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information disclosed by the MHRA under the terms of the FOI Act 2000

(NB: pages start @ page 2. There is no page 1)

LIST OF TABLES

✳ Information withheld under section 43(2)
of the FOI Act, as in our MHRA's view,
disclosure would, or would be likely to prejudice the
commercial interests of TeGenero or associated third parties,
with no overriding public interest in disclosure.

LIST OF FIGURES

GLOSSARY OF TERMS

Term	Explanation
ADCC	antibody-dependent cell-mediated cytotoxicity
BP	British Pharmacopoeia
BSA	Bovine Serum Albumin
CBB	Coomassie Brilliant Blue
CCF	Cell free culture fluid
CDR	complementarity determining region
cGMP	Current Good Manufacturing Practise
CV	Coefficient of variation
CW	Calendar week
DAB	Deutsches Arzneibuch
DTT	Dithiothreitol
ESI-TOF	Electrospray ionization time-of-flight
EU	Endotoxin units
EVA	ethylene-vinyl-acetate
EVOH	ethyl-vinyl-alcohol film
FCC	Food Chemicals Codex
FNU	Formazine-Nephelometric-Unit
FTM	Fluid Thioglycollate Medium
HAP	Hamster Antibody Production
HC	Heavy chain
HCP	Host cell proteins
HED	Human equivalent dose
HIC	Hydrophobic Interaction Chromatography
HP	High performance
HPLC	High Performance Liquid Chromatography
HRPO	Horseradish peroxidase
IEF	IsoelectricFocusing
ICOS	Inducible-Costimulator
Ip	Isoelectric point
IV	Intravenous
LAL	Limulus Amebocyte Lysate
LC	Light chain
LOD	Limit of detection
mOsm	Milliosmole
MRSD	Maximum recommended starting dose
MTX	Methotrexate

MuLV	Murine leukemia virus
MW	Molecular Weight
NLT	Not less than
NMT	Not more than
OPD	O-Phenylenediamine
PBMC	peripheral blood mononuclear cells
pI	Isoelectric point
ppm	Part per million
PPV	Porcine Parvo Virus
Reo-3	Reovirus Type 3
RT	Reverse Transcriptase
RU	Resonance unit
SCB	Safety cell bank
SE	Size exclusion
SIP	Steaming-In-Place
SOP	Standards operating procedure
SPR	Surface plasmon resonance
SSB	Single stranded DNA binding Protein from E. coli
TCR	T cell receptor
TSB	Trypticase Soy Broth
UV	Ultraviolet
WFI	Water for injection

INTRODUCTION

Paragraph withheld under section 43(2) of the FOIAct as, in the MHRA's view, disclose would, or would be likely to, prejudice the commercial interests of TeGenero or associated third parties. Nor does the MHRA consider that the public interest in disclosure outweighs the public interest in maintaining the confidence.

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 will be 10 mg/ml, filled in vials as 400 mg/40 ml. The container is a 50 ml injection vial.

In humans, CD28 is expressed on T cells and it is the most efficient CD receptor that co-stimulates naïve T cells in combination with the T cell receptor (TCR) (Riley and June, 2005). Activation of the CD28 signalling pathway naturally 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.

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 (Dennehy et al., 2003; Luhder et al., 2003).

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 recognize an epitope close to the binding site for the natural ligands, and that this specificity closely correlates with the agonistic activity of anti-CD28 antibodies (Luhder et al., 2003). 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 analyses (Evans et al., 2005).
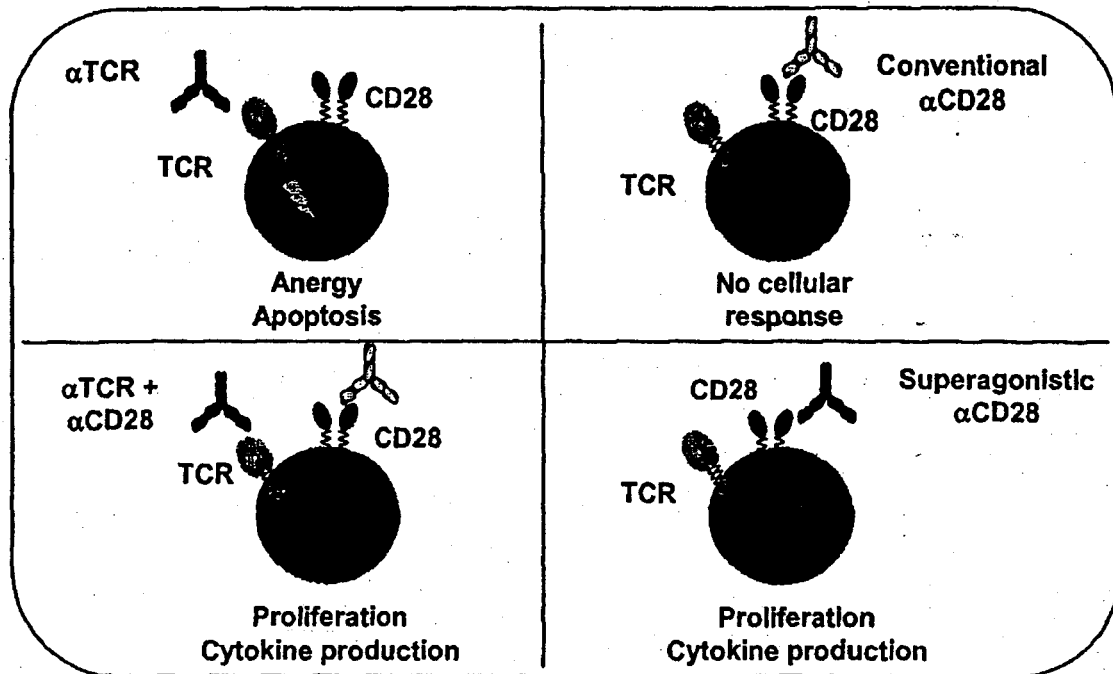
Figure 1: Activation of human T cells in the absence of TCR stimulation

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.

TGN1412 is being developed the treatment of T cell mediated autoimmune diseases and haematological malignancies characterised by functional and qualitative T-lymphocyte deficiency. Based on the underlying characteristic mode of action of this antibody, these two approaches are considered not to be mutually exclusive. In this respect, TGN1412 is different from other mitogenic or T-lymphocyte specific monoclonal antibodies.

The rationale for development of TGN1412 for haematological malignancies is 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 induces both polyclonal T-cell expansion and activation. Furthermore, TGN1412 was shown to indirectly improve deficient antigen-presentation by B-CLL cells (section 2.2.1.2, TeGenero draft study report █████████████████)

The postulated immunomodulatory (anti-inflammatory) properties of TGN1412 are related to its unique 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 rheumatoid arthritis (Section 2.2.1.2), rat experimental autoimmune neuritis (EAN) (Schmidt et al., 2003) and rat experimental autoimmune encephalomyelitis (EAE) (Beyersdorf et al., 2005).

Recent evidence shows that these two mechanisms are mechanistically interdependent and constitute an immunological loop that ensures T-lymphocyte tolerance in the presence of T-lymphocyte competence.

The pre-clinical data package for the agonistic anti-CD28 monoclonal antibody TGN1412 as submitted with the CTA has been developed in accordance with the regulatory requirements outlined in ICH S6 and 286/95 (ICH M3). For a detailed pre-clinical overview of TGN1412 safety and pharmacology studies, please refer to Appendix (section 2.4, Table 1). At this stage of development, the strategy for TGN1412 focusses on both therapeutic approaches (hemato-oncological malignancies as well as autoimmune diseases) co-equally. Therefore, the proposed first in man trial is designed to deliver key information about the immunological mechanism of action in humans.

2.1   QUALITY DATA

2.1.S   DRUG SUBSTANCE

2.1.S.1   GENERAL INFORMATION

2.1.S.1.1   Nomenclature

Antibody Type:	Humanised Anti-CD28 IgG4 monoclonal antibody
International non-proprietary name (INN):	not available at present
Chemical abstracts service (CAS):	not available at present
Company code names:	TGN1412, SM28-01

2.1.S.1.2   Structure

Section withheld under section 43 (2) of the FOI Act - see page 14.

2.1.S.1.3   General Properties

Molecular Characteristics:

✳

Physiochemical Properties

✳

Biological activity:

✳

Sections withheld under section 43(2)
of the FOI Act - see page 14.

2.1.S.2   MANUFACTURE

2.1.S.2.1   Manufacturer

TGN1412 drug substance manufacturing and Quality Control testing is performed on behalf of TeGenero AG by:

         Boehringer Ingelheim Pharma GmbH & Co KG (BIP)

         Birkendorfer Strasse 65

         88397 Biberach/Riss

         Germany

All manufacturing operations are carried out according to Good Manufacturing Practices.

The DS is released by:

[text withheld - redacted]
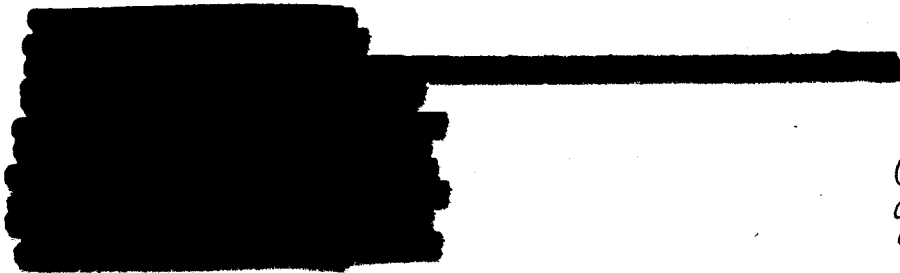
[handwritten annotation: withheld under sections 40 and 38 of the FOI Act as, in the MHRA's view, the public interest in disclosure is not outweighed by public interest in maintaining confidentiality]

2.1.S.2.2   Description of Manufacturing Process and Process Controls

[handwritten annotation: Information from this part of the section to the end on page 36 have been withheld under section 43 (2) of the FOI Act - see page 14.]

2.1.S.3   CHARACTERISATION

2.1.S.3.1   Elucidation of Structure and Other Characteristics

Information from here to end of section on page 45 has been withheld under section 43 (2) of the FOI Act – see page 14.

2.1.S.3.2   Impurities

Information in this section (which continues to page 49 (part) has been withheld under section 43(2) of the FOI Act - see page 14.

2.1.S.4   CONTROL OF DRUG SUBSTANCE

2.1.S.4.1   Specification

Information in this section (only the rest of this page) has been withheld under section 43 (2) of the FOI Act - see page 14.

2.1.S.4.2   Analytical Procedures

Information in this section (which covers pages 50 to 54 inc) has been withheld under section 43 (2) of the FoI Act — see page 14.

2.1.S.4.4   Batch Analyses

Information in this section (which covers pages 55 to 59(part)) has been withheld under section 43(2) of the FOI Act - see page 14

2.1.S.4.5   Justification of Specification

information in this section (which extends
to page 60 (part) has been withheld
under section 43 (2) of the FoI Act —
see page 14.

2.1.S.5   REFERENCE STANDARDS OR MATERIALS

Information in this section (which covers rest of this page to page 63 (part)) has been withheld under section 43 (2) of the FOI Act - see page 14.

2.1.S.6   CONTAINER CLOSURE SYSTEM

TGN1412 drug substance is dispensed into sterile ethylene-vinyl-acetate (EVA) Bio-Process Containers (BPC). These containers are sterilized using gamma irradiation <25 kGy. The container material consists of a 5-layer film, which utilizes a gas barrier ethyl-vinyl-alcohol film (EVOH) in the middle and a pure polymer (EVA) as the inside contact layer. The EVOH provides protection by minimizing gas transmission across the film.

The BPCs are manufactured using no animal derived components. Each BPC is individually labelled with compound name, batch number, date of filling, and filling volume. Storage of drug substance in the described containers is supported by stability study data provided in Section 2.1.S.7.

2.1.S.7 STABILITY

Information in this section (which extends to page 67 inc) is withheld under section 43 (2) of the FOI Act - see page 14.

2.1.P   MEDICINAL PRODUCT

2.1.P.1   DESCRIPTION AND COMPOSITION OF THE MEDICINAL PRODUCT

2.1.P.1.1   Description

Information in this paragraph withheld under Section 43(2) of the FOI Act — see page 14.

2.1.P.1.2   Composition

The unit vial composition is described in Table 32 below.

Table 32:   TGN1412 IV Injection Formulation, Protein Strength 10 mg/ml

Components	Concentration [mmol/L]	Concentration [g/L]	Nominal Amount [mg/vial] V = 40.0 ml
CH$_3$COONa x 3 H$_2$O	▆▆	▆▆	▆▆
NaCl	▆▆	▆▆	▆▆
Tween 20	▆▆	▆▆	▆▆
CH3COOH	▆▆	▆▆	—
TGN1412	▆▆	▆▆	▆▆
Water for injection (WFI)	▆▆	▆▆	▆▆

✳

MW (CH$_3$COONa x 3 H$_2$O) = 136.06 g/mol, MW (NaCl) = 58.44 g/mol, MW (TGN1412) = 150 kD.

To ensure that 40 ml can be withdrawn from the vials, an overfill of 0.8 ml is applied. Thus the vials are filled with 41.3 ml ± 0.5 ml.

✳ withheld under section 43(2) of the FOI Act — see page 14

2.1.P.2   PHARMACEUTICAL DEVELOPMENT

2.1.P.2.1   Components of the Medicinal Product

Information withheld under section 43 (2) of the FOI Act - see page 14

2.1.P.2.2   Medicinal Product

2.1.P.2.2.1   Formulation Development

Information withheld under section 43 (2) of the FOI Act - see page 14.

2.1.P.2.2.2   Overages

No overages are applied.

2.1.P.2.3   Manufacturing Process Development

Standard procedures were used for fill and finish. Please refer to Section 2.1.P.3.3.

2.1.P.2.4   Container Closure System

TGN1412 IV injection will be presented in:

Vial: 50 ml Type I clear glass vial, Ph. Eur. quality

Stopper: Rubber formulation D777-1, siliconized, Teflon-coated

Seal: Aluminium flip-off cap

2.1.P.2.5   Microbiological Attributes

The drug product is filled under aseptic conditions. Drug Product is tested for sterility and endotoxins as part of the release testing (see Section 2.1.P.5).

2.1.P.2.6   Compatibility

A compatibility study investigating the appropriateness of the application system is ongoing and will be completed before start of the clinical program.

2.1.P.3   MANUFACTURE

2.1.P.3.1   Manufacturer

TGN1412 drug product manufacturing and Quality Control is performed on behalf of TeGenero AG by:

Boehringer Ingelheim Pharma GmbH & Co KG

Birkendorfer Strasse 65

88397 Biberach/Riss

Germany

All manufacturing operations are carried out according to Good Manufacturing Practices.

Information witheld under sections 40 and 38 s)are for Act - see page 19.

2.1.P.3.2   Batch Formula

The batch size for TGN1412 IV drug product is approximately 1000 vials. The drug product composition is equivalent to that of the purified bulk drug substance. Manufacture of drug product consists of filling vials under aseptic conditions to the appropriate volume. Changes in the batch size may occur based on manufacturing and clinical needs. The drug product composition is described in 2.1.P.1.

2.1.P.3.3 Description of Manufacturing Process and Process Controls

Information in this section (which extends to page 72 (part)) is withheld under section 43(2) of the FOI Act – see page 14.

2.1.P.3.4   Controls of Critical Steps and Intermediates

Information in this section (which extends to end of this page) is being withheld under Section 43 (2) of the FOI Act — see page 14.

2.1.P.3.5   Process Validation and/or Evaluation

Information being withheld under section 43(2) of the FOI Act - see page 14.

2.1.P.4   CONTROL OF EXCIPIENTS

2.1.P.4.1   Specifications

Information being withheld under section 43(2) of the FOI Act - see page 14.

2.1.P.4.2   Analytical Procedures

All excipients are tested according to current Ph. Eur. quality standards.

2.1.P.4.3   Validation of Analytical Procedures

Analytical testing is performed in accordance with current Ph. Eur.. Therefore validation of assays is not applicable.

2.1.P.4.4   Justification of Specifications

The excipient specifications are justified based on quality standards of the current Ph.Eur..

2.1.P.4.5   Excipients of Human or Animal Origin

Excipients of human or animal origin are not used as excipients for TGN1412 drug product.

2.1.P.4.6   Novel Excipients

No novel excipients are used for TGN1412 drug product formulation.

2.1.P.5   CONTROL OF MEDICINAL PRODUCT

2.1.P.5.1   Specification

Information in this section (which
extends to page 89 (part)) has been
withheld under section 43 (2) of
the FOI Act – see page 14.

2.1.P.6 REFERENCE STANDARDS OR MATERIALS

See section 2.1.S.5.

2.1.P.7 CONTAINER CLOSURE SYSTEM

TGN1412 IV injection will be presented in:

Vial: 50 ml Type I clear glass vial, Ph. Eur. quality

Stopper: Rubber formulation D777-1, siliconized, Teflon-coated

Seal: Aluminium flip-off cap

2.1.P.8 STABILITY

2.1.P.8.1 Stability Program

Information in this section (which extends to page 97 inc) is withheld under section 43 (2) of the FOI Act - see page 14.

2.1.Q   OTHER INFORMATION

2.1.Q.1   PLACEBO

2.1.Q.1.P.1   Description and Composition of the Medicinal Product

Information in this section (which extends to page 100 inc) is being withheld under section 43 (2) of the FOI Act — see page 14.

2.1.Q.1.P.6   Reference Standards or Materials

Not applicable

2.1.Q.1.P.7   Container Closure System

For TGN1412 Placebo the same container closure system is used as for TGN1412 Drug Product:

Vial: 50 ml Type I clear glass vial, Ph. Eur. quality

Stopper: Rubber formulation D777-1, siliconized, Teflon-coated

Seal: Aluminium flip-off cap

2.1.Q.1.P.8   Stability

The composition of the placebo is identical to the drug product with the exception that no drug substance is present in the placebo. Due to this fact, at this stage of development the assigned shelf life of the placebo is deduced from the stability data of the drug product. Hence, a shelf life of 13 months was assigned and the shelf life will be prolonged if justified by future stability data of the drug product.

2.1.A   APPENDICES

2.1.A.1   FACILITIES AND EQUIPMENT

TGN1412 drug substance and drug product are manufactured at Boehringer Ingelheim Pharma GmbH & Co KG (BI) on the Biberach campus. The campus is a multi-purpose facility with buildings for production including fermentation, purification, formulation and aseptic filling, microbiology and quality control as well as storage and warehouse areas. Manufacturing facilities used for commercial production are licensed in the US (license No. 1251), Canada (license No. 228) and registered in the EU (No. HR A 2206). It is being inspected by the relevant authorities regularly.

Currently TGN1412 batches are produced in building G85 (DS) and G55 (DP). Areas and rooms adjacent to the production areas are considered of no concern regarding the integrity of the product, as no highly potent products, e.g. cytotoxics or hazardous substances are handled therein.

Quality control and in-process control testing is performed in multiple buildings on the BI campus, including buildings H102, H104, G101, G61, and P108.

All areas used for manufacture of the bulk drug substance and drug product are designed for ease of cleaning and sanitation. The floor plan is designed to facilitate efficient movement and control of components, personnel, product, and equipment. Adequate floor space has been provided within the facility to ensure segregation of materials and separation of unit operations to prevent cross contamination.

Equipment used for the manufacture of TGN1412 drug product is considered state-of-the-art and is constructed of suitable materials.

SOPs are in place for each type of equipment cleaning operation. Equipment used for multiple products undergoes product changeover before use in a subsequent campaign. The cleaning procedures are validated according to a cleaning validation protocol, which demonstrated adequate removal of residues. Equipment is sterilised prior to use by Steaming-In-Place (SIP) or sterilisation in an autoclave, as appropriate. Equipment sterilisation procedures are validated.

All functional areas in the buildings involved in the manufacture of Drug Product in the aseptic filling rooms are physically segregated from other areas and supplied by a dedicated air handling system working in a recirculation mode. Air pressures in Drug Product production buildings are monitored within each functional area to assure the integrity of critical processing areas.

2.1.A.2   ADVENTITIOUS AGENTS SAFETY EVALUATION

information in this section - which extends to page 104 inc - is being withheld under section 43(2) of the FOI Act - see page 14.

EUROPEAN DIRECTORATE FOR
THE QUALITY OF MEDICINES

CERTIFICATION OF SUITABILITY OF MONOGRAPHS
OF THE EUROPEAN PHARMACOPOEIA

Certificate No. R0-CEP 2001-051-Rev 00

1 *Name of the substance:*
2 BOVINE ALBUMIN
3 (Code Number 84-022)

4 *Name of holder:*
5
6
7

8 *Site of production:*
9
10
11

12 After examination of the information provided on the origin of raw material(s) and type of
13 tissue(s) used and on the manufacturing process for this substance on the site of production
14 mentioned above, USA - IL 60901 Kankakee, we certify that the substance BOVINE ALBUMIN
15 meets the criteria described in the monograph Products with risk of transmitting agents of animal
16 spongiform encephalopathies (no. 1483, Ph. Eur. 4th Ed. and any subsequently revised version).

17 - country of origin: United States of America.

18 - nature of animal tissues used in the manufacture: Bovine blood

19 The submitted dossier must be updated every five years or after any significant modification of
20 the manufacturing method, the country(ies) of origin or the nature of the tissues used that may
21 alter the risk of transmitting animal spongiform encephalopathy agents or require changing the
22 specifications of the monograph.

23 Manufacture of the substance shall take place in accordance with a suitable quality assurance
24 system such as ISO 9002, and in accordance with the dossier submitted.

25 Failure to comply with these provisions will render this certificate void.

26 The certificate is valid provided that there has been no deterioration in the TSE status of the
27 country(ies) of origin of the source material.

Postal Address: 226 Avenue de Colmar (entrance rue Schertz) B.P. 907 — F 67029 Strasbourg Cedex 1
Telephone: 03.88.41.23 47 · Fax 03.88.41.27.71 · E-mail: certification@pheur.org

Figure 31:   **TSE certificate for BSA used for MCB production**

To ███████████   Company/Institute   Boehringer Ingelheim

Date   November 18, 2004

*From ███████████   Total Pages   1

re   Compliance with EMEA/410/01 Rev.2 Section 6.?

This letter is to confirm that the sheep wool-derived cholesterol used in formulation 92-0239 complies with the above referenced Note for Guidance, as the wool is taken from live sheep.

For information on the cholesterol processing, please see the Certificate of Origin for the raw material (number 135-8006).

Best regards,

Information withheld under sections 38 and 40(1) the FOI Act - see page 19

If you did not receive the total number of pages indicated, or if there is a problem with this FAX transmission, please call Technical Services.

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Figure 32:   TSE compliance certificate for cholesterol (Page 1)

28 This certificate is granted within the framework of the procedure established by the European
29 Pharmacopoeia Commission [Resolution AP-CSP (93) 5 as amended] for a period of five years
30 starting from 26 November 2001. Moreover, it is granted according to the provisions of
31 Directive 75/318/EEC amended and Directive 81/852/EEC amended, and the related guidelines.

32 This certificate has 32 lines only.

Strasbourg, 26 November 2001

DECLARATION OF ACCESS (to be filled in by the certificate holder under their own responsibility)

..... as holder of the certificate of suitability

R0-CEP 2001-051-Rev 00 for BOVINE ALBUMIN

hereby authorises .... Boehringer Ingelheim Pharma KG
(name of the pharmaceutical company)

to use the above-mentioned certificate of suitability in support of their application(s) for the following Marketing
Authorisation(s): (name of product(s) and marketing number(s), if known)

Date and Signature

Postal Address: 226 Avenue de Colmar (entrance rue Schertz) B.P. 907 – F 67029 Strasbourg Cedex 1
Telephone: 03.88.41.29.47 - Fax 03.88.41.27.71 - E-mail: certification@pheur.org

Information withheld under sections 38 and 40 of the FOI Act - see page 19.

Figure 33: TSE compliance certificate for cholesterol (Page 2)

Certificate of Origin

Product: Cholesterol

Raw Material Number: 835-8006

Manufacturing Information:
Site: This raw material is manufactured in Singapore

Raw material information:
All wool grease is derived from wool taken from NZ/Australian live sheep. The wool grease is converted to cholesterol by the following process:

1. Wool grease to lanolin anhydrous

   a. Wool grease is bleached and treated with sodium hydroxide to eliminate free fatty acids at temperatures of 70-85°C for five hours.

   b. Solvated at 85°C for one hour

   c. Deodorized with steam at 150-170°C for 10 minutes.

2. Lanolin anhydrous to lanolin alcohol

   a. Treated with sodium hydroxide at 180°C for one hour with pressure of 10kg/cm.

   b. Extracted with organic solvent and further deodorized with steam at 150°C.

3. Lanolin alcohol to cholesterol

   a. Extracted with organic solvent at 100°C (10 hours).

   b. Recrystallized.

   c. Dried at high temperatures for one hour.

Please note: The wool grease is the only material of animal origin used in the process.

Our vendor can only trace their lots back to the manufacturer in Singapore. There is no traceability from Singapore to a particular shipment of wool grease (or a particular export certificate) from New Zealand. There is no Certificate of Suitability for this material.

This document was prepared by:

Date: November 4, 2004

Information withheld vnder sections 38 and 40 d the FOI Act - see page 19.

Figure 34: **Certificate of origin for cholesterol**

2.1.A.3   NOVEL EXCIPIENTS

Not applicable

2.1.A.4   SOLVENTS FOR RECONSTITUTION AND DILUENTS

Not applicable

2.2   NON-CLINICAL PHARMACOLOGY AND TOXICOLOGY DATA

2.2.1   PHARMACODYNAMICS

2.2.1.1   BRIEF SUMMARY

TGN1412 is a humanized agonistic IgG4 antibody directed against the human CD28 antigen expressed on T lymphocytes (T cells). In addition to TGN1412, alternative agonistic anti-CD28 antibody formats have been used in pre-clinical pharmacodynamic studies (Figure 35). The most prominent antibody format for pre-clincial evaluation of agonistic anti-CD28 antibody mode-of-action is the mouse-anti-rat CD28 monoclonal antibody JJ316 (Tacke et al, 1997). Alternatively to TGN1412, an IgG1 variant with an identical variable domain structure (TGN1112) has been used in pharmacology studies in rhesus monkeys and with human cells. Comparability of homologous agonistic anti-CD28 antibody formats has been demonstrated by antigen binding and functional assays. The terms "agonistic" as used throughout this document and "superagonistic" as used in references and publications describe the profound and TCR independent stimulation of T cells and are to be used synonymously.
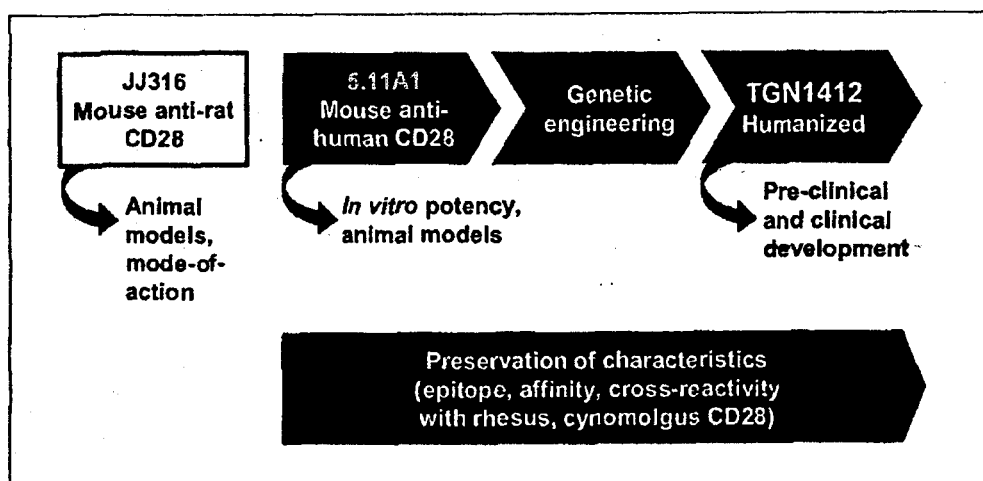
Figure 35:   Use of homologous anti-CD28 antibody formats in pre-clinical studies

The (super-) agonistic principle has been initially described for the anti-rat CD28 specific antibody JJ316. Monoclonal antibody 5.11A1 represents the first anti-human CD28 antibody with agonistic properties. Based on the 5.11A1 sequence, fully humanized anti-CD28 antibody TGN1412 was generated by genetic engineering.

Pre-clinical pharmacology studies have provided evidence that agonistic anti-CD28 antibodies are capable of activating T lymphocytes solely by engaging the co-stimulatory receptor CD28, i.e. independently of stimulation of the T cell antigen receptor (TCR) (Tacke et al., 1997; Luhder et al., 2003; Dennehy et al., 2003). Structural analysis of the CD28 molecule has revealed that agonistic anti-CD28 antibodies bind exclusively to the laterally exposed C″D loop of the immunoglobulin-like domain of CD28 whereas conventional, co-stimulatory antibodies

recognize an epitope close to the binding site for the natural CD80/CD86 ligands (Luhder et al., 2003; Evans et al., 2005).

The T cell activating capacity of TGN1412 was established in *in-vitro* proliferation assays using total peripheral blood mononuclear cells (PBMC) from healthy donors. Co-incubation of PBMC with soluble TGN1412 resulted in a profound, polyclonal T cell proliferation, while conventional, co-stimulatory CD28 specific antibodies were unable to induce substantial cellular proliferation. In further ex vivo experiments, it was demonstrated that TGN1412 induces a profound activation and proliferation of highly purified T cell subsets including CD4 and CD8 T cells, naïve and memory T cells and, significantly, regulatory T cells.

Since F(ab)$_2$ fragments of agonistic anti-CD28 antibodies were not capable to induce a proliferative T-cell response, an intact Fc-region appears to be required for TGN1412 biological activity. Experiments with highly purified T cells and Fc-receptor binding studies underlined the notion that cross-linking via Fc-receptor(s) is required for efficient TGN1412-mediated triggering of T cells.

Complementing the above-mentioned ex vivo studies with primary human T cells, specific *in vitro* and *in vivo* pharmacodynamic studies have been conducted to support the rationale for the development of TGN1412 for the treatment of B-CLL and RA.

Since B-CLL is characterised by a profound functional deficiency of normal T and B cells (Cantwell et al., 1997; Wierda et al., 2000), novel therapeutic approaches aim at immunoreconstitution of the patients immune system, particularly following initial therapy with T-cell depleting agents. In ex-vivo studies with primary PBMC from a broad spectrum of B-CLL donors, it could be demonstrated, that TGN1412 is capable of inducing T-cell expansion and activation, irrespectively of disease stadium and/or concomitant or prior therapy. It was further shown that TGN1412 has the potential to improve antigen-presentation by leukaemic cells and consequently to induce an efficient anti-tumoral T-cell response.

In vivo, functional T cell reconstitution by agonistic anti-CD28 antibody treatment was demonstrated in lethally irradiated, bone marrow reconstituted hosts (Elflein et al., 2003). Significantly, TGN1412 and its IgG1 variant TGN1112 have been shown to induce a transient and well-tolerated T cell expansion in non-human primates. Therefore, it is assumed that TGN1412 has the potential to add significant benefit to B-CLL patients by immunoreconstitution (Application for Designation as Orphan Medicinal Product, 2004).

In autoimmune diseases such as RA, the lack of control of autoreactive T cells by regulatory T cells and the secretion of pro-inflammatory cytokines have been described to play a major role in the pathomechanism of the disease (Sakaguchi et al., 2000; Viglietta et al., 2004; Ehrenstein et al., 2004). *In vitro*, *ex-vivo* and *in vivo* pharmacodynamic studies with TGN1412 and its orthologues indicate that TGN1412 has the potential to add a significant benefit in the treatment of autoimmune disease by induction of anti-inflammatory cytokines, the induction of inhibitory T cell surface molecules, and particularly the control of autoreactive T cells by regulatory T cells (Rodriguez-Palmero et al., 1999; Lin et al., 2003; Beyersdorf et al., 2005b).

Accordingly, agonistic anti-CD28 antibodies were effective in rodent and a non-human primate models of autoimmune diseases of collagen-induced arthritis, in rat experimental autoimmune neuritis (EAN) (Schmidt et al., 2003) and rat experimental autoimmune encephalomyelitis (EAE) (Beyersdorf et al., 2005a). Therefore, pre-clinical evidence suggests that TGN1412 has the potential to add a significant benefit to RA patients by direct interference with proposed patho-mechanisms of T-cell mediated autoimmune diseases.

TGN1412 mediated expansion of conventional and regulatory T-cells is proposed to be mechanistically interdependent and to constitute an immunological loop that ensures T-lymphocyte tolerance in the presence of T-lymphocyte competence. Based on the *in vivo* observations that (a) a TH1 response supporting cellular immunity is maintained when an agonistic anti-CD28 antibody is given to immuno-compromised rats (Elflein et al., 2003) and that (b) an effective anti-inflammatory response is induced concomitant to a generalized CD4 T cell expansion (Beyersdorf et al., 2005a), it is expected that both therapeutic approaches in B-CLL and RA are not per se mutually exclusive.

2.2.1.2   PRIMARY PHARMACODYNAMICS

In vitro studies

Specificity of TGN1412 for human CD28

CD28 has been identified as a cell surface receptor specifically expressed on T cells (Hara, 1985). Specificity of TGN1412 for human CD28 has been shown in various assay systems including flow cytometry and Biacore analysis (TeGenero study report ███████████ TGN1412 does not cross-react with the closely related receptors Cytotoxic T-Lymphocyte-Antigen-4 (CTLA-4) and Inducible Costimulator (ICOS) (Table 62; TeGenero study report ███████████

*[handwritten note: Information marked with * above plus Information in the rest of this section (which extends to page 121 (part)) is withheld under section 43 (2) of the FOI Act - see page 14.]*

2.2.1.3   SECONDARY PHARMACODYNAMICS

In vitro cytotoxicity studies of TGN1112/TGN1412 (TGN01-NC-008)

To assess the cellular cytotoxicity of TGN1412, standard *in vitro* Complement Dependent Cytotoxicity (CDC) and Antibody Cellular Cytotoxicity (ADCC) assays were performed. The depleting anti-CD52 monoclonal antibody alemtuzumab and an IgG1 variant of TGN1412, TGN1112, were used as control.

As compared to the control antibodies, TGN1412 did mediate neither CDC nor ADCC. The results are summarized in Table 64.

Table 64:   **Summary of cytotoxicity assays**

antibody	CDC (huPBMC)	ADCC	ADCC
		(Jurkat CD28+CD52+)	(Jurkat CD28+CD52-)
Alemtuzumab	+	+	-
TGN1112	-	+	+
TGN1412	-	-	-

2.2.1.4   SAFETY PHARMACOLOGY

Safety pharmacology comprises a number of categories of tests and procedures which are intended to provide an assessment of the pharmacological profile of a novel drug in areas other than the intended therapeutic use. Usually, unintended effects on the central nervous system (CNS), cardiovascular system (CV) and respiratory system (RS) are investigated. Since TGN1412 is highly specific for primate CD28 (see section 2.2.1.2), these studies cannot be

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meaningfully conducted in species other than non-human primates and have therefore not been performed. Due to the fact that no cross-reactivity with cardiovascular tissue has been observed for TGN1412 ███████████████████████, a telemetry study in cynomolgus monkeys is not ✳ considered to be reasonable at this stage of development and that it is sufficient to evaluate safety pharmacology endpoints as part of toxicology and pharmacology studies. A short summary of the safety pharmacology assessment derived from toxicology studies are presented below:

Cardiovascular system (CV)

As described above, TGN1412 does not cross react with cynomolgus monkey or human heart tissue ██████████████████████. Effects on the cardiovascular system were evaluated in a ✳ 28-day repeated dose toxicology study in cynomolgus monkeys ████████████████████████████ Eight male and 8 female monkeys received TGN1412 at dose levels of 5 or 50 mg/kg by slow intravenous infusion over a one-hour period, on 4 occasions with a 7 day recovery period between each administration. Dose volumes of 1 or 10 ml/kg body weight were used respectively. No electrocardiogram changes (heart rate, P-R interval, QRS interval and Q-T interval) were observed. No toxicologically significant differences in histology findings were observed in cardiovascular tissues (aorta, heart) between control and treatment group animals. Therefore, it is concluded that treatment with TGN1412 is not expected to adversely affect the cardiovascular system in humans.

Respiratory system (RS)

In cross reactivity studies with cynomolgus and human tissues, it was observed that TGN1412 binds to lymphocytes in lung tissues, in accordance with the target antigen (CD28) distribution. Although no specific functional assessment of the respiratory system has been performed as part of the 28-day toxicology study, no clinical observations were made that would support an unintended effect of TGN1412 on the respiratory system. No treatment related necropsy and histology findings were reported for the respiratory system (trachea, lung). Therefore, it is concluded that treatment with TGN1412 is not expected to adversely affect the respiratory system in humans.

Central nervous system (CNS)

Specific fibrillary staining with TGN1412, considered to represent astrocyte staining, was seen in the brain (cerebrum, cerebellum), spinal cord and pituitary gland of both human and cynomolgus monkey donors. This cross reactivity with CNS tissue may not be of major clinical relevance, since no CNS related observation were reported during toxicology studies, including the 28-day repeated dose toxicology study in cynomolgus monkeys ████████████████████ ✳ ███████ In addition, no histology findings were observed in nervous tissues (eye, brain, optic nerve, sciatic nerve) that could be attributed to treatment with TGN1412. Moreover, as an IgG monoclonal antibody, TGN1412 is not expected to pass the blood-brain barrier under physiological conditions in man. Therefore, it is concluded that treatment with TGN1412 is not expected to adversely affect the central nervous system in humans.

✳ information withheld under Section 43(2)
of the FOI Act - see page 14.

2.2.1.5   PHARMACODYNAMIC INTERACTIONS

Methotrexate (MTX) constitutes the most commonly used DMARD and it has been published that MTX induces apoptosis of activated peripheral T cells (Genestier, 1998; Paillot, 1998). Therefore, the effect of this anti-proliferative and pro-apoptotic DMARD on TGN1412 *in vitro* T cell activating efficacy was tested on resting PBMC or cells pre-stimulated with TGN1412 (TeGenero study report ███████████ Under no culture condition studied and irrespective of the concentration range of this DMARD did MTX interfere with TGN1412-induced T cell proliferation. As MTX did obviously not interfere pharmacodynamically with TGN1412, it has implications for the design of future clinical trials as it indicates that the use of TGN1412 can be combined with MTX administration.

2.2.1.6   DISCUSSION AND CONCLUSION

Information under this section
(which extends to page 125 inc)
is withheld under section 43(2)
of the FOI Act - see page 14.

* information withheld under
section 43(2) of the FOI
Act - see page 14.

2.2.2 PHARMACOKINETICS

2.2.2.1 BRIEF SUMMARY

For protein-based biotechnology derived medicinal products, classical ADME studies are considered not to be required, because pathways of protein degradation are common knowledge. It is assumed that TGN1412, like other antibodies, is catabolized by lysosomal enzymes in the kidney and/or liver into amino acids, which are then reabsorbed. Thus, conventional distribution, metabolism and excretion studies are not intended to be performed for TGN1412.

In contrast to the majority of therapeutic antibodies, TGN1412 does not act directly on malignant or pathological cell/ tissue structures. Due to the mode of action of TGN1412, pharmacodynamic effects are not expected to be solely correlated to plasma/ serum concentrations of the active substance, but rather be dependent on the transient activation of T cells or certain T cell subsets in the peripheral blood and lymphoid tissues.

Serum/ plasma concentrations of surrogate agonistic anti-CD28 antibodies JJ316 and TGN1112 as well as the kinetics of T cell activation have been determined in arthritis prone rats (TeGenero study report ████████████) and in the rhesus monkey (study report ████████████. It was observed that in rats, JJ316 mediated T-cell expansion and activation appears to be faster than in non-human primates treated with either TGN1112 or TGN1412, which is also reflected by a lower estimated half-life of the agonistic anti-CD28 antibody in rats. This difference is expected, as it mirrors the general diversity (genetic background, physiology) of rodents and non-human primates. Therefore, pharmacokinetic (toxicokinetic) characteristics of TGN1412 as determined in cynomolgus monkeys are assumed to be most predictive for human PK.

Toxicokinetics of TGN1412 was assessed in the course of the pilot toxicology study ████████ ████████████and as part of the 28-day repeat-dose toxicology study performed in cynomolgus monkeys ██████████████████. TGN1412 serum concentration vs. time profiles were generally consistent with intravenous injection of a monoclonal antibody medicinal product. Since toxicokinetic profiles of treated animals were somewhat variable, mean values per dose group were calculated on the basis of bioanalytical results from the 28-day repeat dose toxicology study ██████████████████. A terminal elimination half-life of ~8 days after the first injection of 5 mg/kg was estimated for TGN1412 in cynomolgus monkeys. Systemic exposure to TGN1412 increased by up to 20-fold as doses increased from 5 to 50 mg/kg. In addition, there was evidence for increased mean terminal half-life of TGN1412 as the dose increased.

Due to its integration into toxicity testing and its bridging character between non-clinical and clinical studies, the focus of toxicokinetic studies was primarily on the interpretation of toxicity tests and not on characterising the basic pharmacokinetic parameters of the substance studied.

✱ Redacted information being withheld under Sections 38 and 43(2) of the FOI Act – See pages 14 and 19.

2.2.2.2 METHODS OF ANALYSIS

The ELISA method used to detect TGN1412 in serum samples of cynomolgus monkeys (toxicokinetic evaluation) was validated in compliance with the guideline CPMP/ICH/281/95, Note for guidance on Validation of Analytical Procedures ▓▓▓▓▓▓▓▓▓▓. In ✳ principle, TGN1412 was bound to plate-bound sheep anti-human IgG (H+L chain specific) and detected using a mouse anti-human IgG secondary antibody. Cynomolgus monkey serum from individuals and pooled samples were used as matrix for assay optimisation. Linearity was determined by diluting TGN1412 in cynomolgus monkey serum at a concentration outwith the range of the assay into the range of the assay. Intra- and inter-assay accuracy and precision was assessed. In addition, stability of TGN1412 in cynomolgus monkey serum was assessed during storage at -70°C.

The limit of detection was defined as 6 ng/ml. The limit of quantification was defined as 6 ng/ml.

Toxicokinetic parameters were estimated using WinNonlin Model 202 (intravenous infusion dose model, WinNonlin version 4.1, Pharsight Corp., Mountain View, California, USA.). A non-compartmental analysis was performed. Individual plots of the serum concentrations of TGN1412 against target time after dosing were made for each animal and each sampling day, and the toxicokinetic parameters were derived from these data accordingly.

The ELISA method to detect cynomolgus monkey IgG antibodies to TGN1412 (immunogenicity testing) was validated in compliance with the guideline CPMP/ICH/281/95, Note for guidance on Validation of Analytical Procedures ▓▓▓▓▓▓▓▓▓▓▓. In principle, antibodies to ✳ TGN1412 were bound to plate-coated Fab fragments of the anti-CD28 antibody and detected using a goat anti-human IgG Fc specific HRP conjugate. In order to define a positive primate anti-human antibody titre, six pre-immune cynomolgus serum samples were measured. Inter- and intra-assay variability has been assessed. A positive response was defined as serum with a titre of greater than 1:200. A two-fold increase should be applied to occasional samples with very high pre-dose titre to indicate a positive response.

Spiking experiments with TGN1412 have not been conducted in order to investigate potential interference of high TGN1412 serum levels with the traceability of the ELISA method. A titration of anti-TGN1412 antibodies vs. TGN1412 would only be feasible if a standardised serum sample with constant anti-TGN1412 concentration were available. Alternatively, a monoclonal antibody with specificity for TGN1412 could be used for the purpose of extended assay validation. Both methods are currently not available. TeGenero will address the issue of assay interference at a later stage of development. However, anti-TGN1412 antibodies were detected with this validated ELISA method, despite high serum concentrations of TGN1412 in cynomolgus monkeys.

2.2.2.3   ABSORPTION

TGN1412 is administered intravenously and the bioavailability is therefore considered as 100%. No studies have been performed to address absorption of TGN1412.

2.2.2.4   DISTRIBUTION

No specific studies have been performed to address the distribution of TGN1412.

2.2.2.5   METABOLISM

No studies have been performed to address the metabolism of TGN1412. It is assumed that TGN1412, like other antibodies, is catabolized by lysosomal enzymes in the kidney and/or liver into amino acids, which are then reabsorbed.

2.2.2.6   EXCRETION

No studies have been performed to address the excretion of TGN1412.

2.2.2.7   PHARMACOKINETIC DRUG INTERACTIONS

No pharmacokinetic drug interaction studies have been performed.

2.2.2.8   OTHER PHARMACOKINETIC STUDIES

Serum/ plasma concentrations of TGN1412 and its orthologues or varaints (JJ316, TGN1112) have been determined in arthritis prone rats (TeGenero study report ███████████ in the ✳ rhesus monkey (study report ███████████ and as part of the toxicology studies (toxicokinetics) in cynomolgus monkeys ███████████, respectively.

PK of JJ316 in rats

The pharmacokinetic properties of JJ316 were investigated by measurement of free antibody in serum of arthritis prone rats. Rats (n=5) were injected with 5 mg/kg antibody on the day of arthritis induction. Serum samples were taken on days 1, 3 and 6. A C_{max} of approx. ~~100 µg/ml~~ was measured at day 1 after application. JJ316 was not detectable in serum (detection limit: 10 ng/ml) seven days after injection, with an approximate half life estimate of about 2 days.

PK of TGN1112 in rhesus monkeys

Serum concentrations of TGN1112 were determined as part of the pilot safety and tolerability study in rhesus monkeys (TeGenero study report ███████████ In the rhesus monkey, a C_{max} ✳ of 140 µg/ml was measured in serum following i.v. single dose administration of TGN1112 at 5 mg/kg (Figure 39). No statistical analysis of pharmacokinetic parameters has been performed for the rhesus monkey study.

✳ see page 126.
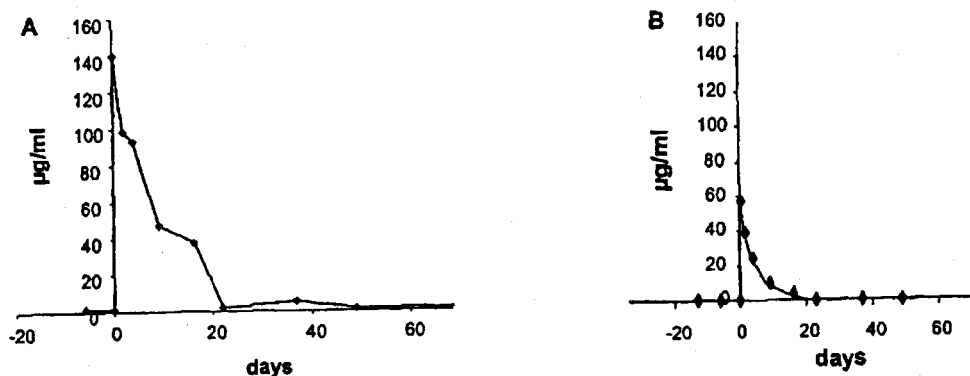
Figure 39: **Pharmacokinetics of an IgG1 variant of TGN1412 in individual rhesus monkeys**

Pharmacokinetics of a IgG1 variant of TGN1412 in the rhesus monkey after (A) a single dose application of 5 mg/kg and (B) a single dose application of 2.5 mg/kg body weight. The antibody was detectable for ~ 20 days.

Toxicokinetics of TGN1412 in cynomolgus monkeys (pilot dose escalation study)

Toxicokinetic investigations were performed as part of the single dose escalation pilot study in cynomolgus monkeys ████████████████████. TGN1412 serum concentration vs. time profiles were generally consistent with intravenous injection of a monoclonal antibody. In the two animals that received single doses of 50 mg/kg TGN1412, C_{max} values of 717 and 777 µg/ml serum were observed. T_{max} was determined to be 2 h post infusion. In serum samples of two animals following administration of four escalating doses of TGN1412 (5 – 10 – 25 – 50 mg/kg in weekly intervals), toxicokinetic analyses indicate that serum concentrations of TGN1412 at the end of the infusion period increased in proportion with the dose level in one animal (Figure 40) and supra-proportionally in the other (not shown). C_{max} values of 627 and 840 µg/ml were observed T_{max} was determined as 2 h and 5 h following infusion of 50 mg/kg TGN1412.
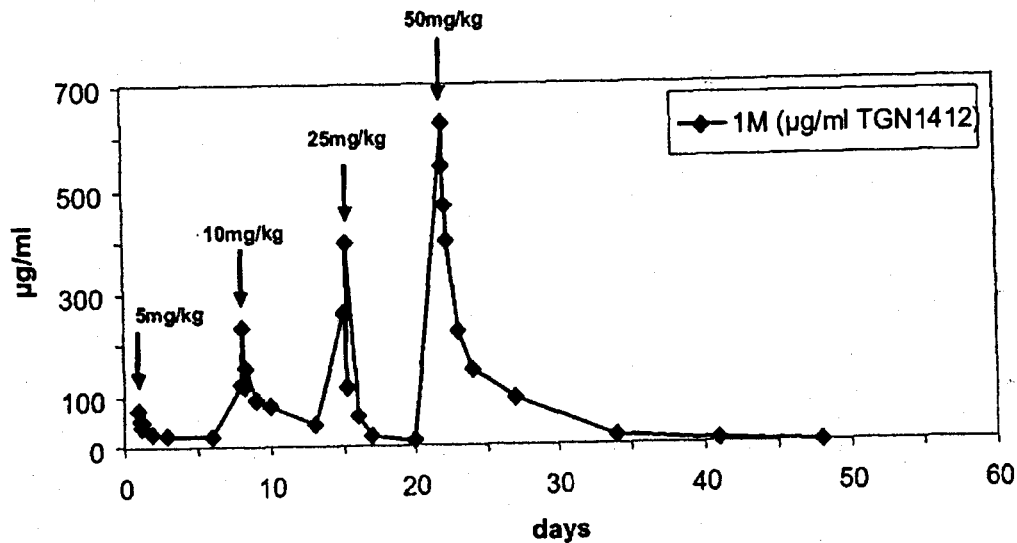
Figure 40: **Toxicokinetic evaluation in a cynomolgus monkey**

Serum concentration vs. time profile of a cynomolgus monkey following intravenous application of escalating doses (5.0 mg – 10.0 mg – 25.0 mg – 50.0 mg/kg) of TGN1412. Serum levels increased proportionally with dose.

Toxicokinetics of TGN1412 in cynomolgus monkeys (28-day repeat dose toxicology study)

Toxicokinetic investigations were also performed as part of the 28-day repeat dose toxicology study performed in cynomolgus monkeys ▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮ The toxicokinetics of TGN1412 following intravenous administration once weekly for four weeks were characterised using serum concentration vs. time data from animals allocated to treatment group 2 (low dose group = 5 mg/kg) and 3 (high dose recovery group = 50 mg/kg). The toxicokinetic parameters C_{max}(obs), T_{max}(obs), $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, Kel, $T_{\frac{1}{2}el}$, CL, Vd, Vcen and Vss were estimated using WinNonlin v4.1 pharmacokinetic software.

TGN1412 serum concentrations vs. time profiles were generally consistent with intravenous injection of a monoclonal antibody. Due to the variability of toxicokinetic profiles observed, mean values per group were calculated and are presented in Figure 41. For TK analysis, serum samples were collected until day 40 after last dosing in recovery group animals. A terminal elimination half live of approximately eight days after the first injection of 5 mg/kg was estimated for TGN1412, consistent with the relatively slow elimination of a large biological molecule such as an antibody.

※ see page 126
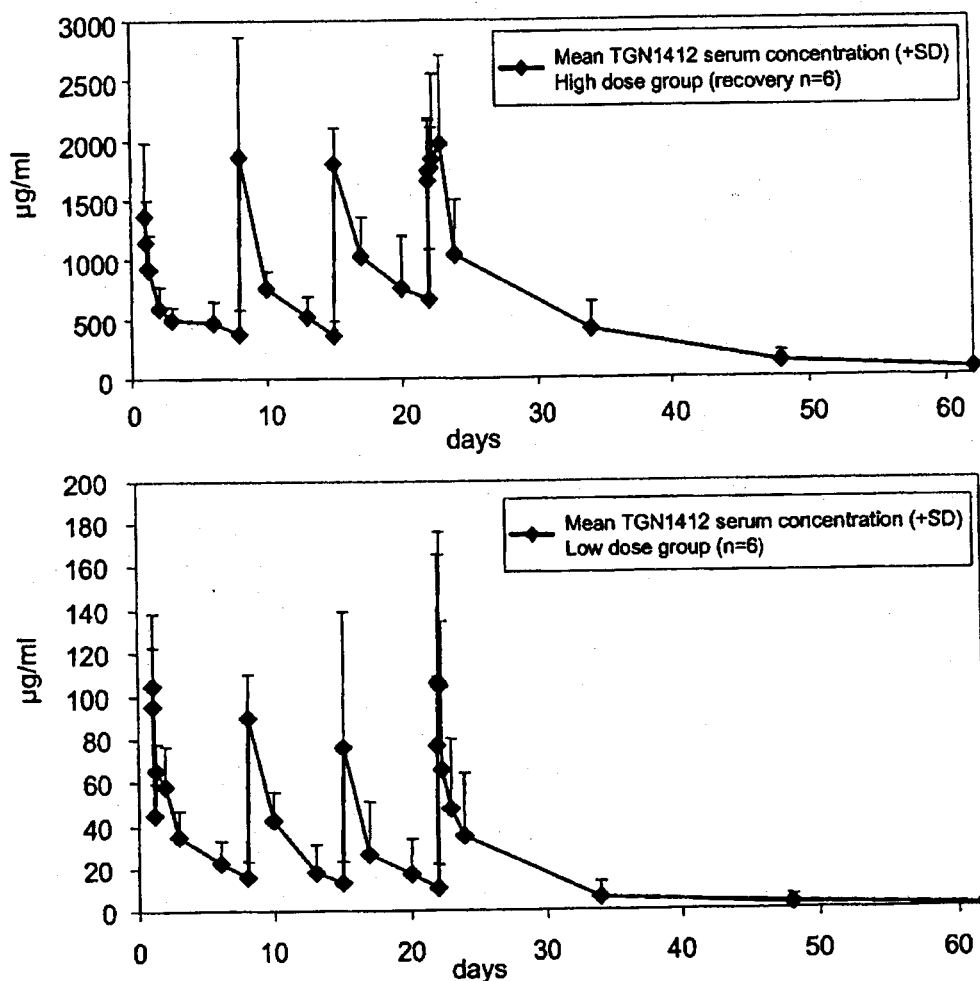
Figure 41: **Toxicokinetic profiles following repeated administration (4 doses in weekly intervals) of TGN1412**

Due to the high variability of TK data, mean values are given (n=6). Values of C_{max}, systemic exposure and values for terminal elimination half-life increased as dose increased. (low dose = 5 mg/kg; high dose = 50 mg/kg).

Systemic exposure to TGN1412 increased by up to approx. 20-fold as dose increased from 5 to 50 mg/kg (Table 4). This was also reflected in estimates of clearance (CL), which decreased as dose increased suggesting that there may be limitations in the elimination mechanisms of TGN1412 at the higher dose administered in this study. In mean estimates of the apparent volume of distribution (Vd), Vd decreased as dose increased from 5 to 50 mg/kg. When the variability associated with the mean is taken into consideration, there were no clear changes in the volume of distribution at steady state (Vss) or of the central compartment (Vcen) as dose increased from 5 to 50 mg/kg. Estimates of T_{max}(obs) also remained largely unchanged as dose of TGN1412 increased from 5 to 50 mg/kg.

No consistent sex-related differences were apparent in any of the toxicokinetic parameters determined for TGN1412. Details of the TK evaluation are provided in ██████████████ ✱
█████

Table 65: Toxicokinetic evaluation of TGN1412 after first (day 1-8) and fourth administration. Mean values for AUC and Cmax are shown.

	N	Mean AUC ($\mu g*h/ml$)		C_{max} ($\mu g/ml$)	
		Day $_{1-8}$	Day $_{1-t}$	1st dose	4th dose
5 mg/kg	6	5,364	27,354	110	125
50 mg/kg (main)	4	90,653	593,561	1,422	2,538
50 mg/kg (recovery)	6	88,495	819,634	1,462	2,235

2.2.2.9 DISCUSSION AND CONCLUSIONS INCLUDING EVALUATION OF TOXICOKINETICS

Due to the well-known pathways of protein degradation, conventional absorption, distribution, metabolism and excretion studies have not been performed and are not intended to be performed for 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 appear to be at least partly reconciled in the kinetics of *in vivo* T cell expansion induced by JJ316 (peak around day 3 post treatment; TeGenero ██████████████ ✱ Tacke et al., 1997; Lin et al., 2003) and TGN1112 (peak around day 15 post treatment, TeGenero *see page* study number ██████████ This difference is expected, as it mirrors the general diversity *126* (genetic background, physiology) of rodents and non-human primates. Therefore, pharmacokinetic (toxicokinetic) characteristics of TGN1412 as determined in cynomolgus monkeys are assumed to be most predictive for human PK.

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

In one animal (8M), relatively low serum concentrations of TGN1412 were observed, which may be attributed to the presence of anti-TGN1412 antibodies (section 2.2.3.8).

No consistent sex-related differences were apparent in any of the toxicokinetic parameters reported for TGN1412.

Systemic exposure to TGN1412 increased by up to ca 20-fold as dose increased from 5 to 50 mg/kg. 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 are not expected to be solely correlated to plasma/ serum concentrations of the active substance, but rather 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 (section 2.2.3.3). 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.

2.2.3   TOXICOLOGY

2.2.3.1   BRIEF SUMMARY

Since the TGN1412 epitope on the CD28 extracellular domain is restricted to humans and non-human primates (section 2.2.1.2), non-human primates (cynomolgus and rhesus monkeys) are considered to be the most relevant species for safety and toxicology studies to assess any potential toxicity of TGN1412 administration to humans. Tabulated summaries of toxicology studies are provided in the Appendix.

The toxicology program included GLP studies of repeat-dose toxicity (section 2.2.3.3), local tolerance (section 2.2.3.7) and immuno-histochemical investigation of cross reactivity with human and cynomolgus monkey tissues (section 2.2.3.8). Validation of analytical methods used for assessment of TGN1412 toxicokinetics and for detection of anti-TGN1412 antibodies were conducted in compliance with GLP (section 2.2.2.2). In addition, a non-GLP study was initially performed in rhesus monkeys to assess the safety and tolerability of single doses of TGN1412 and TGN1112 (section 2.2.3.2). Furthermore, a non-GLP dose range finding pilot toxicity and toxicokinetic study was performed in cynomolgus monkeys. Single doses of 2.5 and 5 mg/kg and repeat doses of 5 and 50 mg/kg had been administered in these studies. The intravenous route of administration was selected to accord with the intended application in clinical trials. Tabulated summaries of conducted toxicology studies are provided in the appendix (section 2.4, tables 2-7).

The results of these studies show that TGN1412 is well tolerated in cynomolgus monkeys at doses up to 50 mg/kg/week 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 major physiological systems (cardiovascular system, respiratory system and central nervous system) were reported for TGN1412. Therefore, 50 mg/kg is considered to be the no-observed-adverse-effect level (NOAEL).

Reproduction and developmental toxicity studies have not yet been performed. Histopathology of reproductive tract tissues has been performed as part of the 28-day toxicity study in cynomolgus monkeys. No treatment-related changes had been observed. Prior to the result of reproductive toxicity studies to be performed later in TGN1412 development, women of child-bearing age (unless sterilized) will not be included in TGN1412 clinical trials.

Due to the species specificity of TGN1412 for humans and non-human primates, the standard battery of genotoxicity and carcinogenicity testing is considered to be inappropriate and has therefore not been performed. The carcinogenic potential of TGN1412 may be assessed as part of future toxicology studies in non-human primates.

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 ███████████████████████ intravenous, perivenous, or intra- ✳ arterial routes of TGN1412 administration were well tolerated and did not produce clinically significant irritation (section 2.2.3.7).

✳ See page 126

Immunotoxicity of TGN1412 was assessed as part of the standard toxicology studies and in non-GLP pharmacology studies (section 2.2.3.8). 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 lead to a transient increase in CD4+ and CD8+ T cell numbers between day 13 and 17 after dosing. The observed immunomodulation is 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 immunosuppression 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 is not regarded as being of clinical importance as exposure of cytoplasmic antigens appears 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 underlines this assumption.

Applying an allometric correction factor of 3.1 in order to calculate the human eqivalent dose (HED, FDA draft guideline "Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers") to the NOAEL of 50 mg/kg in cynomolgus monkeys (=16 mg/kg), the clinical starting dose of 0.1 mg/kg body weight represents a safety margin of 160-fold, which is considered to be sufficient to ensure patient safety.

2.2.3.2   SINGLE DOSE TOXICITY

In general, the single dose toxicity of a novel medicinal product should be evaluated in two mammalian species (usually one rodent and one non-rodent) prior to the first human exposure (ICH-M3 Maintenance of the ICH Guideline on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals finalised in July 1997). Due to the specificity of TGN1412 for human and non-human primate CD28 (see section 2.2.1.2), a standard single dose toxicity testing in rodents was considered to be not appropriate and was therefore not performed.

Single dose toxicity study in *Macaca mulatta* (rhesus monkey) ████████████

The aim of this pilot study was to determine safety and tolerability of the humanized agonistic anti-CD28 monoclonal antibodies TGN1412 and TGN1112 in rhesus monkeys (*Macaca mulatta*). In addition, information on the *in vivo* bioactivity of agonistic anti-CD28 monoclonal antibodies was gained by longitudinal monitoring of peripheral lymphocyte number and their activation state. Demonstration of tolerability and bioactivity of TGN1112 and TGN1412 in

Macaca mulatta was required to justify and initiate a collagen-induced arthritis study using this species. Furthermore, information on the pharmacodynamic and pharmacokinetic properties of TGN1412 and TGN1112 gained in this study was valuable in the design of subsequent toxicity studies.

Binding specificity of TGN1412 and TGN1112 for rhesus monkey CD28 was verified by flow cytometry ██████████████████████ Both antibodies bind rhesus lymphocytes *ex vivo* with comparable efficacy, however, TGN1112 showed superior *ex vivo* pharmacological T cell activating capacity and was therefore more closely examined.

Two animals received TGN1112 at a total dose of 2.5 mg/kg or 5 mg/kg and one animal received TGN1412 at 5 mg/kg. TGN1112 was applied i.v. with an initial injection of 1 mg/kg followed by a second injection of 1.5 or 4 mg/kg three hours later. The third monkey was treated with a bolus injection of TGN1412 at 2.5 mg/kg. Routine health assessment of animals included palpation of lymphnodes (axial, inguinal, abdominal), examination of heart, lungs and oral cavity. Hematological parameters (hemoglobin, hematokrit, erythrocyte counts and thrombocyte numbers) and differential blood counts were determined. The monkey treated with 5 mg/kg of TGN1112 was sacrificed on day 155 post-injection followed by a gross necropsy and histological examination of tissue samples from all major organs. The two remaining animals returned to colony after completion of the study (TeGenero study report ██████████████

All animals tolerated TGN1412 or TGN1112 injection well without any overt side-effects. Hematological parameters were not affected. Histological examination of tissues originating from the rhesus monkey treated with 5 mg/kg TGN1112 showed no abnormalities related to treatment. One animal exhibited focal, chronic granulomatous pneumonia indicative of mite infection, however, according to the pathologist's report the pneumonia was unrelated to treatment with TGN1112 (TeGenero study report ██████████████

A 2-fold transient increase in CD4 and CD8 cell numbers peaking at day 16 was observed after TGN1112 treatment. Rhesus-anti-human antibody (RAHA) responses were determined by ELISA. Both anti-isotype and anti-idiotype responses were observed in treated animals at both dose levels starting around three weeks after treatment.

2.2.3.3 REPEAT-DOSE TOXICITY

Pilot study in cynomolgus monkeys ██████████████████████

A non-GLP pilot study in cynomolgus monkeys (*Macaca fascicularis*; n=4) was conducted to provide a preliminary assessment of the pharmacological activity, toxicity and toxicokinetics of TGN1412 (██████████████ section 2.1.S.2.6) in the non-human primate. The study was intended to establish a suitable high dose for repeated administration of the test compound in further studies. Currently, a maximum dose of 5 mg/kg is planned for early clinical studies. The dosing regimen for the pilot study is shown in Table 66.

One male and one female cynomolgus monkeys (animals 1M and 3F) received TGN1412 at ascending dose levels of 5, 10 and 25 mg/kg, by intravenous infusion over a one hour period each, with a 7 day respite period between each administration. Dose volumes of 1, 2 or 5 ml/kg body weight were used, respectively. In view of absence of any toxicity, the same pair of animals

✳ *See page 126*

as well as another pair of drug-naive animals (animals 2M and 4F) received TGN1412 at a dose level of 50 mg/kg. This was considered the maximum practicable dose at a dose volume of 10 ml/kg (concentration of TGN1412 = 5 mg/ml) for this route of administration. All animals were retained for a subsequent 26-day recovery period.

Table 66: **Treatment schedule cynomolgus pilot study**

Study day	Treatment	Dose level (mg/kg)	Dose volume (ml/kg)	Animal identification number	
				Male	Female
1	TGN1412	5	1	1	3
8	TGN1412	10	2	1	3
15	TGN1412	25	5	1	3
22	TGN1412	50	10	1,2	3,4

A single administration of 50 mg/kg TGN1412 was well tolerated by the cynomolgus monkeys. Therefore, this dose level was considered to be the no-observed-adverse-effect level (NOAEL) in this study. In addition, a cumulative dose of 90 mg/kg body weight did not cause adverse events in the treated animals. Between days 13 and 19, the axillary and inguinal lymph nodes were enlarged for animals 1M and 3F, which may be interpreted as a pharmacodynamic effect of TGN1412. Body weight gain and food consumption were unaffected by treatment. No plasma biochemical changes were detected during the study. Individual animals showed a trend towards decreased neutophil counts after treatment with TGN1412. Necropsy revealed no organ weight changes or macroscopic findings related to treatment.

Flow cytometry was conducted on 3 pretrial occasions and on days 1, 8, 15, 22, 27, 34, 41 and 48, using cell surface suited to determine lymphocyte subset frequencies and activation states.

Figure 42 shows results of the flow cytometry analysis in one animal. CD4 and CD8 positive T lymphocytes were expanded transiently and showed a clear peak around day 15 post infusion (A). This T cell expansion was paralleled by an increase in T cell numbers expressing CD69 and CD25 (B and D). TGN1412 could be detected on the surface of CD4+ T cells (C). This observation correlated inversely with availability of free TGN1412 binding sites (C). A modest decrease in NK cell numbers was observed following injection of TGN1412, which returned to baseline after completion of dosing (day 22). B cell numbers were slightly increased (F) after injection of TGN1412 between days 30 and 40 post infusion.
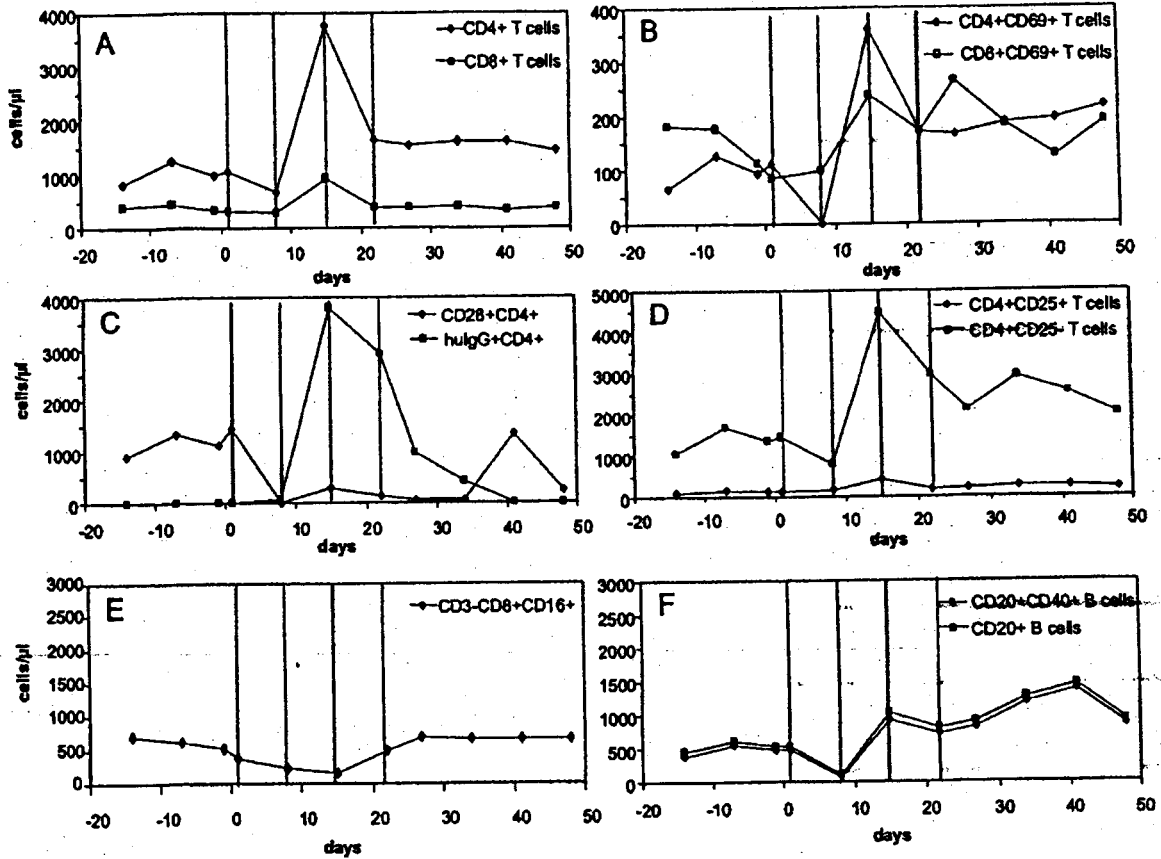
Figure 42: Flow cytometry analysis of lymphocyte subsets in a cynomolgus monkey (1M)

Flow cytometry analysis of leukocyte subsets in a male cynomolgus monkey (1M) following intravenous injection of escalating doses (5.0mg – 10.0 mg – 25.0 mg – 50.0 mg/kg) of TGN1412.

Toxicokinetic evaluation during the pilot study in cynomolgus monkeys showed that TGN1412 serum concentration vs. time profiles were generally consistent with intravenous injection of the drug (section 2.2.2.8). In addition, it was observed that serum concentrations of TGN1412 increased in proportion with the dose level.

Immunogenicity of TGN1412 was assessed by determination of anti-TGN1412 antibodies in serum samples taken at various timepoints following administration. Only one animal (1M) showed a substantial titer of anti-TGN1412 antibodies three weeks after administration (section 2.2.3.8).

The fact that TGN1412 was pharmacologically active in this species – demonstrated by peripheral T cell expansion and activation – confirmed the selection of the cynomolgus monkey as a suitable species for further toxicology studies.

28-day repeated dose toxicology study in cynomolgus monkeys ██████████████ ✳

The study was designed on the basis of results from the above-mentioned pilot study in cynomolgus monkeys. The objective of this study was to assess the potential toxicity of weekly intravenous administrations of TGN1412 (████████████ section 2.1.S.2.6) in the ✳ cynomolgus monkey over a 4 week treatment period and to assess the reversibility of any treatment related findings during a subsequent 6 week recovery period. The study was performed in accordance to GLP guidelines.

Eight male and 8 female cynomolgus monkeys received TGN1412 at dose levels of 5 or 50 mg/kg by slow intravenous infusion over a one hour period on 4 occasions with a 7 day recovery period between each administration. Dose volumes of 1 or 10 ml/kg body weight were used respectively. A control group consisting of 5 male and 5 female cynomolgus monkeys received the vehicle alone at a dose volume of 10 ml/kg body weight. After 4 weeks, 2 males and 2 females from the control and 50 mg/kg groups were sacrificed and the remaining animals were retained for a subsequent 41 (females) or 42 (males) day recovery assessment. Treatment was conducted according to the tabulated scheme below (Table 67).

Table 67: Dosing scheme of 28-day toxicology study in cynomolgus monkeys

Group	Treatment (mg/kg)	Dose volume (ml/kg)	Animal number			
			Main study		Recovery study	
			males	females	males	females
1	0	10	2	2	3	3
2 (low dose)	5	1	-	-	3	3
3 (high dose)	50	10	2	2	3	3

In the low dose group, six animals were treated with TGN1412. All animals were included in the recovery study, since no adverse effects were expected at a dose of 5 mg/kg on the basis of the pilot study results. In the high dose group, four animals were necropsied after completion of dosing and six additional animals were included in the recovery study.

✳ see page 126.

General monitoring was carried out during the study and included food intake, body weight, hematology, clinical chemistry, urinalysis, ophthalmology, ECG and general behaviour. The injection sites were observed for the presence of any visible reactions (local tolerance).

Terminal observations (autopsies) were conducted on all animals. Quantification of TGN1412 and anti-TGN1412 antibodies in serum samples was conducted using validated ELISA methodologies to obtain PK / immunogenicity data.

There were no treatment-related deaths and no clinical signs attributable to treatment. Body weight performance and food intake were unaffected by treatment. There were no ocular or electrocardiogram changes. There were no hematological or plasma biochemical findings or any change in urinary composition during 4 weeks of treatment. Organ weights were unaffected by treatment and there were no treatment-related necropsy findings after 28 days.

Histopathological findings revealed phlebitis and periphlebitis and perivascular or subcutaneous hemorrhage at the injection sites and dermatitis and/or subcutaneous inflammation at the tail of some animals. There were no toxicologically significant differences between treated and control animals.

One male receiving 5 mg/kg TGN1412 (7M) was killed on day 3 on humane grounds, since signs of persistent liquid faeces were observed. Colon and caecum swabs obtained at necropsy revealed the presence of *Campylobacter jejuni*. Another male control group animal (No. 4) exhibited similar signs of liquid faeces, and the presence of *C. jejuni* and *Salmonella* spp Group C were determined. At the request of the veterinarian, all animals were screened for any enteric pathogens over a 3 day period. A number of animals both from control and treatment groups were positive for *C. jejuni* and/or *Salmonella* spp Group C.

Because of the high prevalence of infections with *C. jejuni* in immature laboratory primates (Morton et al., 1983), the fact that infections and/or abnormal faeces were observed in control and treatment group animals during both the pre-trial and treatment period was considered not to be a limiting factor to the interpretation of the results or to the validity of the study. It was concluded that the death of the male animal was clearly unrelated to treatment.

In summary, a NOAEL of 50 mg/kg was defined for TGN1412 in cynomolgus monkeys.

Toxicokinetic investigations

Toxicokinetic investigations were conducted as part of the 28-day toxicology study in cynomolgus monkeys (section 2.2.2.8). TGN1412 serum concentration vs. time profiles were generally consistent with intravenous injection of the drug. Systemic exposure to TGN1412 increased by up to ca 20-fold as dose increased from 5 to 50 mg/kg. Estimates of the apparent terminal elimination half-life of TGN1412 were variable: a mean half-life of ~8 days was calculated on the basis of mean serum concentrations after first infusion of 5 mg/kg TGN1412. There was evidence of an increase in mean estimates as dose increased. This was reflected in mean estimates of the apparent volume of distribution (V_d), which decreased as dose increased from 5 to 50 mg/kg. There were no clear changes in the volume of distribution at steady state (V_{ss}) or of the central compartment (V_{cen}) as dose increased from 5 to 50 mg/kg. Estimates of $T_{max(obs)}$ also remained largely unchanged as dose of TGN1412 increased from 5 to 50 mg/kg.

In one animal (8M), relatively low serum concentrations of TGN1412 were observed, which may be attributed to the presence of anti-TGN1412 antibodies. Overall, in four out of 16 treated animals anti-TGN1412 antibodies were detected.

No consistent sex-related differences were apparent in any of the toxicokinetic parameters reported for TGN1412.

Flow cytometric evaluation

As part of the toxicology study, flow cytometric evaluation of peripheral blood lymphocyte subset composition and activation was performed. Whole blood samples were collected from control and treatment group animals for the analysis of various phenotypic cell surface markers.

Contingent on a rather high degree of variability in pre-trial data, the flow cytometric data assessed imply that TGN1412 transiently expanded both CD8+ and CD4+ T cells in male and less pronounced in female animals. Expansion appeared to optimally occur at the 5 mg/kg dose level. A possible increase in absolute CD25+CD4+CD14- T cell was observed in three animals in the treatment groups and appeared to correlate with overall CD4+ T cell counts. There was no evidence for a dose dependent increase of activated CD69+CD4+ or CD69+CD8+ T cell numbers. Although up-regulation of CD69 is a feature of TGN1412 stimulation *in vitro*, no elevated CD69 expression levels were found on the T cell surface *in vivo* in cynomolgus monkeys.

There was a high variability in NK cell numbers observed in control and treatment group animals with a tendency to increase during the recovery period after TGN1412 treatment. TGN1412 appeared to also induce a B cell expansion that persisted longer than the observed T cell expansion in individual animals.

The observed effects of TGN1412 on peripheral blood lymphocytes appeared not to correlate with observed serum concentrations of cytokines (IL-2, IL-4, IL-5, IL-6, TNFα, IFNγ) as determined as part of the toxicology study ▬▬▬▬▬▬▬▬▬▬▬▬ *See page 126*

From determination of free CD28 binding sites, initial dosing of TGN1412 saturated most if not all CD28 binding sites. Recovery of free CD28 binding sites was observed at several timepoints during dosing and in the recovery period. It is not clear whether these observations are due to downmodulation and/or cycling of the CD28 molecule as a consequence of TGN1412 binding or saturation of CD28 binding sites that prevents detection by the competitive detection antibody TGN1112-FITC.

In conclusion, TGN1412 expanded CD4+ and CD8+ T cells efficiently in male animals at the 5 mg/kg dose level. An increase in CD25+CD4+ T cell numbers appears to correlate with the increase in absolute CD4+ cell counts. There were less optimal responses observed in females and at the 50 mg/kg dose level. The observed changes in absolute T cell numbers are an expected pharmacodynamic response to TGN1412 treatment.

2.2.3.4   GENOTOXICITY

No genotoxicity studies have been performed since the standard battery of genotoxicity studies is not expected to deliver meaningful results for TGN1412. Due to the fact that TGN1412 acts by extracellular binding to the T cell surface molecule CD28, it is not anticipated that TGN1412 has a genotoxic effect.

2.2.3.4.1   In vitro

No data available

2.2.3.4.2   In vivo

No data available

2.2.3.5   CARCINOGENICITY

Standard long-term carcinogenicity studies in rodents are not expected to deliver meaningful results for TGN1412 due to its species specificity for human and non-human primate CD28. In addition, long term studies with a humanized protein such as TGN1412 in animals may be difficult due to the immunogenicity of the drug. There is no evidence from available pharmacology and toxicology studies that TGN1412 has mutagenic or carcinogenic potential.

2.2.3.6   REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Reproduction and developmental toxicity studies with TGN1412 have not yet been performed due to the fact that such studies would be limited to non-human primates. A reproductive toxicity study may be conducted in the homologous rat model or in cynomolgus monkeys at a later timepoint in development.

In cross reactivity studies with human and cynomolgus monkey tissues ██████████████ ✴
██████████intracytoplasmic staining with TGN1412 that was considered to be specific was recorded in some keratinised epithelial cells in the cervix of cynomolgus monkeys and also in cytotrophoblast cells in the placenta of humans. This intracytoplasmic staining was not regarded as being of clinical importance as exposure of cytoplasmic antigens may be a result of tissue sectioning and no treatment related histology findings were reported for the genital system (testis, epididymis, ovary, uterus, vagina) in the 28-day repeated dose toxicology study in ✴ cynomolgus monkeys ███████████████████████.

Due to the fact that dedicated reproductive and developmental toxicity studies have not yet been performed, women will not be included in the proposed phase I clinical trial.

In order to support later clinical development of TGN1412 for the treatment of B-CLL and RA, it is planned to conduct reproductive toxicology studies in the rat and/or in the cynomolgus monkey prior to any administration of TGN1412 in females.

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2.2.3.7   LOCAL TOLERANCE

<u>28-day repeated dose toxicity study in cynomolgys monkeys</u> ███████████   ✳

Eight male and 8 female cynomolgus monkeys received TGN1412 at dose levels of 5 or 50 mg/kg, by slow intravenous infusion via the tail vein or other suitable vein over a one hour period, on 4 occasions with a 7 day recovery period between each administration. The injection site was observed for the presence of any visible reactions. Histopathological findings revealed phlebitis and periphlebitis and perivascular or subcutaneous haemorrhage at the injection sites and dermatitis and/or subcutaneous inflammation at the tail in some animals. There were no toxicologically significant differences between treated and control animals.

<u>Local tolerance study in rabbits</u> ███████████   ✳

The purpose of this study was to determine the local tolerance of the test article TGN1412 in comparison to saline 0.9% in New Zealand white female rabbits. Therefore, the undiluted test article formulation and the control were applied intravenously (i.v.), intraarterially (i.a.) and paravenously (p.v.) to a total of four female rabbits.

Body weight was determined on study day -3, 0, and 4. On study day 0, the animals were observed shortly after administration. On study day 1, 2, 3 and 4, clinical observations were performed once a day. Erythema and edema formation were evaluated by the scoring system first published by Draize (1959). All other clinical findings such as pain were traced with respect to severity. Necropsies were performed on study day 4. All application sites were preserved. Different areas of the application sites were histologically evaluated by a pathologist.

In summary, no substance-related clinical findings and no drug-related histopathological findings were observed after intravenous, intraarterial and paravenous administration of test article or saline. The paravenous injection of 10 mg/ml TGN1412 may cause pain reactions in single cases.

Based on the clinical and histopathological observations, it is concluded that a single intravenous, intraarterial or paravenous application of 10 mg/ml TGN1412 in a volume of 0.5 ml is well tolerated.

2.2.3.8   OTHER TOXICITY STUDIES   ✳

<u>Human and non-human primate tissue cross reactivity</u> ███████████

A tissue cross reactivity study has been performed to determine the cellular localisation of TGN1412 in a range of normal human and cynomolgus monkey tissues. This study was intended to identify sites, in addition to target sites, with which the antibody cross-reacts and to aid in interpretation of pre-clinical toxicity studies. Tissues were examined from 3 unrelated human donors and 3 cynomolgus monkeys.

For each batch of staining, samples of positive and negative tissue controls were used for the staining procedure. Tissue samples of human thymus previously shown to express CD28 were used as positive controls. Tissue samples of human heart, previously shown not to express CD28, were used as negative controls.

Specific membrane bound staining of lymphocytes was seen in the primary and secondary lymphoid organs and also in the Mucosal Associated Lymphoid Tissue (MALT) of the stomach, small intestine and large intestine. The distribution of lymphocyte staining recorded appeared consistent with the expected distribution of T cells within lymphoid tissue. This lymphoid staining is considered consistent with target antigen specificity and does not represent tissue cross reactivity.

Specific fibrillary staining, considered to represent astrocyte staining, was seen in the brain, spinal cord and pituitary gland of both human and cynomolgus monkey donors. This cross-reactivity with tissue of the central nervous system (CNS) may be due to the IgG4 isotype of TGN1412 and not specific for the CD28 antigen since staining, although to a lesser degree, was also observed in tissue sections stained with control antibody. In addition, staining of astrocytes may be intracytoplasmic as a result of tissue sectioning procedures. CNS tissue cross-reactivity was not associated with CNS related adverse clinical symptoms/toxicology findings in cynomolgus monkeys as assessed in the 28-day repeat-dose toxicology study ███████████. Moreover, as an IgG antibody, TGN1412 is not expected to cross the blood-brain barrier under physiological conditions. Consequently, cross reactivity with CNS tissues is not considered to be of major relevance for the assessment of TGN1412 safety.

Intracytoplasmic staining that is considered to be specific was also recorded in some keratinised epithelial cells in the cervix of cynomolgus donors and also in cytotrophoblast cells in the placenta of humans. Both these cell types produce certain members of the cytokeratin family, which may cross-react with the test antibody. However, this intracytoplasmic staining is not regarded as being of clinical importance as exposure of cytoplasmic antigens may be a result of tissue sectioning.

Minimal specific staining of cytotrophoblast cells was also seen in the placenta of some human donors. Placental tissue was not evaluated from cynomolgus donors. Due to the artefactual exposure of intracytoplasmic antigens however, neither was considered to be of major clinical significance.

Immunotoxicity studies with TGN1412

Potential adverse effects of TGN1412 on the immune system were evaluated as part of the standard toxicity studies and in additional immunotoxicity studies.

Standard toxicity studies (STS)

In the main 28-day repeated dose toxicology study ██████████████████, no adverse haematological changes (e.g. pancytopenia, leukopenia, lymphopenia) have been observed that could be attributed to treatment with TGN1412. In the pilot dose escalation study, individual animals showed a trend towards decreased neutrophil counts after treatment with TGN1412. However, this finding was not confirmed in the main toxicology study, where neutrophil counts were highly variable in control and treatment group animals, irrespective of TGN1412 dose. Therefore, the observed changes in neutrophil counts are likely to be related to stress, rather than being attributable to treatment with TGN1412.

Since neutropenia is a common complication in B-CLL (Tsiodras et al., 2000), neutrophil counts will be closely monitored during clinical trials in order to ensure maximum patient safety.

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No TGN1412 treatment related alterations in primary or secondary lymphoid organ weights or histology (thymus, spleen, lymph nodes and bone marrow) were observed in cynomolgus monkeys during the 28-day repeated dose toxicology study. In the pilot study, a transient reversible enlargement of axillary and inguinal lymph nodes was observed in two cynomolgus monkeys between day 13 and 19 after treatment with increasing doses of TGN1412, which are interpreted as a pharmacodynamic effect of TGN1412 treatment.

No changes in globulin levels and albumin/ globulin levels were observed following treatment with TGN1412.

There was no evidence for an increased incidence of infections or carcinogenicity in animals treated with agonistic anti-CD28 monoclonal antibodies in rodent or primate models. Therefore, it is concluded that TGN1412 does not mediate a generalised immunosuppression.

Additional immunotoxicity studies

T cell subsets and function

In addition to the assessment of haematological changes as part of the standard toxicology studies, immunophenotyping was conducted by flow cytometric analysis in order to assess changes in the absolute counts and percentages of lymphocyte subsets (see section 2.2.3.3). In accordance with its capability to induce *ex vivo* T cell proliferation, a transient increase in CD4+ and CD8+ T cells was observed in cynomolgus monkeys after treatment with TGN1412. Therefore, T cell expansion is an expected pharmacodynamic effect of TGN1412.

An increase in absolute CD25+CD4+ T cell numbers was observed in cynomolgus monkeys after treatment with TGN1412 and appeared to correlate with overall CD4+ T cell counts. In the main toxicology study, no evidence for a dose dependent increase of activated CD69+CD4+ or CD69+CD8+ T cell numbers was found. Although up-regulation of CD69 is a feature of TGN1412 *in vitro*, no elevated CD69 expression levels were found on the T cell surface *in vivo* in cynomolgus monkeys. It is therefore concluded that TGN1412 is capable of inducing a profound T-cell expansion in the absence of a massive (pathogenic) T-cell activation marked by CD69 expression.

The effect of the agonistic anti-CD28 antibody JJ316 on T-cell reconstitution and function was investigated in an orthologous rat model. In lethally irradiated, bone marrow reconstituted hosts, JJ316 (1 mg total dose, ~ 5 mg/kg administered by intravenous route) was shown to accelerate polyclonal T cell repopulation by a small inoculum of mature, allotype-marked T cells (Elflein et al. 2003). In this model, recovery of CD4+ T cells was superior to that of CD8 T cells. Expanded T cells had maintained T cell receptor diversity and were functional *in vitro* and *in vivo*. *In vitro*, it was demonstrated that T cells from treated animals were capable to mount an efficient proliferative response to allogeneic stimulator cells. *In vivo*, the response to foreign MHC antigens was shown by efficient skin graft rejection after treatment with agonistic anti-CD28 antibody.

In the study by Elflein et al. (2003), the responses to a T-cell dependent protein model antigen, keyhole limpet hemocyanin (KLH) were maintained. High and similar titers of KLH-specific antibodies were detected in both treatment and control groups. In addition, KLH response was not affected by treatment with agonistic anti-CD28 treatment as measured by proliferation and

ability of CD4+ lymphoblasts to produce IFN-γ and IL-4 following restimulation with KLH. Both treatment and control group animals yielded comparable frequencies of cells producing IFN-γ, whereas the frequency of IL-4 producers was elevated in the treatment group.

T cell function was also tested in rhesus monkeys by a KLH recall assay (TeGenero study report ██████████████). A single injection of TGN1112 (2.5 mg/kg) did not alter the recall response to KLH in *in vitro* proliferation assays after stimulation of PBMC with KLH, indicating that the frequencies and function of primed T cells were not affected by TGN1112 treatment.

Overall, these observations indicate that agonistic anti-CD28 triggered expansion of CD4+ T cells maintains antigen-specific T-cell memory.

The initially observed well-tolerated T-cell expansion induced by JJ316 application was further studied and shown to be accompanied by the predominant expression of anti-inflammatory (TH2) cytokines, most notably of IL-10 (Rodriguez-Palmero et al., 1999), rather than an acute secretion of pro-inflammatory cytokines such as observed with anti-TCR antibodies.

Importantly and in accordance with these findings, it was subsequently shown that treatment of rats with JJ316 lead to a profound, transient and over-proportional expansion of functional CD4+CD25+CTLA-4+ regulatory T cells (Lin and Hunig, 2003).

Most recently, there is evidence that the effect of agonistic anti-CD28 antibodies on the expansion and function of regulatory and conventional T cells may depend on the dose applied (section section 2.2.1.2; Beyersdorf et al., 2005a). It could be demonstrated that as little as 0.1 mg of JJ316 per animal (0.5 mg/ kg body weight) lead to a significant increase in the percentage and absolute numbers of regulatory CD25+ cells among CD4+ cells both in peripheral lymph nodes and in the spleen, whereas CD4+CD25- cells did not show a significant change in numbers with both these dosages. Only upon injection of 1 mg or more of JJ316, a significant and very pronounced increase in conventional CD4+CD25- T cell numbers was observed, which is in accordance with previous observations (Tacke et al., 1997).

Therefore, application of low doses of JJ316 into normal Lewis rats is sufficient to induce *in vivo* expansion of regulatory T cells without the generalized lymphocytosis observed with high doses of the antibody.

Autoimmune models

A number of pharmacodynamic studies were conducted in rodent and nonhuman primate models of autoimmune disease. In accordance with the findings in normal Lewis rats, no generalized lymphocytosis was observed when relatively low doses (~ 1 mg/kg) of JJ316 were applied to arthritis-prone rat, but, importantly, disease symptoms and surrogate parameters were inhibited with similar efficacy at low doses (0.3 mg/kg) as in high dose treated animals (5 mg/kg).

In rat adjuvant arthritis (TeGenero study report ████████████; Beyersdorf et al., 2005a), rat EAN (Schmidt et al., 2003), and rhesus monkey collagen-induced arthritis (TeGenenero study report ████████████ models, aggravation of disease was never observed irrespective of the dose of JJ316 or TGN1112 applied. In contrast, the agonistic anti-CD28 antibody JJ316 failed to confer a protective effect in the rat CIA model ████████████████████. Instead, it was observed that a dose of 3mg/kg apparently worsened disease symptoms (joint swelling and function), whereas lower doses showed no effect (section 2.2.1.2).

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The pathogenesis of rodent CIA appears to be mainly caused by B cells and complement (Brand et al., 2003), while auto-aggressive TH1 CD4 T cells appear to be the main mediators of autoimmunity in rat adjuvant arthritis (Wauben et al., 1994) or rhesus monkey collagen-induced arthritis (Bakker et al., 1991). Since agonistic anti-CD28 antibodies have the potential to transiently stimulate B cells via T cell help (Tacke et al., 1997), and B cells and auto-antibodies contribute significantly to the pathogenesis of rat CIA, it is concluded that treatment of CIA rats with high doses of agonistic anti-CD28 antibody may have resulted in an amplification of the humoral autoimmune response initiated by injection of collagen. This interpretation is supported by the observation that aggravation of disease, although to a lesser degree than with JJ316, was also observed in animals, which had received a control antibody.

In conclusion, no aggravation of T-cell mediated autoimmune disease symptoms was observed in any of the pre-clinical studies performed. No exacerbation of autoimmune disease was observed even at doses previously shown to induce a generalised lymphocytosis. However, it cannot be excluded that TGN1412 might have the potential to aggravate autoimmune disease in patients when antibody, B-cell or complement mediated patho-mechanisms play a major role.

Effects on other lymphocytes

As shown by Tacke et al. (1997) and in accordance with the findings described above, the stimulatory effect of agonistic anti-rat CD28 antibody JJ316 on B cells and immunoglobulin levels is apparently an indirect effect of T cell stimulation since it did not occur in thymectomized animals.

In cynomolgus monkey toxicology studies, a trend towards increased B-cell numbers was observed in animals treated with TGN1412 ██████████████████████ In general, this observation is in accordance with pharmacology studies performed in rats as described above. In order to detect potential B-cell mediated adverse or autoimmune effects induced by TGN1412 in humans, absolute and relative B-cell counts, B cell differentiation and activation as well as immunoglobulin levels, rheumatoid factor and ANA antibodies will be closely monitored during the proposed phase I clinical trial.

In the main toxicology study, there was a high variability in NK cell numbers observed in control and treatment group animals with a tendency to increase during the recovery period. However, a decrease in NK cell numbers was observed in the pilot toxicology study after treatment with TGN1412. Therefore, the observed changes could not be clearly attributed to TGN1412. In order to ensure maximum safety of treated subjects in the proposed clinical trial, NK cell numbers will be closely monitored in addition to routine safety assessments.

Cytokine secretion

Analysis of cytokine secretion was conducted as part of early pharmacology studies. *In vivo*, the polyclonal T cell expansion by agonistic anti-CD28 antibodies was shown to be accompanied by the expression of anti-inflammatory cytokines, most notably of IL-10 (Rodriguez-Palmero et al., 1999) rather than an acute secretion of pro-inflammatory cytokines.

As part of the pilot study in rhesus monkeys, analysis of serum cytokine levels was performed (TeGenero study report ████████████ After a single injection of 2.5 mg/kg TGN1112, no detectable levels of IFN-γ, IL-5 and IL-6 were found. In a second rhesus monkey, IFN-γ, IL-6

✳ See page 126

and IL-10 serum levels were analysed. No substantial changes were observed throughout the study. These data imply that injection of agonistic anti-CD28 monoclonal antibodies do not results in an acute systemic cytokine release.

Analysis of cytokine secretion was also performed as part of the 28-day repeat-dose toxicology study in cynomolgus monkeys. Systemic cytokine release was assessed in serum using the cytokine bead array (CBA) technique. This has the ability to measure 6 cytokines simultaneously (IL-2, IL-4, IL-5, IL-6, TNFα and IFNγ) from a single sample. The samples measured were at the time points pre-trial, day 1+ 2h, day 1+24h, day 17 and day 62. Samples from treatment as well as control group animals were analysed. The individual standard curve range for a given cytokine defined the minimum and maximum quantifiable levels, i.e. 20 pg/ml and 5,000 pg/ml, with the detection of lower values possible by extrapolation using the 4-parameter logistic curve fit option.

Animals in each group exhibited measurable levels of IL-2, IL-4, IL-5, IL-6, TNFα and IFNγ in the pg/ml range. After administration of the first dose of TGN1412 on day 1, peak serum concentrations were detected after 2 hours (IL-2, IL-6) or 24 hours (IL-5). All measurements at days 17 and 62 showed pre-trial cytokine levels. Table 68 provides a summary of the mean peak serum concentrations of the measured cytokines.

Table 68: **Mean peak serum concentrations in each dosing group after administration of TGN1412 in cynomologus monkeys**

Cytokine	Mean peak cytokine level (range) pg/ml		
	Control group (0 mg/kg)	Low dose group (5 mg/kg)	High dose group (50 mg/kg)
IL-2	37 (20-60)	25 (0-84)	100 (25-211)
IL-4	12 (0-18)	13 (8-18)	17 (0-40)
IL-5	6 (3-7)	49 (6-139)	107 (11-458)
IL-6	7 (0-22)	68 (32-101)	128 (24-390)
TFN-alpha	20 (11-26)	20 (15-27)	22 (19-26)
IFN-gamma	18 (0-35)	23 (19-32)	33 (17-93)

Following administration of 5.0 mg/kg TGN1412, IL-5 and IL-6 showed transiently and moderately elevated serum levels. Serum concentrations of IL-2, IL-4, TNFα and IFNγ were generally in the range of control group values. Administration of 50 mg/kg TGN1412 resulted in transiently increased serum levels of IL-2, IL-5 and IL-6. IL-4 secretion appeared not to be affected by TGN1412 treatment. Most notably, no increased TNFα or IFNγ serum levels were observed after administration of either dose of TGN1412.

In summary, treatment of cynomologus monkeys with TGN1412 resulted in transient and moderately elevated serum levels specifically of IL-2, the inflammatory cytokine IL-6 and the anti-inflammatory (TH2 type) cytokine IL-5. Increased secretion of these cytokines appeared to be dependent on the dose administered. IL-2 serum levels were only elevated in high dose group animals. Serum levels of two additional major pro-inflammatory cytokines, TNFα and IFNγ,

were not substantially elevated after first dosing with TGN1412. Elevated cytokine levels in individual animals did not correlate with increased numbers of (activated) T cells or other leukocyte subsets.

These data sharply contrast the induction of high concentrations of pro-inflammatory cytokines including IFNγ and TNFα in serum, resulting the induction of high TNFα and IFNγ levels in non-human primates (Hwei et al., 1999) and in a clinically apparent cytokine release syndrome (CRS) in humans (Abramowicz et al., 1989) upon administration of agonistic anti-CD3 antibodies.

Since only a weak increase in cytokine levels was observed at 5 mg/kg TGN1412 in cynomolgus monkeys, no CRS is expected in a dose range of 0.1 to 5.0 mg/kg to be administered in the proposed phase I clinical trial. In order to ensure maximum safety of treated individuals during early clinical trials with TGN1412, subjects will be closely monitored for first-dose CRS.

Antibody investigation (immunogenicity)

The induction of antibodies to TNG1412 was investigated in context of the pilot dose escalation study and the 28-day repeat dose toxicology study in cynomolgys monkeys ▓▓▓▓▓▓▓▓ ▓▓▓▓▓▓▓▓▓▓▓▓▓▓Blood samples were obtained for the detection of the presence of antibodies to TGN1412 for all animals on one occasion during the pretrial period and then on various timepoints following adminstration. *See page 176*

In the pilot study, only one of four animals showed a substantial anti-TGN1412 antibody titre (animal 1M, Figure 43). Antibodies against TGN1412 were detected approximately three weeks following initiation of dosing.
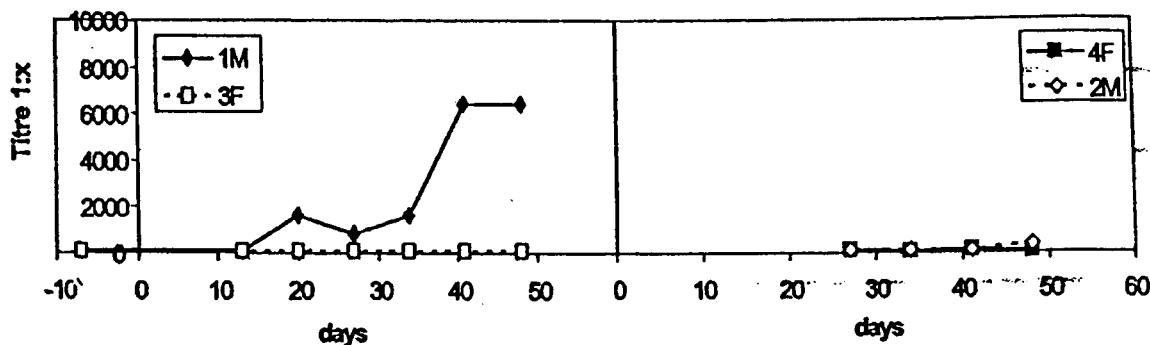

Figure 43: Immunogenicity (serum titres of anti-TGN1412 antibodies) of TGN1412 in the cynomolgus monkey

Animals 1M and 3F were treated with four weekly escalating doses of TGN1412 (5 – 10 – 25 – 50 mg/kg). Animals 2M and 4F received a single dose of 50mg/kg on day 22.

In the 28-day toxicity study, four out of 16 treated animals showed substantial titres of anti-TGN1412 antibodies in serum (two males and one female [Nos. 6, 8 and 20] receiving 5 mg/kg and one male [No. 11] receiving 50 mg/kg). These were observed 3 to 4 weeks after start of dosing.

In summary, in the cynomolgus monkeys treated with TGN1412, a rather high percentage (25%) of animals developed anti-TGN1412 antibodies. Despite the high degree of homology between human and non-human primate immunoglobins, species differences may explain the observed immunogenicity. Thus, the finding that TGN1412 induces substantial antibody responses in rhesus monkeys is not unexpected and is in accordance with results obtained for other humanized antibodies. It should be noted that the degree of immunogenicity of humanized antibodies in non-human primates is not predictive for its immunogenicity in humans.

2.2.3.9   DISCUSSION AND CONCLUSIONS

Due to the specificity of TGN1412 for human and certain non-human primates, safety and toxicity of the agonistic anti-CD28 monoclonal antibody TGN1412 has been assessed after single and repeated doses in rhesus and cynomolgus monkeys. Because of the immunomodulating potential of TGN1412, assessment of immunotoxicity was performed as part of pharmacology and toxicology studies. For certain studies, an agonistic antibody with specificity for rat CD28 (JJ316) or an IgG1 variant of TGN1412 (TGN1112) was used. Detailed discussions are provided within the respective sections 2.2.3.1-8.

Reproduction and developmental toxicity studies have not yet been performed, but no treatment related histology findings were reported for the genital system in the 28-day repeated dose toxicology study in cynomolgus monkeys. As a safety precaution, it is not intended to include pregnant women or women of childbearing potential (unless sterilised) into clinical trials in this early stage of development.

Cross reactivity of TGN1412 was investigated with human and cynomolgus monkey tissues. Specific reactivity of TGN1412 with structures other than expected to express the target CD28 were only reported for central nervous tissue (most likely astrocytes) and cervix (cynomolgus monkey) or placenta (human) as intracytoplasmic staining, respectively. Since in the cynomolgus monkey studies, there was no correlation of these findings with histo-pathological, necropsy or clinical findings, it is not considered to be of clinical importance for the proposed phase I study.

Local tolerance was assessed as part of the toxicology studies in cynomolgus monkeys (intravenous route of administration) and rabbits (intravenous, perivenous and intra-arterial route of administration). Minor local reactions at the injection sites of treated cynomolgus monkeys or rabbits were considered not to be related to treatment with TGN1412 but to the dose administration procedure

No adverse effects on major physiological systems (cardiovascular system, respiratory system and central nervous system) were reported for TGN1412.

Administration of TGN1412 to non-human primates led to a transient reversible increase in CD4+ and CD8+ T cell numbers. The observed immunomodulation is an expected pharmacodynamic effect of TGN1412. There was no evidence for an unintended induction of

substantial pro-inflammatory cytokine release or of autoimmune disease in animals treated with any agonistic anti-CD28 monoclonal antibody.

In summary, the results of non-clinical studies in rodents and non-human primates have not revealed any potentially serious toxicities that would preclude the use of TGN1412 in a healthy subject. TGN1412 was well tolerated at doses up to 50 mg/kg/week for at least four consecutive weeks in cynomolgus monkeys.

Consequently, a "No observed adverse effect level" (NOAEL) of 50 mg/kg was defined in a pivotal 28-day repeated dose toxicology study in cynomolgus monkeys.

Calculation of the phase I trial starting dose was primarily based on safety data (NOAEL in cynomolgus monkeys) and the procedure described in the draft FDA guideline "Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers" (http://www.fda.gov/cber/gdlns/dose.htm#i). For the cynomolgus monkey NOAEL, an allometric correction factor of 3.1 was applied in order to calculate the HED, i.e. $50 \div 3.1 = 16$ mg/kg/day. If the default safety factor of 10 is then applied, the MRSD (maximum recommended starting dose) is thus estimated to be 1.6 mg/kg/day. Thus, the proposed starting dose of 0.1 mg/kg/day is considered to be rather conservative (safety factor 160).

2.2.4   LIST OF STUDY REPORTS

List of reports (which extends to
page 153) is withheld under
section 43(2) of the FOI Act –
see page 14

2.3 CLINICAL DATA

2.3.1 CLINICAL PHARMACOLOGY

To date, there are no data in humans available

2.3.1.1 BRIEF SUMMARY

Not applicable

2.3.1.2 MECHANISM OF PRIMARY ACTION

Not applicable

2.3.1.3 SECONDARY PHARMACOLOGICAL EFFECTS

Not applicable

2.3.1.4 PHARMACODYNAMIC INTERACTIONS

Not applicable

2.3.2 CLINICAL PHARMACOKINETICS

To date, there are no data in humans available.

2.3.2.1 BRIEF SUMMARY

Not applicable

2.3.2.2 ABSORPTION

Not applicable

2.3.2.3 DISTRIBUTION

Not applicable

2.3.2.4 ELIMINATION

Not applicable

2.3.2.5 PHARMACOKINETICS OF ACTIVE METABOLITES

Not applicable

2.3.2.6 PLASMA CONCENTRATION-EFFECT RELATIONSHIP

Not applicable

2.3.2.7. DOSE AND TIME-DEPENDENCIES

Not applicable

2.3.2.8   SPECIAL PATIENT POPULATIONS

Not applicable

2.3.2.9   INTERACTIONS

Not applicable

2.3.3   HUMAN EXPOSURE

To date there are no data in humans available.

2.3.3.1   BRIEF SUMMARY

Not applicable

2.3.3.2   OVERVIEW OF SAFETY AND EFFICACY

Not applicable

2.3.3.3   HEALTHY SUBJECT STUDIES

Not applicable

2.3.3.4   PATIENT STUDIES

Not applicable

2.3.3.5   PREVIOUS HUMAN EXPERIENCE

Not applicable

2.3.4   BENEFITS AND RISK ASSESSMENT

The agonistic anti-CD28 monoclonal antibody TGN1412 represents a novel therapeutic approach intended to therapeutically balance the immune system in diseases associated with severe abnormalities in T lymphocyte number and/or function. Based on its unique mode-of-action and pre-clinical data demonstrating *ex vivo* expansion of human T cells, *in vivo* expansion and activation of T lymphocytes in animal models and therapeutic efficacy in multiple disease models, TGN1412 is anticipated to have beneficial properties for the treatment of B-CLL and/or RA.

In the proposed first-in-man TGN1412-HV clinical trial, TGN1412 will be administered to healthy subjects to assess the safety and tolerability of ascending single intravenous doses of TGN1412 in separate cohorts of healthy volunteers and to determine the pharmacokinetics of TGN1412 (primary objectives). As secondary objectives, the effect of TGN1412 on lymphocyte subsets and on the cytokine profile as well as the induction of anti-TGN1412 antibodies will be determined. The study is designed as a single-centre, randomised, double-blind, placebo-controlled, single ascending-dose escalation trial.

Based on the proposed TGN1412 mode-of-action and on its demonstrated pre-clinical safety in non-human primates (see section 2.2.1), a detailed analysis of its (immunological) safety and pharmacodynamic effects in immunocompetent humans is thought to justify the selection of healthy subjects as the target population for a TGN1412 first-in-man trial.

In particular, a study in healthy subjects yielding valuable information on TGN1412 safety and pharmacology is believed to be warranted prior to studies in patients because

i)   the target antigen CD28 is expressed comparably in healthy subjects and in RA and B-CLL patients, respectively, implying that safety, PK and PD data can be at least partially transferred to B-CLL and/or RA patients

ii)   immunological safety of TGN1412 is given most likely in healthy subjects since no adverse effects have been observed in healthy animals in multiple pre-clinical studies including non-human primates while immunologically adverse effects cannot be completely excluded in subjects with an existing immunopathogenic predisposition

iii) interpretation of safety and pharmacology data in healthy subjects will not be compromized 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 hematopoietic compartment, and

iv)   healthy subjects as outlined in the study protocol 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.

Since the immunomodulatory effects of TGN1412 are thought to depend on the selected dose, an important aspect of this first in man trial will be to examine the effect of different doses of TGN1412 on the immune system. This will help to guide the design of future clinical trials in patients.

Benefits for the healthy subject trial population are not expected at the individual level. Therefore, section 2.3.4 focuses on the discussion of potential risks associated with the application of TGN1412 to humans and safety measure taken in the TGN1412-HV clinical trial design to take these risks into account.

2.3.4.1   Potential risks for humans predicted from pre-clinical trials

Since specificity of TGN1412 is restricted to CD28 expressed on T cells from humans and non-human primates (section 2.2.1.2), safety and toxicology studies in non-human primates (cynomolgus and rhesus monkeys) are 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 regimen. The results of these studies showed that TGN1412 was well tolerated at doses up to 50 mg/kg/week for at least 4 consecutive weeks.

However, a possibility for the occurrence of the following mode-of-action related or unintended effects of TGN1412 in humans must be taken into account during the conduct of clinical trials.

Risks associated with TGN1412 mode-of-action

Despite a moderate increase of IL-2, IL-5 and IL-6 serum levels in TGN1412-treated cynomolgus monkeys as assessed in the 28-day repeat dose toxicology study, no signs of a first-dose cytokine release syndrome were observed in pre-clinical studies. However, it cannot be excluded that a first-dose effect on cytokine release may occur in humans treated with TGN1412.

T cell memory and TH1 as well as TH2 effector functions were not perturbed after treatment with agonistic anti-CD28 antibodies. Single or multiple applications of agonistic anti-CD28 antibodies did not result in any detectable uncontrolled T cell expansion or, on the other hand, generalized immunosuppression. Therefore, risks for pathogenic T lymphocyte differentiation, activation and/or expansion or clinically relevant immunosuppression resulting e.g. in the re-activation of latent EBV or increased incidences of infections is considered to be very low.

Transient lymphadenopathy and splenomegaly reflecting TGN1412 pharmacodynamic activity have been observed in some preclinical studies after treatment with agonistic anti-CD28 antibodies and may therefore be expected to occur in the proposed clinical study. In addition, a tendency towards low neutrophil counts has been observed in individual cynomolgus monkeys following application of escalating doses of TGN1412 and therefore cannot completely be ruled out to occur in humans.

In multiple animal models for autoimmunity including a rhesus monkey CIA model, TGN1412 orthologues and TGN1412 variants exerted substantial curative and/or prophylactic effects. An exception was the results of a rat collagen-induced arthritis study, which raises the possibility that high doses of TGN1412 may aggravate a pre-existing autoimmunity, especially when disease pathogenesis is primarily promoted by B cells, immunoglobulin and/or complement. Such risk not only applies for RA but also for B-CLL patients. Since induction of autoimmune disease symptoms has never been observed in any of the studies conducted with healthy rats or non-human primates, the risk that exposure of healthy subjects to a single dose of TGN1412 may lead to a pathogenic induction of autoimmunity is considered to be very low.

Risks generally associated with administration of therapeutic monoclonal antibodies

Risks generally associated with protein-based medicinal products including anaphylactic reactions and hypersensitivity also apply for TGN1412. TGN1412 administration may lead to the induction of anti-TGN1412 antibodies that, if massive, may be accompanied by side-effects related to the formation of immune complexes. However, such findings have never been observed in pre-clinical studies and are therefore considered to be very unlikely.

In local tolerance studies, intravenous, perivenous, or intraarterial routes of administration of TGN1412 were well tolerated and did not produce clinically significant irritation. However, the occurrence of local reactions related to the intended intravenous route-of-administration cannot be generally excluded in humans.

Although staining of CNS and placental tissues was observed for TGN1412 in immunohistochemical studies, respective organs were found not to be target organs for toxicity in non-human primate studies. In addition, TGN1412 is not expected to pass the blood-brain barrier under physiological conditions. Therefore, the risk of CNS related side effects in human subjects is considered to be very low. Women will not be included in the proposed phase I clinical study.

Conclusion

In conclusion, the results of non-clinical studies in rodents and non-human primates have not revealed any potentially serious (immuno-) toxicities that would preclude the use of TGN1412 in a healthy subject. Nevertheless, the risks related to TGN1412 mode-of-action or generally associated with protein-based therapeutics outlined above warrant the safety and immunomonitoring measures for the proposed TGN1412-HV trial as detailed in the study protocol and as outlined in the following section.

2.3.4.2 Measures taken to minimize subject risk in TGN1412-HV trial

In order to ensure maximum safety of treated subjects, the proposed TGN1412-HV phase I trial has been carefully designed. Special caution has been exercised to safeguard the volunteers against potential immunological risks.

TGN1412 dose selection

The proposed TGN1412-HV trial design is expected to establish the safety and tolerability of TGN1412 in humans. Healthy subjects will be administered a single i.v. dose of TGN1412 (0.1 mg/kg – 0.5 mg/kg – 2.0 mg/kg – 5.0 mg/kg bodyweight).

Calculation of the phase I trial starting dose was primarily based on safety data (NOAEL in cynomolgus monkeys) and the procedure described in the draft FDA guideline "Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers" (http://www.fda.gov/cber/gdlns/dose.htm#i). For the cynomolgus monkey NOAEL, an allometric correction factor of 3.1 was applied in order to calculate the HED (human eqivalent dose), ie $50 \div 3.1 = 16$ mg/kg/day. If the default safety factor of 10 is then applied, the MRSD (maximum recommended starting dose) is thus estimated to be 1.6 mg/kg/day. Thus, the proposed starting dose of 0.1 mg/kg/day is considered to be rather conservative (safety factor 160).

Pharmacological activity of TGN1412 and its surrogate has been observed in rhesus and cynomolgus monkeys at between 2.5 und 25 mg/kg body weight. A NOEL (no observed effect level) of <0.3 mg/kg body weight was derived from preclinical studies conducted in healthy and arthritic (adjuvant arthritis) rats using the rat CD28 specific homolgous antibody JJ316. Optimal pharmacological responses were achieved between 1 and 5 mg/kg body weight. Therefore, the dose range in the proposed phase I trial is considered to both ensure maximum patient safety due to the conservative safety factor of 160 (see above) and enable investigation of basic PD/PK characteristics of TGN1412, relevant for future clinical trials to be conducted in patient populations.

General measures

Care will be taken to ensure that participating subjects match the TGN1412-specific inclusion/exclusion criteria.

To assure maximum subject safety, dose escalation to the next level will only proceed following satisfactory review of safety data covering at least fourteen days following each dosing by an independent Data Safety Monitoring Board.

The following standard tests will be performed to evaluate the safety of TGN1412: close monitoring of adverse events, vital signs including body temperature, hematology, serum chemistry and urinalysis, ECG, assessments for injection site reactions and physical examinations.

Measures relating to TGN1412 administration

In order to minimize infusion-related events, TGN1412 drug product will be administered by slow i.v. infusion. In the unlikely event of infusion-related symptoms, appropriate clinical measures (e.g. histamine antagonists, glucocorticoids) will be taken.

Measure relating to potential immunotoxicies

It should be noted that the safety and tolerability of an immunomodulatory monoclonal antibody (albeit humanized), such as TGN1412, might not surface in the form of adverse events or abnormal results from the standard tests mentioned above but necessitates comprehensive immunomonitoring.

In consideration of potential immunotoxicities that may be related to TGN1412 mode-of-action, flow cytometric analyses of lymphocyte subsets and activation states, pro- and anti-inflammatory cytokine serum levels, serum immunoglobulin levels and in vitro T cell functionality tests will be performed.

In order to evaluate the risk of unintended immunoactivation or immunosuppression by TGN1412, the following additional immunological markers will be closely monitored: CRP, total IL-8, complement activation, rheumatoid factor, anti-nuclear antibodies and EBV viral load.

In the unlikely event that single-dose application of TGN1412 elicits pronounced immunosuppression via massive induction of anti-inflammatory cytokines and/or regulatory T cells, appropriate clinical counter-measures (e.g. application of antibiotics) must be considered.

In the unlikely event that single-dose application of TGN1412 elicits pronounced autoimmunity or anaphylaxis by unintended activation of pathogenic T cells, other leukocytes, mast cells or other mediators of an autoimmune/inflammatory/anaphylactic response, appropriate clinical counter-measures (e.g. application of glucocorticoids, anti-histamines etc.) must be considered.

In the unlikely event that TGN1412 elicits a cytokine release syndrome, appropriate clinical counter-measures must be considered.

<u>Measure relating to unintended side-effects</u>

In a tissue cross-reactivity study with human and cynomolgus monkey tissue, staining of astrocytes in CNS was observed (see above). In the unlikely event that TGN1412 elicits neurological symptoms, symptomatic treatment will be considered.

Anti-TGN1412 antibodies may be generated after TGN1412 application. In the very unlikely event that such anti-idiotypic reactions are accompanied by clinical signs (e.g. serum sickness), appropriate counter-measures will be taken.

2.3.4.3 Risk-benefit analysis for future trials with B-CLL and/or RA patients

As outline in section 2.2.1, pre-clinical evidence from *ex vivo* and *in vitro* experiments as well as multiple animal models including non-human primates suggest that TGN1412 has the potential to be safe and efficacious in the treatment of patients suffering from RA and/or B-CLL.

Although the pharmacodynamic dose-response relationship in healthy subjects may differ from that in patients due to a different activation state of the immune system, different T cell activation/differentiation states, potential drug interactions with pre/co-medication and/or altered immunogenicity, it is expected that TGN1412-mediated agonistic triggering of CD28 in healthy subjects will result in an intended effect on PD parameters such as T cell numbers, T cell/lymphocyte subsets composition and cytokine levels that may be used as surrogate markers for clinical response in future clinical trials.

The proposed phase I clinical trial in healthy subjects is expected to significantly contribute to the safety of TGN1412 in future patient trials because based on the knowledge gained from the immunomonitoring performed and PK-PD relationship analyses, it may be avoided that patients are treated with a sub-optimal or unsafe entry-dose, that sub-optimal dose-intervals are chosen, and that TGN1412 efficacy in patients is monitored with sub-optimal biomarkers.

A justification of significant benefit for the treatment of B-CLL is outlined in the Application for Designation as Orphan Medicinal Product (2004). A detailed risk-benefit analysis of TGN1412 application to B-CLL and/or RA patients in future clinical trials will depend on the outcome the proposed TGN1412-HV phase I clinical trial and will therefore be provided at a later time point of TGN1412 development.

2.4 APPENDIX

Table 1: Overview pre-clinical pharmacology and toxicology studies

This information (will cover pages
161 to 171 inc) is withheld under
section 43 (2) of the FOI Act -
see page 14.

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