INVESTIGATIONS INTO ADVERSE INCIDENTS DURING CLINICAL TRIALS OF TGN1412

1. INTRODUCTION

This is a summary report into the Medicines and Healthcare products Regulatory Agency (MHRA) investigations regarding the Serious Adverse Events experienced during the entry into human study of the monoclonal antibody TGN1412. It summarises the inspections performed by the MHRA and other European authorities and the testing performed to date 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, which 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

2. KEY ORGANISATIONS INVOLVED IN THE TRIAL

Role	Name	Location
Sponsor	TeGenero AG	Wurzburg, Germany
Manufacturer	Boehringer Ingelheim	Germany
GLP Studies	XXXXXXXXLaboratories	XXXXXXXXXXX
Contract Research Organisation / Phase I Clinical Pharmacology Unit	Parexel	Northwick Park Hospital, Harrow, UK

3. TRIGGERED INSPECTIONS OF FACILITIES

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

Facility	Inspection Type	Date of Inspection	Inspector(s)
TeGenero AG, Germany	GCP	17th of March 2006	German Regulatory Authority
Parexel, Northwick Park Hospital, Harrow, UK	GCP	16th, 17th and 27th March 2006	XXXXXXXXXX MHRA
Parexel	GMP	14th and 16th March 2006	XXXXXXXXXX MHRA
XXXXXXXXXXXX	GLP	20th-21st March 2006	XXXXXXXXXX MHRA
Boehringer Ingelheim, Germany	GMP	22nd – 24th of March 2006	German Regulatory Authority, observed by XXXXXXXXXX MHRA

4. INSPECTION FINDINGS

4.1 GCP Inspection of TeGenero AG (Final Report Awaited)

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.

4.2 GCP Inspection of Parexel (Report Reference GCP 12689 / 0306)

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.

However, a number of discrepancies were identified which are detailed in Appendix 1 and for which Parexel are required to provide a response to the MHRA with evidence of appropriate corrective action.

4.3 Parexel GMP Inspection (Report Reference 12689-25747-0003)

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.

4.4 XXXXXXXXXXXXXXXXX GLP Inspection XXXXXXXXXXXXX

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 –

XXXXXXXXXXXXXXX: "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.

5. PRODUCT TESTING

Following the incident a series of tests were performed by 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). A copy of the protocol, which includes tests to date, is included in Appendix 2.

6. CONCLUSIONS

This investigation indicates that the adverse incidents did not involve errors in the manufacture of TGN1412 or in its formulation, dilution or administration to trial participants. The MHRA therefore concludes that an unpredicted biological action of the drug in humans is the most likely cause of the adverse reactions in the trial participants. Monoclonal antibodies are a relatively new type of biological drug although there are a number of them already licensed and in use. However, TGN1412 is a new class of monoclonal antibody which has a stimulatory mode of action affecting certain types of cell in the immune system. In this case the resulting activity seen in humans was not predicted from apparently adequate pre-clinical testing. This is a complex scientific issue which raises important scientific and medical questions about the potential risks associated with this type of drug and how to make the transition from pre-clinical testing to trials in humans.

Footnote:

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APPENDIX 1

GCP INSPECTION OF PAREXEL - FINDINGS

As a result of the inspection the following discrepancies were identified:

1. **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.

2. **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**.**

3. **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.

4. **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. (Although events had suggested that this was the case.)

5. **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.

6. **Medical Cover;**

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

APPENDIX 2

TGN1412 PRODUCT ANALYSIS TESTING

1.0 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 Standards and 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 a UK Official Medicines Control Laboratory NIBSC were selected by MHRA to perform most of the analysis required on the product. However, tests which NIBSC could not perform were carried out at three other laboratories. XXXXXXXXXXXXXXX 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.

2.0 Samples available for testing.

2.1 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

2.2 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 No. 5646LO04.

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

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

3.0 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.

3.1 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.

Biocor (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 was contaminated with micro-organisms.

3.2 Non Company release specification tests.

Rabbit Pyrogen Test:- To confirm that product did not contain pyrogenic material.

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.

4.0 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 can found in Table 1.

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Table 1

Results of Analysis obtained and performing Laboratory

Test	Tox Trial Material ex 80L Pilot Batch Frozen Run 90695/90710 Ref Std DS 01 Ex Boehringer Ingelheim	IMP 2000L C.T Batch Refrigerated Run 100301-01 (Lot E5646LO04) Parexel CPRU	2000L C.T Batch Run 100301-01 Frozen (Lot E5646LO04) Std Material DS 02 Ex Boehringer Ingelheim	CPRU material frozen & thawed (to mimic treatment of tox material)	Placebo (Acetate Buffer) (Lot E5653LO01	Laboratory Selected
UV Assay	Not Performed	10.12mg/ml No Absorption 320 -340nm Free insoluble aggregates. Diln in saline no increase Abs @ 320 -340nm Does not aggregate when diluted	Not Performed	Not Performed		NIBSC
Endotoxins		< 0.3IU/mL				NIBSC
SDS PAGE (NIBSC In-House	Not Performed	Bands consistent with Human MAb intact IgG. + bands for half molecules IgG4	Not Performed			NIBSC
SDS-PAGE	Bands consistent with purified Human MAb intact IgG. + bands for half molecules IgG4	Bands consistent with purified Human MAb intact IgG. + bands for half molecules IgG4	Bands consistent with purified Human MAb intact IgG. + bands for half molecules IgG4	Similar to other samples– purified human IgG4		NIBSC
Isoelectric focussing	Differences in some bands with IMP product Not considered Clinically relevant	Bands consistent with Human MAb Identical to DS 02 Minor differences DS 01 Not considered clinically relevant	Identical to IMP product	Similar to other samples		NIBSC

Test	Tox Trial Material ex 80L Pilot Batch Frozen Run 90695/90710 Ref Std DS 01 Ex Boehringer Ingelheim	IMP 2000L C.T Batch Refrigerated Run 100301-01 (Lot E5646LO04) Parexel CPRU	2000L C.T Batch Run 100301-01 Frozen (Lot E5646LO04) Std Material DS 02 Ex Boehringer Ingelheim	CPRU material frozen & thawed (to mimic treatment of tox material)	Placebo (Acetate Buffer) (Lot E5653LO01	Laboratory Selected
Size Exclusion HPLC (NIBSC In-House)	Major peaks at the positions expected for purified IgG. Small amount of dimer. No evidence of larger aggregates	Profile consistent with human MAb. Small amount of dimer No evidence of larger aggregates	Major peaks at the positions expected for purified IgG. Small amount of dimer. No evidence of larger aggregates	Not Performed		NIBSC
Size Exclusion HPLC (Company)	Profile similar to final product Single peak (IgG) minor pk (IgG Dimer)	Single peak (IgG) minor pk (IgG Dimer)	Profile similar to final product Single peak (IgG) minor pk (IgG Dimer)	Not Performed		NIBSC
Cell Binding	Binds strongly to the CD28 construct used in the assay	Binds strongly to the CD28 construct used in the assay	Binds strongly to the CD28 construct used in the assay	Similar to other samples		NIBSC
Rabbit Pyrogens	Not Performed	TGN1412 passed rabbit pyrogen test with no pyrogenic response recorded	Not Performed	Not Performed		XXXXXXXXX
TVC/Sterility	Not Performed	Sterility- no growth after 14 days TVC – 0 cfu/ml each for bacteria & fungi After 14 days	Not Performed	Not Performed	Sterility- no growth after 14 days TVC – 0 cfu/ml each for bacteria & fungi After 14 days	XXXXXXXXXX

Test	Tox Trial Material ex 80L Pilot Batch Frozen Run 90695/90710 Ref Std DS 01 Ex Boehringer Ingelheim	IMP 2000L C.T Batch Refrigerated Run 100301-01 (Lot E5646LO04) Parexel CPRU	2000L C.T Batch Run 100301-01 Frozen (Lot E5646LO04) Std Material DS 02 Ex Boehringer Ingelheim	CPRU material frozen & thawed (to mimic treatment of tox material)	Placebo (Acetate Buffer) (Lot E5653LO01	Laboratory Selected
Toxicity Guinea Pig	No Evidence of Toxicity	No Evidence of Toxicity	No Evidence of Toxicity	Not Performed		XXXXXXX
Toxicity Mouse	No Evidence of Toxicity	No Evidence of Toxicity	No Evidence of Toxicity			XXXXXXX
Chemical Toxicity Screen		Negative for contaminants/poisons Including anions (Azide, Nitrite and Fluoroacetate)				FDA W/C 3/4/06

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Table 2

Clinical Samples from TGN1412 Trial Subjects in storage at NIBSC

A:- **Samples ex**Parexel CPRU

Evidence Bag Identifier	Material	Storage Temperature	Storage Area	Laboratory Notebook Reference
MPSD 40343037	Blood Samples (16)	-20°C	SB010	HAEM 0036
MPSD40343048	Bottles and Syringes containing residues of administered solutions (8)	-20°C	SB010	HAEM 0036
MPSD 40336899	Patient Samples (76)	-80°C	BT063	HAEM 0036
E6912743	Blood Samples (12)	-80°C	BT063	HAEM 0036

B:- Samples ex XXXXXXX

Evidence Bag Identifier	Material	Storage Temperature	Storage Area	Laboratory Notebook Reference
MPSB 20108332	Serum (6)	-20°C	SB010	HAEM 0036
MPSC 30311310	Serum (6)	-20°C	SB010	HAEM 0036
MPSB 20108331	Serum (6)	-20°C	SB010	HAEM 0036
MPSB 20108330	Serum (6)	-20°C	SB010	HAEM 0036
MPSC 30311307	Serum (18)	-20°C	SB010	HAEM 0036
MPSB 20108333	Serum (6)	-20°C	SB010	HAEM 0036
MPSB 20108309	Serum (17)	-20°C	SB010	HAEM 0036
MPSB 20108313	Serum (6)	-20°C	SB010	HAEM 0036
MPSB 20108312	Serum (13)	-20°C	SB010	HAEM 0036
MPSB 20108311	Serum (6)	-20°C	SB010	HAEM 0036
MPSB 20108314	Blood (12)	-20°C	SB010	HAEM 0036
MPSB 20108310	Serum (3)	-20°C	SB010	HAEM 0036
MPSB 20108320	Blood (8)	-20°C	SB010	HAEM 0036
MPSB 20108307	Serum (8)	-20°C	SB010	HAEM 0036
MPSB 20108308	Serum (1)	-20°C	SB010	HAEM 0036
C5651678	Urine (11)	-20°C	SB010	HAEM 0036
MPSC 30311309	Urine (11)	-20°C	SB010	HAEM 0036
MPSC 30311308	Urine (17)	-20°C	SB010	HAEM 0036

B:- Samples ex XXXXXXX Continued

Evidence Bag Identifier	Material	Storage Temperature	Storage Area	Laboratory Notebook Reference
MPSB 20108318 *	Blood Samples (6)	4 - 8°C	H048	HAEM 0036
MPSB 20108315 *	Blood Samples (6)	4 - 8°C	H048	HAEM 0036
MPSB 20108316	Blood Samples (6)	4 - 8°C	H048	HAEM 0036
MPSB 20108317 *	Blood Samples (11)	4 - 8°C	H048	HAEM 0036
MPSB 20108319	Blood Samples (17)	4 - 8°C	H048	HAEM 0036

NB Samples marked * were received at, and initially stored at 4 - 8°C to ensure suitable preservation of the material for further analysis. Samples were centrifuged for 5 minutes at 2,500 RPM, the supernatant solution was removed and saved in sterile tubes, previously labelled with the same original identification numbers. These samples were then stored at -20°C in storage area SB010.