropriate experts (see next recommendation). Advance warning would reduce the possibility of prolonging the assessment process.

6. For appraisal of applications for trials of higher risk agents, as defined by the nature of the agent, its degree of novelty, its intended pharmacological target, and its intended recipient, the regulator should have access to additional opinion from independent, specialist experts with research knowledge of their fields.
7. An Expert Advisory Group (EAG) of the Commission on Human Medicines, or a similar body, might undertake this role with a core membership of appropriate experts and the ability to co-opt additional expertise as the need dictates.
8. Consideration should be given to introducing some flexibility in the time-scale of clinical trial appraisal in exceptional cases of unusual complexity.

We emphasise “exceptional cases of unusual complexity”; there is no intention to introduce unjustified delay in the regulatory process in the development of new medicines. The European Clinical Trials Directive (article 6, paragraph 7) (Directive 2001/20/EC, below) already allows for time extension in the appraisal of gene therapy, somatic cell therapy and products containing genetically modified organisms. This might be a model for other highly innovative medicines that bring new science into therapeutics.

Determining and administering the initial doses in man:

9. Special consideration should be given to new agents for which the primary pharmacological action, for the proposed therapeutic effect, cannot be demonstrated in an animal model. The underpinning case for dose selection in any trial of this kind should include a clear rationale for the proposed mechanism of action and also for the safety and efficacy of the substance in its intended clinical use.
10. A broader approach to dose calculation, beyond reliance on ‘No Observable Effect Level’ or ‘No Observable Adverse Effect Level’ in animal studies, should be taken. The calculation of starting dose should utilise all relevant information. Factors to be taken into account include the novelty of the agent, its biological potency and its mechanism of action, the degree of species-specificity of the agent, the dose-response curves of biological effects in human and animal cells, dose-response data from *in vivo* animal studies, pharmacokinetic and pharmacodynamic modelling, the calculation of target occupancy versus concentration and the calculated exposure of targets or target cells in humans *in vivo*.

The ‘Minimal Anticipated Biological Effect Level’ (MABEL) approach is one good model for achieving this. (See BIA/ABPI report and stakeholder submission.)

11. If different methods give different estimates of a safe dose in humans, the lowest value should be taken as the starting point in first-in-man trials and a margin of safety introduced in calculation of the actual starting doses in man.
12. When it is likely that pre-clinical information, for any reason, may be a poor guide to human responses *in vivo*, the starting doses in first-in-man trials should be calculated to err on the side of caution. Further dose increases should proceed with caution since the initial dose may have been particularly low and there may be a steep dose-response curve.
13. Careful consideration should be given to the route and the rate of administration of the first doses in first-in-man trials, with careful monitoring for an adverse or exaggerated response.

For example, in the case of first human exposure to a higher risk agent given intravenously, a slow infusion over several hours may be more appropriate than a slow bolus over several minutes. This would allow monitoring for an adverse response and stopping the infusion if clinically indicated.

14. The trial design, including number of subjects, decisions on starting doses and the dose escalation regime, should be made on a case-by-case basis, and should be scientifically and statistically justifiable, taking account of all relevant information.

(See stakeholder submission from The Royal Statistical Society)

15. New agents in first-in-man trials should be administered sequentially to subjects with an appropriate period of observation between dosing of individual subjects. The interval of observation between sequential dosing of subjects should be related to the kind of adverse reactions that might be anticipated based on the nature of the agent, its target and the recipient, as well as the potential pharmacokinetics and pharmacodynamics of the agent.

There should be a suitable interval between doses to different individuals as well as between doses to the same individual, and both should be justified on the basis of available evidence.

16. A similar period of monitoring should occur between sequential dosing of the subjects during dose escalation. (*as above*)
17. The decision whether to conduct a first-in-man trial in healthy volunteers or in volunteer patients should be carefully considered and fully justified, taking into

account all factors relevant to the safety of the subjects and the value of the scientific information that is likely to be obtained.

In general, there is no anticipated benefit to a patient in a first-in-man trial of a new medicine. Therefore, risk to benefit assessment is not usually a major factor in deciding whether such trials should be performed in volunteer patients or in healthy subjects.

In the field of cancer medicine there may be exceptions since patients who experience a beneficial response to a trial agent are often able to continue treatment with it. In this case the balance of risk and benefit may become a factor in deciding that patients are more appropriate subjects of a clinical trial, especially patients that have not responded to available therapies and when the trial medicine has predictable cellular toxicity (which may, in fact, be its intended pharmacological effect).

However, the paramount factors should always be the rights, safety, and well-being of the volunteers, whether patients or healthy individuals, and the value of what can be learned from the clinical trial.

The clinical environment for first-in-man studies

18. Principal Investigators who are responsible for the care of subjects in first-in-man trials should always be appropriately qualified, and satisfy themselves that they know enough about the agent, its target and mechanism of action to be in a position to make informed clinical judgements.

The development of a national professional accreditation system for Principal Investigators conducting first-in-man clinical trials should be strongly encouraged.

19. In first-in-man studies where there is a predictable risk of certain types of severe adverse reaction, a treatment strategy should be considered beforehand. This should include the availability of specific antidotes where they exist and a clear plan of supportive treatment, including the pre-arranged contingency availability of ITU facilities.

20. First-in-man studies of higher risk medicines should always be conducted in an appropriate clinical environment supervised by staff with appropriate levels of training and expertise, with immediate access to facilities for the treatment and stabilisation of individuals in an acute emergency, and with pre-arranged contingency availability of ITU facilities in reasonable proximity.

There should always be an adequate staffing level in first-in-man studies and adequate 24-hour cover when volunteers are kept in overnight.

All clinical sites conducting such trials should have standard operating procedures for emergency situations, and staff should maintain expertise in implementing these procedures through regular drills. Trial subjects should always be clearly informed about what to do if they experience symptoms of an adverse reaction during or after a clinical trial.

Developing expertise

21. The availability of ‘hands-on’ experience in the planning and conduct of clinical trials should be widened. For example postgraduate training programmes could have within them a secondment period to commercial organisations or a training period in specialist centres within the NHS and Universities (see next recommendation).

The need to train a new generation of doctors in the skills needed for the assessment of safety and efficacy of medicines was highlighted in the UK’s Academy of Medical Sciences 2005 report “Safer Medicines”. Similar needs were identified in “Sustaining the skills pipeline in the pharmaceutical and biopharmaceutical industries”, a recent report of a study by the ABPI.

This will need co-operation between higher education funding bodies and institutions, the NHS and industry. Given that major stakeholders agree on the need to fill this skills gap with a new generation of Clinical Pharmacologists and related professionals, it should be possible to address it, and we encourage this in the interests of the safety of future first-in-man clinical trials.

22. The feasibility of developing specialist centres for phase one clinical trials of higher risk agents and advanced medicinal products should be explored.

The development of a national inspection and accreditation system for clinical centres that undertake first-in-man studies of higher risk agents should be encouraged. The accreditation should be open to all centres that fulfil defined criteria, in both the public and private sectors.

Further reading

‘Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412’
G. Suntharalingam, M. Perry, S. Ward, S. Brett, A. Castello-Cortes, M. Brunner, and N. Panoskaltzis.

N Engl J Med. 2006, 355:1018-1028.

The European Clinical Trials Directive 2001/20/EC
(http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/vol-1/dir_2001_20/dir_2001_20_en.pdf)

EudraLex Volume 10 –Clinical Trials
(<http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/homev10.htm>)

European Union Guidelines -
<http://www.emea.europa.eu/htms/human/humanguidelines/background.htm>

ICH E8 - General Considerations for Clinical Trials (CPMP/ICH/291/95)
(<http://www.emea.europa.eu/pdfs/human/ich/029195en.pdf>)

ICH S6 Pre-clinical Safety Evaluation of Biotechnology-Derived Products
(CPMP/ICH/302/95)
(<http://www.emea.europa.eu/pdfs/human/ich/030295en.pdf>)

ICH M3 (R1) Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (CPMP/ICH/286/95).
(<http://www.emea.europa.eu/pdfs/human/ich/028695en.pdf>).

ICH International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) <http://www.ich.org/cache/compo/276-254-1.html>

Academy of Medical Sciences 2005 report “Safer Medicines”
(<http://www.acmedsci.ac.uk/p102.html>).

“Sustaining the skills pipeline in the pharmaceutical and biopharmaceutical industries”,
ABPI. (<http://www.abpi.org.uk/Details.asp?ProductID=285>).

