INVESTIGATOR´S BROCHURE

TGN1412
HUMANIZED AGONISTIC ANTI-CD28 MONOCLONAL ANTIBODY

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Edition Number:	1.1			
Replaces Edition:	No.:	1	Date:	19.12.2005

SPONSOR'S SIGNATURE PAGE

I confirm that TeGenero AG has approved the Investigator's Brochure on TGN1412 (Humanized Agonistic Anti-CD28 Monoclonal Antibody) (Edition No. 1.1, Version Number No. 1, dated: 13.12.2005) and agree that this may be issued to the relevant authorised study personnel, independent Ethics Committees and Regulatory Authorities.

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CHANGES FROM PREVIOUS VERSION OF INVESTIGATOR'S BROCHURE (IB)

Previous Edition:	No.:	1	Date:	25.07.2005

The changes presented in this edition of the IB are summarised by chapter/section below:

3.5	Results of the in-use compatibility study added
3.4	Results from ongoing stability testing updated
4.4	Information on pre-clinical pharmacodynamic interactions added
4.5	Detailed information on pre-clinical pharmacokinetics and toxicokinetics added
4.5.2	Information on TGN1412 tissue cross reactivity integrated in new section 4.6.9 "Other toxicology studies"
4.5.3	Fig. 9 and table 6 on TGN1412 toxicokinetics in cynomolgus monkeys added; numbering of subsequent figures and tables adapted
4.6.1	Text adapted
4.6.4.1	Fig. 12 adapted. Immunogenicity data are presented in the context of section 4.6.9.2 "Immunotoxicology studies"
4.6.5-8	Section numbers adapted 4.6.5 Genotoxicity 4.6.6 Carcinogenicity 4.6.7 Reproductive and developmental toxicity 4.6.8 Local tolerance
4.6.8	Information on local tolerance in rabbits added
4.6.9.2	Information on cytokine release in cynomolgus monkeys following repeated administration of TGN1412 added
7	New references added

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1 SUMMARY

1.1. Intention of this Investigator's Brochure

This Investigator's Brochure (IB) is a summary of the non-clinical development of the agonistic anti-CD28 monocloncal antibody TGN1412 that is being developed by TeGenero for the treatment of haematological malignancies such as B-cell chronic lymphocytic leukaemia, B-CLL, and autoimmune/inflammatory diseases such as rheumatoid arthritis, RA. It is designed to provide the Investigator(s) and others involved in a first-in-man clinical trial with information to facilitate the understanding of the key objectives of the clinical study as well as to promote compliance with the key features of the protocol. The IB has been designed to provide a concise, simple, objective, balanced and non-promotional guidance that may enable the physicians or potential investigator(s) to make their own risk-benefit assessment of the overall design of the proposed clinical study.

1.2. Properties of TGN1412 drug product

TGN1412 is a genetically engineered and recombinantly expressed humanized monoclonal antibody of an IgG4-κ isotype that specifically reacts with the CD28 molecule on T lymphocytes. TGN1412 drug product for early clinical trials was manufactured by Boehringer Ingelheim GmbH & Co. KG according to cGMP standards. For details, please refer to Section 3.

1.3. TGN1412 proposed mode-of-action

TGN1412 is a first-in-class agonistic anti-CD28 monoclonal antibody that has been generated to therapeutically balance the immune system in diseases associated with life-threatening abnormalities in T-lymphocyte number and/or function. It is being developed for certain chronic autoimmune/inflammatory diseases such as RA or certain haematological malignancies such as B-CLL.

CD28 is a co-stimulatory receptor expressed on the cell surface of CD4 T lymphocytes (T cells) and on a large fraction of CD8 T cells. It efficiently co-stimulates resting T cells in combination with a signal from the T cell antigen receptor (TCR). Activation of the CD28 signaling pathway naturally requires simultaneous triggering of the TCR by antigen and of CD28 by its physiological membrane-bound ligands B7-1 or B7-2. TGN1412 bypasses the requirement for TCR triggering and activates T cells irrespective of their TCR specificity. This novel mode of T cell activation has been termed "agonistic" or "superagonistic", respectively. Both terms are used synonymously throughout this document and the literature cited.

TGN1412 induces a pronounced ex vivo expansion and activation of human T cells in the absence of additional stimuli. In pre-clinical animal models, in vivo application of TGN1412 or orthologous antibodies lead to a well-tolerated expansion and activation of T cells that is characterized by the absence of any detectable pro-inflammatory reactions. Moreover, TGN1412 variants have proven to have anti-inflammatory properties in animal models for autoimmune diseases. Therefore, depending on the state of the patient's immune system, the dose of TGN1412 to be applied and/or other parameters, TGN1412 has the potential to be beneficial for patients who suffer either from decreased T cell numbers and function (as in B-CLL) or from autoimmune disease(s) in which auto-aggressive T cells play an important role in disease pathogenesis (as in RA). For details, please refer to Section 2.

1.4. Medical rationale for the treatment of B-CLL and RA

On the one hand, TGN1412 will be used to reconstitute a collapsed T cell compartment in the context of haemato-oncological malignancies (e.g. 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 impacts both T- and B-lymphocyte subsets. At the T cell level, TGN1412 induces a profound polyclonal expansion and activation, as measured by absolute and relative T cell counts

and expression of activation markers such as CD25, CD69, CD40L, and CD134 (Ox-40). On the tumour cell level, TGN1412 indirectly mediates up-regulation of CD80 and CD86 via the CD40/CD40L pathway. The improved antigen-presentation by malignant B-cells results in an efficient recognition and elimination of leukaemic B cells by tumour-specific T cells in vitro. In addition, it could be demonstrated that apoptosis of leukaemic B cells was induced via CD95 (fas) and TRAIL pathways after co-culture with TGN1412.

Therefore, preclinical evidence suggests that TGN1412 has the potential to add a significant benefit to B-CLL patients by improvement of T cell numbers and function as well as induction of a long-lasting anti-tumour T cell response.

On the other hand, it could be demonstrated that superagonistic anti-CD28 antibodies orthologous to TGN1412 have anti-inflammatory properties in animals. In healthy animals, treatment with superagonistic anti-CD28 antibodies leads to a T cell expansion and activation dominated by the induction of anti-inflammatory cytokines such as IL-4 and IL-10 and the over-proportional expansion of regulatory T cell which play a key role in the control of pathogenic and auto-aggressive T cell responses. Consequently, in relevant animal models for RA, e.g. the rat adjuvant arthritis model or the rhesus monkey collagen-induced arthritis model, TGN1412 orthologues were effective in the prophylaxis and/or treatment of disease.

Therefore, preclinical evidence from multiple animal models including non-human primates suggests that TGN1412 has the potential to add a significant benefit to RA patients by induction of anti-inflammatory cytokines, expansion of regulatory T cells and/or other mechanisms that interfere with patho-mechnisms of disease.

1.5. Nonclinical pharmacology and toxicology

Since the TGN1412 epitope on the CD28 extracellular domain is restricted to humans and non-human primates, 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. A number of safety and efficacy studies with TGN1412 in non-human primates have been conducted using single-dose and multiple-dose regimen. The results of these studies show that TGN1412 is well tolerated in non-human primates at doses up to 50mg/kg/week for four consecutive weeks. No TGN1412-related signs of toxicity, hypersensitivity or systemic immune system deviation were observed in these studies. In a local tolerance study, intravenous, perivenous, or intra-arterial routes of TGN1412 administration were well tolerated and did not produce clinically significant irritation.

Non-clinical reproductive and developmental toxicity studies have not yet been performed. It is not known whether TGN1412 can cause fetal harm when administered to a pregnant woman or affect reproductive capacity. 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.

Non-clinical pharmacology studies showed that TGN1412 has a predictable, well-defined pharmacokinetic profile following infusion of doses of 5 to 50mg/kg body weight. Maximum serum concentration (Cmax) and area under the curve (AUC) have been largely proportional to dose and stable concentrations are observed with repeated dosing.

1.6. Proposed first-in-man clinical trial

Until December 2005, no human subjects have been exposed to TGN1412. The design and choice of trial population of this first-in-man clinical phase-I trial is based on the need to initially demonstrate the safety of TGN1412 in man. The safety and immunological outcome measures in this trial, which may also answer questions concerning the mechanism of action of TGN1412

should help guiding the choice of dose and dose frequency for subsequent single- and multiple-dose studies in B-CLL and/or RA patients.

The study is designed as a single-centre, double-blind, randomised, placebo-controlled, dose-escalation trial, including 32 healthy male subjects, who will be divided into four groups of eight subjects each. In each group, six subjects will receive verum and two subjects placebo (random ratio: 3:1). Intravenous (i.v.) doses of 0.1, 0.5, 2.0 and 5.0 mg/kg body weight are planned to be investigated. These i.v. doses will be administered as short-term infusion. Dose escalation to the next dose level will proceed following satisfactory review of safety data from at least fourteen days following each administration. This review will be done by a Data Safety Monitoring Board.

Primary objectives of the proposed study will be the assessment of the safety and tolerability of ascending single intravenous doses TGN1412 in separate cohorts of healthy volunteers and the determination of the pharmacokinetics of single intravenous doses of TGN1412. Secondary objectives will be the determination the effect of acute administration of TGN1412 on lymphocyte subsets, the assessment of the cytokine profile following acute administration of TGN1412 and the assessment of anti-TGN1412 antibodies up to seven weeks post-dose.

An important aspect of this first-in man-trial will be to examine the effect of different doses of TGN1412 on immunological parameters such as lymphocyte subset composition and activation state, T cell functionality, serum cytokine profile etc.. This will help to further examine the mechanism of action of this novel agent and to provide a high degree of comfort concerning its pharmacodynamic effects in humans before moving into a diseased population.

1.7. Clinical management of the study

Clinical management of the study will be according to the study protocol. In short, a healthy volunteer trial population will be selected according to well-defined eligibility (inclusion/exclusion) criteria that take into account, inter alia, the immune status of the subjects. Each subject in each treatment group will be randomly assigned to receive active drug or placebo. All medication will be administered intravenously by a study physician. All concomitant medications taken during the study will be recorded. Although not to be expected after TGN1412 administration, a cytokine release syndrome or other immunological complications may occur after infusion of TGN1412. In this case, high-dose glucocorticoids, anti-histaminic drugs as well as other appropriate treatment shall be considered for symptom relief, as appropriate. Any adverse events will be assessed and reported according to the CRO's standard operating procedures (SOP).

2 INTRODUCTION

2.1. Cellular distribution and function of CD28

CD28 is a disulfide-linked homodimeric member of the immunoglobulin superfamily. Soon after its discovery in 1985 (Hara T, 1985), it was recognized as an important co-stimulatory receptor on resting, naive T cells. CD28 expression is restricted to lymphocytes: 60% to 80% of peripheral blood $CD3^+$ T lymphocytes express CD28 with almost all $CD4^+$ T lymphocytes being $CD28^+$ and a variable fraction (approximately 50%) of $CD8^+$ T lymphocytes being $CD28^+$ (Riley and June, 2005). According to some investigations, CD28 can also be expressed by plasma cells and myeloma cells (Jego et al., 1999; Shapiro et al., 2001).

CD28 has two ligands, B7-1 (CD80) and B7-2 (CD86), which are expressed on antigen-presenting cells. The physiological function of the interaction of B7-1/2 with CD28 is the co-stimulation of T cells: CD28-derived signals synergize with signals from the TCR to promote T lymphocyte proliferation, metabolism, cytokine production, resistance to apoptosis, and long-term expansion (Sharpe and Freeman, 2002). Recently, it has also been shown that B7:CD28 interactions are critical for the generation and/or maintenance of regulatory T cells which maintain peripheral T lymphocyte tolerance (Bluestone and Abbas, 2003; Bluestone and Tang, 2004).

Based on the key role of CD28 and B7 molecules in the activation and differentiation of T lymphocytes and adaptive immunity, these molecules have become attractive targets for immunotherapeutic approaches in the treatment of caner or autoimmune/inflammatory diseases (Riley and June, 2005). In light of the multitude of reagents developed against CD28 and related receptors, it is worth mentioning that the proposed TGN1412 therapeutic mode-of-action is considered to be unique and not shared by any of the other therapeutic approaches targeting members of the CD28 or B-7 family (see below).

The rest of this section (to page 20) has been withheld under section 43(2) of the FOI Act as, in the MHRA's view, disclosure would, or would be likely to, prejudice the commercial interests of TeGenero or associated third parties. In the Agency's view the public interest in disclosure is not outweighed by the public interest in withholding the information.

3 PHYSICAL, CHEMICAL, AND PHARMACEUTICAL PROPERTIES AND FORMULATION

3.1. Description

TGN1412 is a humanised monoclonal antibody directed against the human CD28 antigen. The molecule was genetically engineered by transfer of the complementarity determining regions (CDRs) from heavy and light chain variable region sequences of a monoclonal mouse anti-humanCD28 antibody (5.11A1, Luhder et al., 2003) into human heavy and light chain variable region frameworks. Humanised variable regions were subsequently recombined with a human gene coding for the IgG4 gamma chain and with a human gene coding for a human kappa chain, respectively.

The key functional property of TGN1412 is its capability to activate T cells independently of triggering of the antigen receptor, TCR, and to induce T cell proliferation when given to human PBMCs in solution. Just like antibody 5.11A1, from which it was derived, TGN1412 binds to a particular epitope on CD28 located close to the plasma membrane, the so called C"D-loop. This site is different from the binding site for CD80 and CD86, the natural ligands of CD28 (Luhder et al., 2003). Specific binding to a homologous epitope was also demonstrated for JJ316, the functionally equivalent antibody against rat CD28. Antibodies against rat or human CD28 without agonistic properties were shown to bind to clearly distinct epitopes (Luhder et al., 2003). For all antibodies analysed, agonistic activity correlated with binding to the C"D-loop. Binding to the C"D-loop also was sufficient to transfer agonistic activity. Therefore, the unique way of binding to the CD28 molecule is considered to be the cause of the effect of TGN1412 on T cell activation.

The rest of this section (to page 23) has been withheld under section 43(2) of the FOI Act as, in the MHRA's view, disclosure would or would be likely to, prejudice the commercial interests of Tegenero or associated third parties. In the Agency's view the public interest in disclosure is not outweighed by the public interest in withholding the information.

4 NONCLINICAL STUDIES

4.1. Primary pharmacodynamics

4.1.1. Specificity and mode-of-action of TGN1412

Specificity of TGN1412 for human CD28 has been assessed with various model assays. Most importantly, the topological requirements of agonistic antibodies have been investigated by epitope mapping using mutated CD28 molecules (Luhder et al., 2003). It was shown that agonistic anti-CD28 monoclonal antibodies 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. Recently, the critical involvement of the C"D loop for CD28 superagonist binding has been confirmed by X-ray crystallography (Evans et al., 2005). The affinity of TGN1412 to recombinant human CD28 construct has been determined by Biacore analysis to be 1.88×10^{-9} M (Kd) [redacted] * [redacted]. By flow cytometry, TGN1412 stained human PBMC cell suspensions in a pattern characteristic and specific for CD28. Moreover, TGN1412 bound to cell lines transfected with the human CD28 gene, but not with genes encoding the two most closely related receptor, cytotoxic T lymphocyte antigen-4 (CTLA-4) and inducible co-stimulator (ICOS) (study report [redacted] * [redacted] These data show that TGN1412 is specific for CD28 (see section 4.5). In addition to TGN1412, orthologous antibody formats have been used in pre-clinical studies (fig. 4). 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).

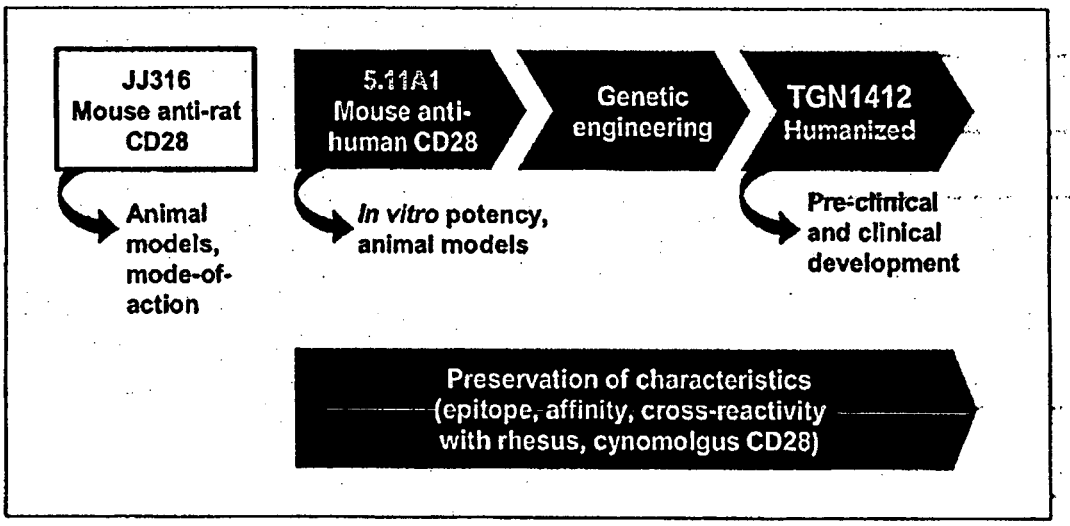

Figure 4: The (super-) agonistic principle has been initially described for the anti-rat CD28 specific antibody JJ316. Monoclonal antibody 5.11A1 represents the first anti-human CD28 antibody with agonistic properties. Based on the 5.11A1 sequence, fully humanised anti-CD28 antibody TGN1412 was generated by genetic engineering.

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.

* withheld under section 43(2) of the FOI Act - see page 11

Sections 4.1.2 to 4.1.5 (page 24 to this page) have been withheld under section 43(2) of the FOI Act as, in the MHRA's view, disclosure would, or would be likely to, prejudice the commercial interests of TeGenero or associated third parties. In the Agency's view the public interest in disclosure does not outweigh the public interest in withholding the information.

4.2. Secondary pharmacodynamics

4.2.1. In vitro cytotoxicity

To assess the cellular cytotoxicity of TGN1412, standard Complement Dependent Cytotoxicity (CDC) and Antibody Dependent Cellular Cytotoxicity (ADCC) assays were performed. The monoclonal antibody alemtuzumab (Campath-1H) directed against human CD52 and TGN1112, were used as controls. The results are summarized in table 5 (study report [redacted]

* see page 31

As expected, the incubation of human PBMC with Campath-1H resulted in a significant CDC, while TGN1412 and TGN1112 did not mediate CDC. ADCC was investigated using modified human Jurkat cell lines. While Campath-1H mediated ADCC against a CD52+ variant, TGN1112 mediated ADCC against all CD28+ cells lines and TGN1412 exerted no ADCC towards the tested Jurkat cells lines.

Table 5: Summary of cytotoxicity assays.

mAb	CDC (huPBMC)	ADCC (Jurkat CD28+ CD52+)	ADCC (Jurkat CD28+ CD52-)
Campath	+	+	-
TGN1112	-	+	+
TGN1412	-	-	-

4.3. 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 are investigated. Usually, two acute rodent safety pharmacology studies are required to assess potential changes of behaviour and respiratory system. These studies cannot be conducted in non-human primates. Since TGN1412 is highly specific for primate CD28 (see section 4.5.1), studies in rodents or dogs are not expected to deliver meaningful results. Due to the fact that no cross-reactivity with cardiovascular tissue has been observed for TGN1412 [redacted] *, it is believed that a telemetry study in cynomolgus monkeys is not reasonable at this stage of development and that it is sufficient to evaluate safety pharmacology endpoints as part of toxicology and pharmacology studies.

4.3.1. Cardiovascular system (CV)

As described above, TGN1412 does not cross react with cynomolgus monkey or human heart tissue. In addition, no electrocardiogram changes (heart rate, P-R interval, QRS interval and Q-T interval) were observed in the main 28-day repeated dose toxicology study in cynomolgus monekys. 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.

4.3.2. 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.

4.3.3. 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,

* Withheld under section 43(2) of the FOI Act - see page 11

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. Therefore, it is concluded, that treatment with TGN1412 is not expected to adversely affect the central nervous system.

4.4. 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 [redacted]. Under no culture condition studied and irrespective of the concentration range of this DMARD did MTX interfere with TGN1412-induced T cell proliferation. These results may have implications for the design of future clinical trials as they indicate that the use of TGN1412 may be combined with MTX administration.

* see page 31

4.5. Pharmacokinetics (PK)

Classical conventional adsorption, distribution, metabolism and excretion (ADME) studies have not been conducted for the agonistic anti-CD28 monoclonal antibody TGN1412, because pathways of protein degradation are common knowledge. It is believed that TGN1412, like other antibodies, is catabolized by lysosomal enzymes in the kidney and/or liver into amino acids which are then reabsorbed.

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

4.5.1. PK of JJ316 in the rat

In the rat adjuvant arthritis model, a Cmax of approx. 100 µg/ml was measured in serum following i.v. treatment with a dose of 5 mg/kg JJ316. JJ316 was not detectable 7 days after injection. A dose-dependent lymphocytosis was observed in lymph nodes and spleen between day 2 and day 4 post injection. The maximum level of T cell activation and proliferation was detected on day 3 post injection of JJ316. In line with earlier observations in healthy animals, (Lin and Hunig, 2003), an expansion of CD4+CD25+ T cells was observed.

4.5.2. PK of TGN1112 in rhesus monkeys

In the rhesus monkey, a Cmax of 140µg/ml was measured in serum following i.v. administration of TGN1112, an IgG1 variant of TGN1412, at 5 mg/kg. TGN1112 was detectable for approx. 20 days post injection. A lymphocyte subset PK analysis revealed that the peak level of T cell expansion (CD3+ cells) was around day 20. It could be demonstrated that CD4+ and CD8+ T cells were expanded approximately equally well, whereas CD20+ B cells were not affected by TGN1112 treatment (section 4.5.3.1).

4.5.3. *Toxicokinetics (TK) of TGN1412 in cynomolgus monkeys*

Toxicokinetics of TGN1412 were assessed in the course of a pilot dose-range finding study and as part of the 28-day repeat-dose toxicology study performed in cynomolgus monkeys. TGN1412 serum concentrations vs. time profiles were generally consistent with intravenous administration of a monoclonal antibody. Due to the variability of toxicokinetic profiles observed, mean values per group were calculated. They are presented in Figure 9. For TK analysis, serum samples were collected until day 40 after last dosing in recovery group animals. A terminal elimination half-lifve 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.

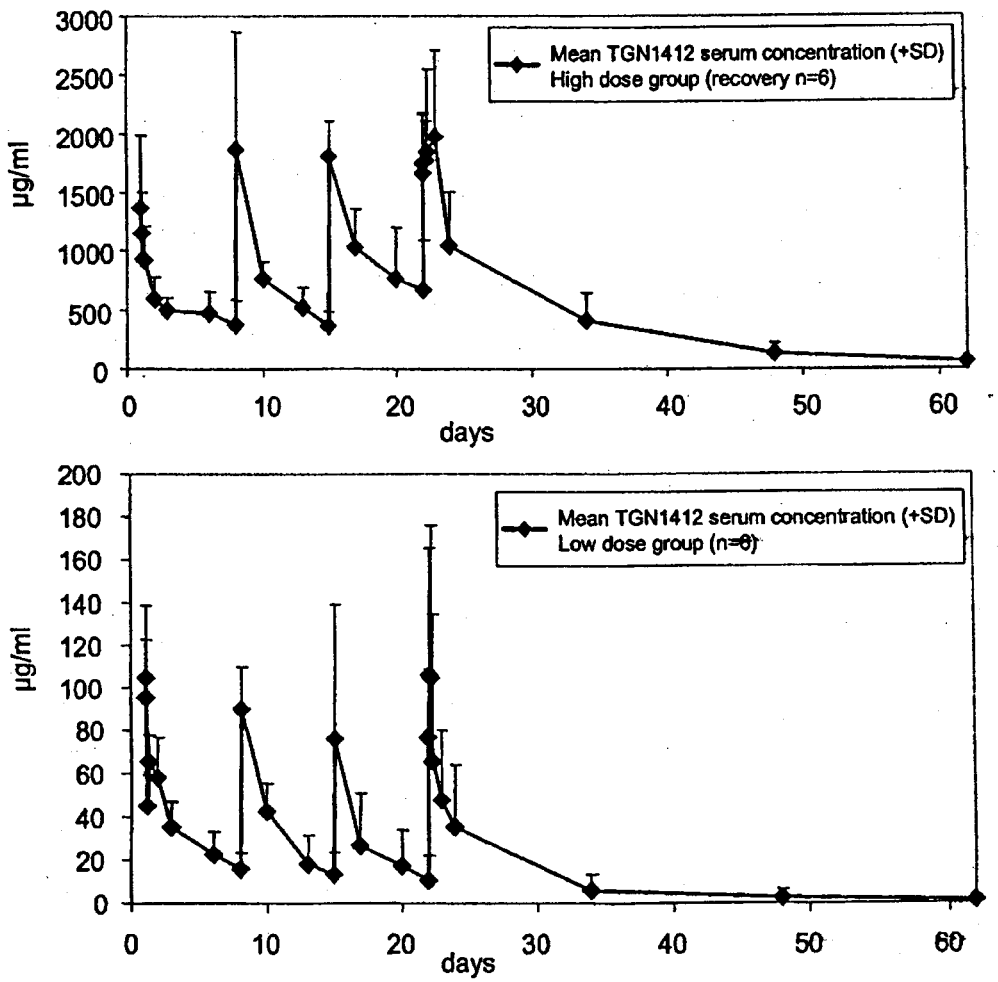

Figure 9: Toxicokinetic profiles following repeated administration (4 doses in weekly intervals) of TGN1412. Due to the high variability of TK data, mean values are given (n=6). Values of Cmax, systemic exposure and values for terminal elimination half-life increased as dose increased (low dose = 5 mg/kg; high dose = 50 mg/kg).

Systemic exposure to TGN1412 increased by up to approx. 20-fold as the dose increased from 5 to 50 mg/kg (Table 6). 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.

Table 6: Toxicokinetic evaluation of TGN1412 after first (day 1-8) and fourth administration. Mean values for AUC and Cmax are shown.

	N	Mean AUC (µg*h/ml)		C_{max} (µg/ml)	
		Day 1-8	Day 1-4	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

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

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

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

4.6. Toxicology

4.6.1. Selection of animal model

Binding of TGN1412 and TGN1112 to T cells of several primate species has been investigated. Both antibodies specifically react with human, rhesus and cynomolgus T cells but not with marmoset lymphocytes. TGN1412 does not cross-react with rat or mouse CD28. The results of the binding studies are summarized in table 7.

Table 7: Summary of binding studies (JJ316 = anti-rat CD28 antibody; 5.11A1 = mouse anti-human CD28 antibody; TGN1112 = humanised anti-human CD28 antibody (IgG1); TGN1412 = humanized anti-human CD28 antibody (IgG4)); n.d. = not determined

Antibody	Jurkat cell line		Rat	Mouse	Non-human primate			Human
	CD28+	CD28-	PBMC	Human C''D loop transplant	rhesus	cynomolgus	marmoset	PBMC
JJ316	n.d.	n.d.	+	n.d.	n.d.	n.d.	n.d.	-
5.11A1	+	-	-	+	n.d.	+	n.d.	+
TGN1112	+	-	-	+	+	+	-	+
TGN1412	+	-	n.d	+	+	+	n.d.	+

These findings are supported by a homology analysis on the basis of the C''D loop amino acid sequence. A homology of 100% could be found when C''D loop sequences of human, cynomolgus and rhesus monkey origin were compared, whereas the marmorset C''D differs in 2 out of 6 amino-acids. The rodent C''D loop is characterised by a very low or no homology to human CD28 C''D loop. The C''D loop sequence of dog, cat and woodchuck exhibits two amino-acid mutations in the binding epitope. Consequently, homology studies indicate that TGN1412 is not expected to react with the CD28 molecule from species other than primates.

4.6.2. Single-dose toxicity

In general, the single dose (acute) toxicity of a novel medicinal product should be evaluated in two mammalian species (usually two different rodent species) prior to the first human exposure (ICH-M3). Due to the specificity of TGN1412 for human and non-human primate CD28, a standard single dose toxicity testing in rodents was considered to be not appropriate.

4.6.3. Pilot study in rhesus monkeys

A pilot study to assess safety, pharmacodynamics, pharmacokinetics and tolerability of TGN1412 (n=1) and the IgG1 variant TGN1112 (n=2) has been conducted in rhesus monkeys (macaca mulatta, TeGenero study report [redacted] 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. * see page 31.

TGN1412 or TGN1112 were injected i.v. at a total dose of 2.5 mg/kg or 5 mg/kg body weight. Routine health assessment of animals included palpation of lymphnodes (axial, inguinal, abdominal), examination of heart, lungs and oral cavity. All treated animals tolerated

TGN1412 or TGN1112 injection well. Haematological parameters (haemoglobin, haematokrit, erythrocyte counts and thrombocytes) were not affected by antibody treatment.

A 2-fold transient increase in CD4 and CD8 cell numbers peaking at day 16 was observed after TGN1112 treatment (Fig. 10). Rhesus-anti-human antibodies (RAHA) were determined by ELISA. Only a moderate RAHA response was observed in treated animals, appearing around day 20. Both anti-isotype and anti-idiotype responses were observed in the animal treated with 5 mg/kg, whereas anti-isotype and anti-idiotype response in the animal treated with 2.5 mg/kg was very low.

It was observed that TGN1412 had a significant lower pharmacological activity in rhesus monkeys as compared to TGN1112. This may be explained by different affinities and/or FcR-binding properties of the two antibody formats. For toxicology studies with TGN1412, the cynomolgus monkey was selected as the more appropriate non-human primate species.

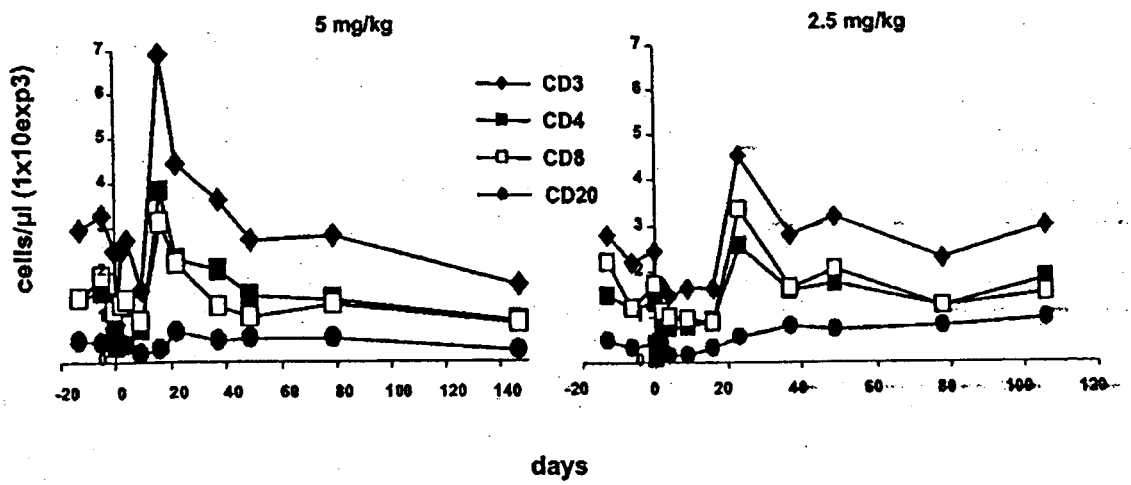

Fig. 10: Lymphocyte subset kinetics in rhesus monkeys after treatment with a dose of either 5mg/kg or 2.5mg/kg of an IgG1 variant of TGN1412 (TGN1112). The animal receiving 5 mg/kg of TGN1112 showed a transient increase (> 2-fold) in peripheral CD4 and CD8 T cell numbers which peaked at day 16. Similarly, expansion of peripheral T cells, involving both CD4+ and CD8+ subsets, was observed in the animal treated with 2.5 mg/kg of TGN1112 around day 23 post-injection. In addition, this animal exhibited a delayed, 2-fold rise in B cell numbers which started to become apparent on day 78 and lasted until day 106.

4.6.4. Repeated-dose toxicity

4.6.4.1. Pilot dose-range finding study in cynomolgus monkeys

A 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 in the non-human primate [redacted] 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. In a previous pre-clinical pharmacology study with rhesus monkeys, administration of 2.5 mg/kg of TGN1412 (IgG4) and 2.5 and 5 mg/kg of the IgG1 isotype variant TGN1112 had been well tolerated. No signs of toxicity had been observed. Therefore, a dose of 5 mg/kg was used as the lowest dose for this pilot dose ranging study. The dosing regimen is shown in table 8.

* see page 31

Table 8: Treatment schedule cynomolgus pilot study

Day of Study	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 pair of one male and one female cynomolgus monkeys (animals 1M and 3F) received TGN1412 at dose levels of 5, 10 or 25 mg/kg, by intravenous infusion over a one hour period, 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 limited signs of toxicity, the same pair of animals as well as another pair of naïve 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 for this route of administration. All animals were retained for a subsequent 26 day recovery assessment.

In essence, the data show that single administration of up to 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. No plasma biochemical changes were detected during the study. Necropsy revealed no organ weight changes or macroscopic findings related to treatment.

Due to the T cell activating capacity of TGN1412, determination of the general effect on the immune system plays an important role in pre-clinical development. On the other hand, differentiation between unintended immunotoxicological effects and intended pharmacodynamic effects represents a challenge for the preclinical development of TGN1412. Therefore, leukocyte counts and changes in lymphocyte subsets and their activation status were carefully monitored by flow cytometry during toxicology studies. The following marker combinations were used in the pilot study in cynomolgus monkeys:

This tabulation has been withheld under section 43(2) of the FOI Act as, in the MHRA's view, disclosure would or would be likely to prejudice the commercial interests of TeGenero or associated third parties. In the Agency's view the public interest in disclosure is not outweighed by the public interest in withholding the information.

Flow cytometry was conducted on 3 occasions pretrial and on days 1, 8, 15, 22, 27, 34, 41 and 48, using the cell surface markers listed above. An example for TGN1412 mediated immunomodulation is presented in figure 9. CD4 and CD8 positive T lymphocytes were expanded substantially and showed a clear peak around day 15 post infusion (Fig. 11A). The profound T cell expansion was paralleled by cellular activation as measured by CD69 and CD25 (Fig. 11, B + D). However, the modulation of T cell subsets is clearly attributed to the primary pharmacodynamic activity of TGN1412.

TGN1412 could be detected on the surface of CD4+ T cells (Fig. 11C). This observation correlated inversely with the availability of free TGN1412 binding sites (Fig. 11C). A decrease in NK cell numbers was observed following injection of TGN1412 (Fig. 11E), which returned to baseline after completion of dosing (day 22). B cell numbers were somewhat increased (Fig. 11F) after injection of TGN1412.

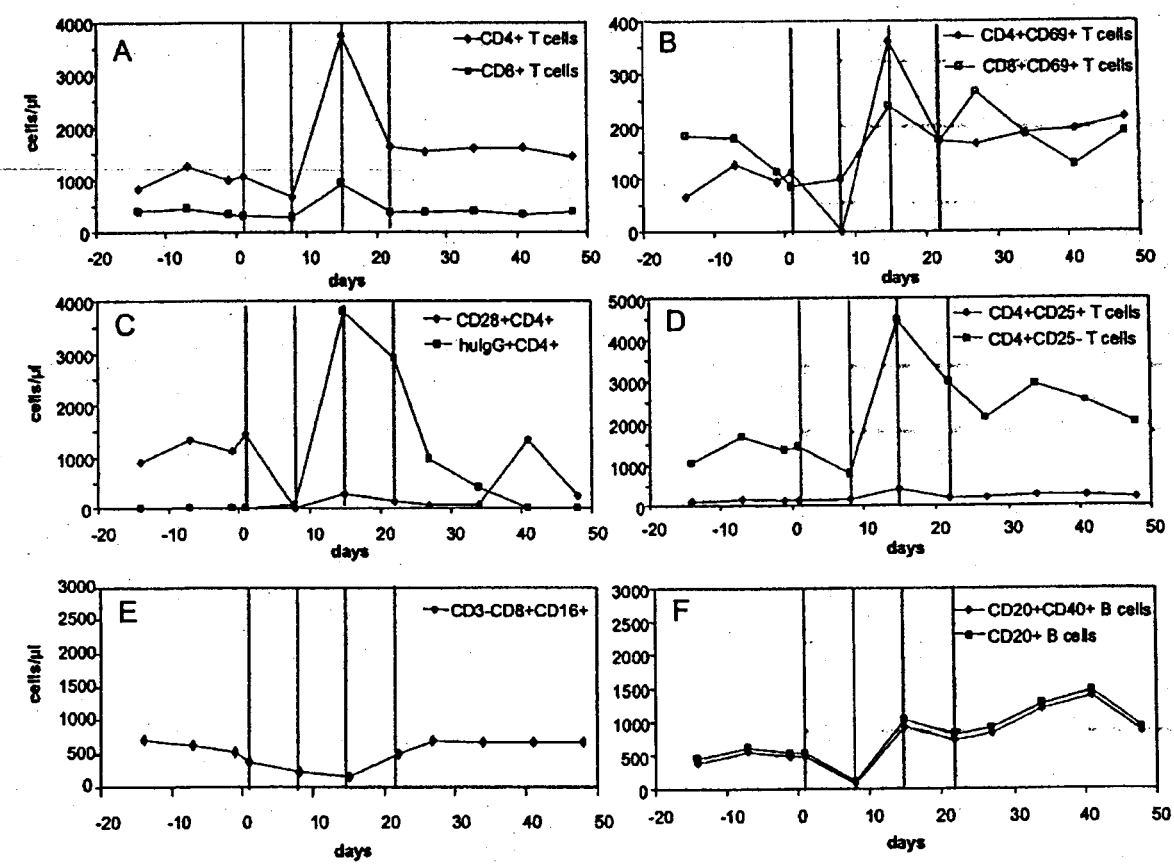

Fig. 11: Flow cytometry analysis of leukocyte subsets in a male cynomolgus monkey (1M) following intravenous injection of weekly (days 1, 8, 15, 22) escalating doses (5.0mg – 10.0mg – 25.0mg – 50.0mg/kg) of TGN1412. (A) CD4 and CD8 positive T lymphocytes were expanded substantially and showed a clear peak. This T cell expansion was paralleled by cellular activation as measured by CD69 and CD25 (B + D). TGN1412 could be detected on the surface of CD4+ T cells (as determined by anti-huIg; C). This observation correlated inversely with availability of free TGN1412 binding sites (CD28+CD4+; C). A descrease in NK cell numbers was observed following injection of TGN1412,

which returned to baseline after completion of dosing (day 22). A tendency towards increased B cell numbers was observed (F) after injection of TGN1412.

Toxicokinetic investigations indicated that serum concentrations of TGN1412 at the end of the infusion period increased in proportion with the dose level (Fig. 12). During the observation period, serum concentrations were lower than Day 22 values and concentrations gradually decreased for all animals during this period.

The immunogenicity of TGN1412 was determined by measurement of primate-anti-human-antibodies, using a validated ELISA methodology. In both cynomolgus studies, immunogenicity of TGN1412 seemed to be very low. In the pilot study, only one out of four animals (1M) showed a significant titer of anti-TGN1412 antibodies 40 days post infusion (see section 4.6.9.2).

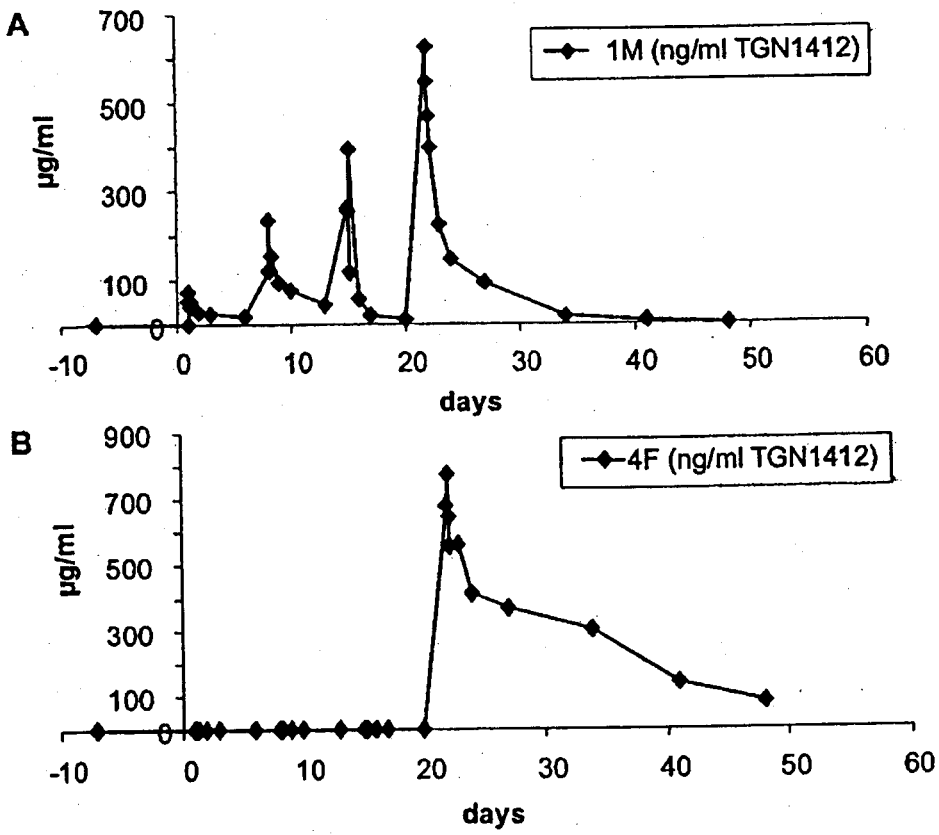

Fig 12. (A) Toxicokinetic evaluation in a male cynomolgus monkey following intravenous application of escalating doses (5.0mg – 10.0mg – 25.0mg – 50.0mg/kg) of TGN1412. Serum levels increased proportionally with dose. (B) Toxicokinetic evaluation in a female cynomolgus monkey following intravenous application of a single dose of 50.0mg/kg of TGN1412 on day 22. (B) Anti-TGN1412 antibody formation. No anti-TGN1412 immune response was found in animal 4F.

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 toxicology studies.

4.6.4.2. 28-day repeated dose toxicology study in cynomolgus monkeys

A 28-day repeated dose toxicology study in cynomolgus monkeys was conducted (n=26). Four weekly doses of TGN1412 were administered intravenously by short infusion [redacted] Treatment was conducted according to the tabulated schedule below:

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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

The study was designed on the basis of results from the above-mentioned pilot study in cynomolgus monkeys in order to define a "No-Observed-Adverse Effect Level" (NOAEL) and a potential "Maximum Tolerated Dose" (MTD) of TGN1412. In the control group and the high dose group, 10 female and 10 male monkeys were treated by weekly intravenous administration of TGN1412 (short infusion). Two male and two female monkeys were sacrificed on day 28, after they have had received the last of four doses TGN1412. The six remaining animals were followed-up in a recovery/observation period of six weeks. This procedure was chosen in order to demonstrate reversibility of possible toxicology findings after completion of the dosing period. In the low dose group, 6 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.

General monitoring was carried out during the study and included food intake, body weight, haematology, clinical chemistry, urinalysis, ophthalmology, ECG and general behaviour. The injection site was 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 a validated ELISA methodology to obtain PK/ immunogenicity data data.

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 respite period between each administration. Dose volumes of 1 or 10 mL/kg body weight were used respectively. A control group consisting of five male and five female cynomolgus monkeys received the vehicle alone, TGN1412-Placebo vehicle buffer, at a dose volume of 10 mL/kg body weight. After four weeks, two males and two females from the control and high dose groups were killed and the remaining animals were retained for a subsequent 41 (females) or 42 (males) day recovery assessment.

There were no treatment-related deaths and no signs attributable to treatment. Body weight performance and food intake were unaffected by treatment. There were no ocular changes during week 4. No electrocardiogram changes were observed following treatment with TGN1412. There were no haematological or plasma biochemical findings or any change in urinary composition during week 4 of treatment. Organ weights were unaffected by treatment and there were no treatment-related necropsy findings after 28 days.

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

Generally, the immunogenicity of TGN1412 was low in cynomolgus monkeys. Only four out of 16 treated animals showed substantial titres of anti-TGN1412 antibodies in serum. These were observed 3 to 4 weeks after start of dosing.

Overall, it was concluded that the once weekly one-hour slow intravenous infusion of TGN1412 to cynomolgus monkeys at dose levels up to 50 mg/kg (the maximal practicable dose for this route of administration) for four weeks was not associated with any toxicologically significant changes and this dose level was therefore considered to be the no-observed-adverse-effect level (NOAEL) as identified in this study.

Toxicokinetic (TK) evaluation

Toxicokinetic investigations were conducted as part of the 28-day toxicology study in cynomolgus monkeys. 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.

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.

Flow cytometry evaluation

Contingent on a rather high degree of variability in pre-trial data, the flow cytometric data assessed imply that TGN1412 expanded both CD8+ and CD4+ T cells in male and and less pronounced in female animals. Expansion appeared to optimally occur at the 5 mg/kg body weight 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 a high variability in NK cell numbers observed in control and treatment group animals with a tendency to increased values during the recovery period. In addition, TGN1412 appeared to also induce a B cell expansion that persisted longer than the observed T cell expansion, especially in male animals.

From determination of free CD28 binding sites, it appeared that initial dosing saturated most if not all CD28 binding sites. Recovery of free CD28 binding site was observed at several timepoints during dosing and in the recovery period. It is not clear, whether lack of free binding sites was 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 antibody TGN1112-FITC.

In conclusion, TGN1412 expanded CD4+ and CD8+ T cells efficiently in male animals at the 5 mg/kg dose level. Increase in CD25+CD4+ T cell numbers appeared to correlate with 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.

4.6.5. 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.

4.6.6. 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.

4.6.7. 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 [redacted] 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 [redacted]

* see page 31

4.6.8. Local tolerance

4.6.8.1. 28-day repeated dose toxicity study in cynomolgys monkeys

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.

4.6.8.2. Local tolerance study in rabbits

Local tolerance of TGN1412 in comparison to saline 0.9% was tested in New Zealand white female rabbits. TGN1412 and control were applied intravenously (i.v.), intraarterially (i.a.) and paravenously (p.v.) to a total of four female rabbits.

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 TGN1412 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 was concluded that a single intravenous, intraarterial or paravenous application of 10 mg/ml TGN1412 in a volume of 0.5 ml is well tolerated.

4.6.9. Other toxicology studies

4.6.9.1. Human and non-human primate tissue cross-reactivity

A tissue cross reactivity study has been performed to determine the cellular localisation of antibody in a range of normal human and cynomolgus monkey tissues [redacted] This identifies sites, other than target sites, with which the antibody cross-reacts and aids in interpretation preclinical toxicity studies. Tissues were examined from 3 unrelated human donors and 3 cynomolgus monkeys.

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For each batch of staining, samples of positive and negative tissue controls were used. 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 of the stomach, small intestine and large intestine. The distribution of lymphocyte staining recorded was consistent with the expected distribution of T cells within lymphoid tissue. This lymphoid staining reflects 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. However, central nervous system (CNS) tissue cross reactivity was not associated with CNS related adverse clinical symptoms/toxicology findings in cynomolgus monkeys. 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 of 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 is an artefact 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.

4.6.9.2. Immunotoxicicology studies

The potential adverse effects of TGN1412 on the immune system were evaluated as part of the standard toxicity studies and as 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 neutophil 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.

No treatment related alterations in immune system organ weights and histology (thymus, spleen, lymph nodes and bone marrow) were observed in cynomolgus monkeys during the 28-day repeated dose toxicology study that could be attributed to treatment with TGN1412. 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 maybe interpreted as a pharmacodynamic effect of the agonistic anti-CD28 monoclonal antibody.

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

Finally, there was no evidence for an increase incidence of infections nor carcinogenicity in animals treated with agonistic anti-CD28 monoclonal antibodies. Therefore, it is concluded that TGN1412 does not mediate a generalised immunosuppression.

Additional immunotoxicity studies

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 specific cell types and activation markers (see section 4.5.4.2.2). In accordance with its capability to induce T-cell proliferation, a transient, reversible 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 cells 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.

There is evidence that agonistic anti-CD28 monoclonal antibodies may indirectly affect the B-cell compartment, since a trend towards increased B-cell numbers was observed in animals treated with TGN1412 in toxicology studies performed in cynomolgus monkeys. In general, this observation is confirmed by findings from pharmacology studies performed in rats using an orthologous antibody with specificity for rat CD28 (Tacke et al., 1997). Consequently, absolute and relative B-cell counts, immunoglobulin levels, rheumatoid factor and ANA antibodies will be closely monitored during clinical trials in order to detect potential B-cell mediated adverse or autoimmune effects of TGN1412.

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, decrease NK cell numbers were observed in the pilot toxicology study after treatment with TGN1412. Therefore the observed changes could not be clearly attributed to TGN1412.

Free CD28 binding sites appeared to be very quickly saturated after dosing of animals with TGN1412. Recovery of free CD28 binding site was observed at several timepoints during dosing and in the recovery period.

The effect of the agonistic anti-CD28 mAb on the T-cell dependent antibody response (TDAR) and cell-mediated immunity was investigated in an orthologous rat model. In lethally irradiated, bone marrow reconstituted hosts, an agonistic anti-rat CD28 monoclonal antibody (1mg total dose, ~ 5mg/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. The responses to a protein model antigen, keyhole limpet hemocyanin (KLH), applied in vivo three weeks after T cell reconstitution were analysed. 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 [redacted] A single injection of an IgG1 variant of TGN1412 (TGN1112, 2.5 mg/kg) did not alter the recall response to KLH: The animal was immunized with KLH and received a booster immunization 2 weeks prior to injection of TGN1112. The recall response to KLH 14 days before and 23 days after administration of TGN1112 was analyzed in in vitro proliferation assays after stimulation of PBMC with KLH for 60 hours. The recall response to KLH before and after treatment with TGN1112 was similar at all KLH concentrations tested indicating that the frequencies and function of primed T-cells were not affected by TGN1112 treatment. *

These observation confirms the assumption, that agonistic anti-CD28 triggered expansion of CD4+ T cells increases the Th2 compartment while leaving Th1 function intact. Importantly, T-cell memory was maintained after agonistic anti-CD28 treatment.

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 [redacted] 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 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. *

* see page 31

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 5000 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 9 provides a summary of the mean peak serum concentrations of the measured cytokines.

Table 9: Mean peak serum concentrations in each dosing group after administration of TGN1412 in cynomolgus monkeys

Cytokine	Mean peak cytokine level (range) in 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 (Hsu 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 [redacted] 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 31

In the pilot, only one of four animals showed a substantial anti-TGN1412 antibody titre (animal 1M, Figure 13). Antibodies against TGN1412 were detected approximately three weeks following initiation of dosing.

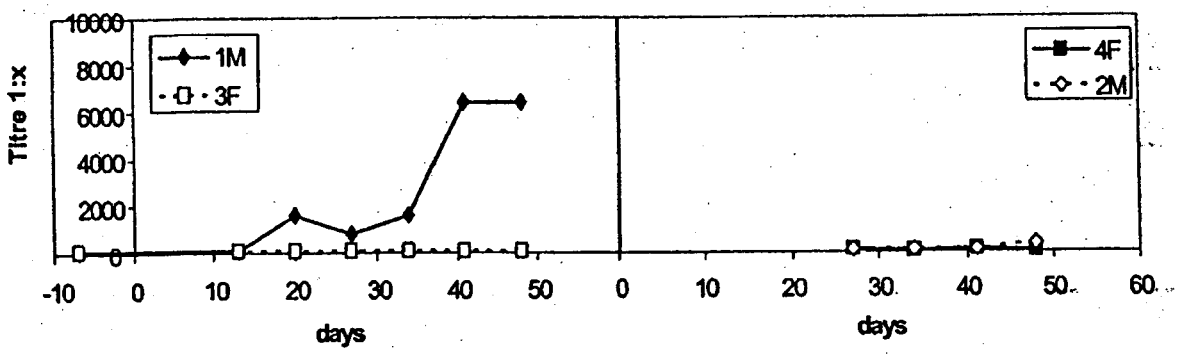

Figure 13: Immunogenicity (serum titres of anti-TGN1412 antibodies) of TGN1412 in the cynomolgus monkey. Animals 1M and 3F were treated with four weekly escalating doses of TGN1412 (5 – 10 – 25 – 50 mg/kg). Animals 2M and 4F received a single dose of 50mg/kg on day 22.

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.

4.6.10. Summary of toxicology studies

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. TGN1412 was well tolerated at doses up to 50 mg/kg/week for at least four consecutive weeks in cynomolgus monkeys. No adverse effects on major physiological systems (cardiovascular system, respiratory system and central nervous system) were reported for TGN1412. 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.

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

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.

Overall, 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 healthy subjects. Based on a NOAEL of 50 mg/kg body weight, the clinical starting dose of 0.1 mg/kg body weight represents a safety margin of 500-fold, which is considered to be sufficient to ensure patient safety. The maximum dose in this clinical trial is 5.0 mg/kg body weight, still being 10-fold lower than the observed NOAEL in pre-clinical toxicology studies.

5 EFFECTS IN HUMANS

5.1. Overview

As of December 2005 no human subjects have been exposed to TGN1412. No data are available from ongoing clinical studies.

The design and choice of trial population of this first-in-man clinical phase-I trial is based on the need to initially demonstrate the safety of TGN1412 in man. The safety and immunological outcome measures in this trial, which may also answer questions concerning the mechanism of action of TGN1412 should help guiding the choice of dose and dose frequency for subsequent single- and multiple-dose studies in B-CLL and/or RA patients. In addition, serum drug concentrations and anti-TGN1412 antibody formation will be measured to determine the rate and extend of escalating doses of TGN1412 in men.

The study is designed as a single-centre, double-blind, randomised, placebo-controlled, dose-escalation trial, including 32 healthy male subjects, who will be divided into four groups of eight subjects each. In each group, six subjects will receive verum and two subjects placebo (random ratio: 3:1). Intravenous (i.v.) doses of 0.1, 0.5, 2.0 and 5.0 mg/kg body weight (b.w.) are planned to be investigated. These i.v. doses will be administered as short-term infusion. Dose escalation to the next dose level will proceed following satisfactory review of safety data from at least fourteen days following each administration. This review will be done by a Data Safety Monitoring Board.

Primary objectives of the proposed study will be the assessment of the safety and tolerability of ascending single intravenous doses TGN1412 in separate cohorts of healthy volunteers and the determination of the pharmacokinetics of single intravenous doses of TGN1412. Secondary objectives will be the determination the effect of acute administration of TGN1412 on lymphocyte subsets, the assessment of the cytokine profile following acute administration of TGN1412 and the assessment of anti-TGN1412 antibodies up to seven weeks post-dose.

An important aspect of this first-in man-trial will be to examine the effect of different doses of TGN1412 on immunological parameters such as lymphocyte subset composition and activation state, T cell functionality, serum cytokine profile etc.. The results will help to further examine the mechanism of action of this novel agent and to provide a high degree of comfort concerning its pharmacodynamic effects in humans before moving into a diseased population.

5.1.1. First-in-man clinical study

The Company's classical approach to clinical development is merited by the fact that TGN1412 is acting by a novel mechanism of action which is mediated via immunomodulation. An important aspect of this first-in-man trial will be to examine the effect of different doses of TGN1412 on the status of the immune system and in particular on different lymphocyte subsets. The results will help to further examine the mechanism of action of this novel agent and to provide a degree of comfort concerning its pharmacodynamic effects in humans before moving into diseased populations..

5.1.1.1. Subject population

The first-in-man trial will include 32 healthy male subjects of normal body weight and aged between 18 and 40 years of age.

5.1.1.2. Trial design

The study is designed as a single-centre, double-blind, randomised, placebo-controlled, dose-escalation trial, including 32 healthy male subjects who will be divided into four groups of eight subjects each. In each group, six subjects will receive TGN1412 and two subjects placebo (random ratio: 3:1). Intravenous (i.v.) doses of 0.1, 0.5, 2.0 and 5.0 mg/kg body weight (b.w.) are planned to be investigated. These i.v. doses will be administered as short-term infusion. Dose escalation to the next dose level will proceed following satisfactory review of safety data by a Data Safety Monitoring Board from at least fourteen days following each administration. In view of the possibility of anti-TGN1412 antibody formation, patients will be followed for 6 weeks post TGN1412 administration.

This proposed trial design is expected to minimize subject risk.

5.1.2. Pharmacokinetics (PK) and product metabolism in humans

No data are available on TGN1412 pharmacokinetics in humans. Since TGN1412 format is a IgG4 isoytpe, it is assumed that TGN1412 will be catabolized in humans via standard proteolytic pathways in the liver and elsewhere.

The intended single dose pharmacokinetics analysis will enable a preliminary determination of the relationship between dose and plasma concentration, and volume of distribution, clearance and half-life. Blood samples will be taken at regular intervals over the predicted time of TGN1412 systemic exposure (as determined from animal studies). The peak concentration of TGN1412 in the plasma (Cmax) and time to peak concentration (Tmax), the overall systemic exposure ($AUC_{0-\infty}$), the half-life (T½), volume of distribution and clearance will be determined.

The half-life of TGN1412 measured after single dose intravenous application may assist in determination of the dosing interval. Knowledge of the pharmacokinetic parameters for TGN1412 obtained from early trials will enable selection of doses for subsequent proof-of-concept /dose ranging trials.

In case of generation of anti-TGN1412 antibodies, the PK of TGN1412 may be altered, for example by increasing or decreasing clearance. In this event, the impact of anti-TGN1412 antibodies on pharmacokinetic parameters should be carefully evaluated.

For subsequent clinical trials, is recognized that the pharmacokinetic profile of TGN1412 in B-CLL or RA patients may substantially differ from that in healthy subjects. The PK profile will need to be evaluated in patients, too, to determine comparability.

5.1.3. TGN1412 safety and efficacy

No data are available on TGN1412 safety and efficacy in humans.

The primary objective of the proposed first-in-man trial is to establish the safety and tolerability of TGN1412 in man by evaluation of ascending single doses of TGN1412. In addition, this trial will guide decision making for dose selection in subsequent studies.

The following tests are considered essential for evaluating the safety of this novel agent: adverse events (AEs), laboratory analyses including specific immunological methods, haematology, blood chemistry, urine analysis, vital signs, ECGs and physical examination. Evaluation of AEs will include assessments for injection site reactions.

It should be noted that the safety and tolerability of an immunomodulatory monoclonal antibody (albeit humanised), such as TGN1412, might not surface in the form of overt AEs or abnormal results from the standard battery of tests mentioned above. Indeed, a maximum

tolerated dose (MTD) may not be identified on this basis but rather on basis of the effects of TGN1412 on the immune system. Thus, safety evaluations particular to TGN1412 will include the immunologic parameters:

- Number and phenotype analysis of lymphocyte subsets
- Serum levels of selected inflammatory cytokines
- C5a as a marker of complement activation
- Anti-TGN1412 antibody formation
- Epstein-Barr viral load
- Rheumatoid factor and anti-nuclear-antibodies (ANA)

5.1.4. Marketing experience

No marketing experience exists for TGN1412.

5.2. Summary of data and guidance for the investigator

TGN1412 has been tested preclinically in various in vitro and in vivo models which support the concept of immunomodulating and immunoactivating activity. The immuno-therapeutic treatment with TGN1412 is believed to result in effective anti-tumour and anti-rheumatic activity also in humans. Due to a unknown dose-response (pharmacokinetics/ pharmacodynamics) relationship, a single dose escalation phase I clinical study in healthy volunteers is planned to be conducted prior to exposure of RA and/or B-CLL patients.

Preliminary experiences in pre-clinical use suggest general safety but caution has to be taken for infusion-related reactions, cytokine release and/or anaphylactic reactions.

5.2.1. Preclinical data

Safety, pharmacodynamics and pharmacokinetics of the agonistic anti-CD28 monoclonal antibody TGN1412 and its orthologues has been assessed after single and repeated doses in various animal model systems. Overall, it could be demonstrated that agonistic anti-CD28 monoclonal antibodies mediate a well tolerated expansion and activation of T cells in rodent and non-human primates. It is assumed, that TGN1412 may be used to reconstitute a collapsed immune system in haemato-oncology indications such as B-CLL. Furthermore, the capability of agonistic anti-CD28 monoclonal antibodies to mediate an anti-inflammatory immune response by modulation of certain T-cell subsets (e.g. regulatory T cells) and cytokines supports the assumption that TGN1412 has a potential to be effective in the treatment of autoimmune diseases such as rheumatoid arhtritis.

The results of toxicology studies show that TGN1412 was well tolerated in cynomolgus monkeys at doses up to 50mg/kg/week for four consecutive weeks (NOAEL = 50mg/kg). 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.

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.

5.2.2. *Clinical data*

5.2.2.1. *Adverse events*

No clinical adverse events have been reported so far since the proposed trial is a first- in-man study. This chapter will be updated during the conduct of further studies.

5.2.2.2. *Contraindications*

No contraindications have been reported since this IB describes a first-in-man study.

5.2.2.3. *Precautions*

Based on the pre-clinical studies conducted so far, TGN1412 is expected to be well tolerated in humans and not to elicit any adverse events. However, based on theoretical considerations on immunological effects of agonistic anti-CD28 antibodies and on TGN1412 tissue-crossreactivity data, the following unintended effects may theoretically be encountered during a first-in-man-trial. Appropriate measurements of clinical precaution shall be taken:

Immunosuppression: 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 counter-measures (e.g. application of antibiotics) must be considered.

Autoimmunity & anaphylaxis: 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 counter-measures (e.g. application of glucocorticoids, anti-histamines etc.) must be considered.

Cytokine release: Activation of T lymphocytes by other pharmacological approaches, e.g. the anti-CD3 monoclonal antibody OKT-3, elicits a "cytokine storm" characterized by an increase in systemic inflammatory mediators. In the unlikely event that TGN1412 also induces massive cytokine release, appropriate counter-measures must be taken.

Neurology: In a tissue cross-reactivity study with human and cynomolgus monkey tissue, TGN1412 reacted with astrocytes in central nervous tissue. Despite the fact that this had no neurologically apparent effect in any cynomolgus monkey, it is advised that subjects be monitored for neurological symptoms.

Anti-TGN1412 antibodies: Since TGN1412 is a monoclonal antibody, possible complications from the generation of anti-isotype as well as anti-idiotype antibody reactions must be taken into account and, if necessary, appropriate counter-measures must be taken.

5.2.2.4. *Drug interactions*

Specific drug-interaction studies have not been conducted.

5.2.2.5. *Carcinogenesis, mutagenesis, and impairment of fertility*

Long-term studies in animals have not been performed to evaluate the carcinogenic potential. It is not known whether TGN1412 can impair fertility in humans.

5.2.2.6. *Overdosage*

Repeated doses of up to 50 mg/kg body weight have been administered to cynomolgus monkeys without any direct toxic effect. In the unlikely case of overdosage, it is recommended that the patient be monitored for any signs or symptoms of adverse reactions or effects and appropriate symptomatic treatment instituted immediately.

5.2.2.7. Compatibility

Diluted TGN1412 solutions may be prepared in EVA (ethylvinylacetat, PVC-free) infusion bags. No physical biochemical compatibility studies have been conducted to evaluate the co-administration of TGN1412 with other agents. TGN1412 should not be infused concomitantly in the same intravenous line with other agents. The solution for infusion should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If visibly opaque particles, discoloration, or other foreign particles are observed, the solution should not be used.

5.2.2.8. Formulation, Dosage and Administration

The formulation of TGN1412 drug product consists of 20 mM acetate, 139 mM sodium chloride and 0.02% Tween 20. The pH was adjusted to 5.5 and the product concentration will be 10 mg/mL, filled in vials as 400 mg/40 mL. The container is a 50 R (V = 50 mL) injection vial composed of glass type I with a teflon-coated butyl-rubber stopper and an aluminium flip-off cap. All primary packaging materials comply with requirements of the Pharmacopoeia Europe and the US Pharmacopoeia.

For the proposed first-in-man study, TGN1412 is supplied as a concentrated solution to be diluted before i.v. infusion. TGN1412 drug product is a clear, colourless liquid.

TGN1412 will be diluted in 0.9% NaCl in the ratio 1:5, i.e., the 10-mg/mL concentrate will be reconstituted to give an infusion concentration of 2 mg/mL. The infusion solution will be administered by means of a perfusor with a speed of 1 – 5 mL/ min. The infusion rate may be reduced to a lower rate, if a higher rate was not well tolerated.

6 ABBREVIATIONS

5.11.A1	Mouse agonistic anti-human CD28 mAb
ADCC	Antibody Dependent Cellular Cytotoxicity
AE/ SAE	(Serious) Adverse Event
ANA	Anti-Nuclear Antibodies
APC	Antigen Presenting Cell
AUC	Area Under the Curve
B-CLL	B cell chronic lymphocytic leukaemia
BrdU	Bromodeoxyuridine
BSA	Bovine Serum Albumine
BSE/TSE	Bovine (Transmissible) Spongiform Encephalopathy
bw	body weight
CD	Cluster of Differentiation
CDC	Complement Dependent Cytotoxicity
CDR	Complentarity Determining Region
CFU	Colony Forming Units
CHO	Chinese Hamster Ovary
CIA	Collagen-Induced Arthritis
CL	Clearance
Cmax	Maximal Concentration
COMP	Committee for Orphan Medicinal Products
CPMP/ CVMP	Committee for Proprietary (Veterinary) Medicinal Products
CRP	C-reactive Protein
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
DMARD	Disease Modifying Anti-Rheumatic Drug
EAE	Experimental Autoimmune Encyphalomyelitis
EAN	Experimental Autoimmune Neuritis
ECG	Electrocardiogram
ED	Effective Dose
EDQM	European Directorate for the Quality of Medicines
ELISA	Enzyme Linked Immunosorbent Assay
EMEA	European Medicines Agency
ESI-TOF	Electro-Spray Ionisation Time-of-Flight
EU	Endotoxin Units
EVA	Ethyl-Vinyl-Acetate

FcR	Fc-Receptor
FIM	First-in-man
FoxP3	Forkhead box transcription factor P3
GBS	Guillain-Barre Syndrom
GMP	Good Manufacturing Practice
HC	Heavy Chain
HP	Hydroxylysylpyridinoline
HP-SEC	High-Performance Size Exclusion Chromatograpy
i.a.	intra-arterial
i.p.	intra-peritoneal
i.v.	intravenous
IB	Investigator's Brochure
ICOS	Inducible Costimulator
IFN	Interferon
IgG	Immunglobulin G
IL	interleukin
JJ316	Agonistic anti-rat CD28 monoclonal antibody
kg	kilogram
KLH	Keyhole Limpet Antigen
L	Liter
LC	Light Chain
LP	Lysylpyridinoline
M	Mol
mAb	monoclonal Antibody
MCB	Master Cell Bank
MCB	Master Cell Bank
mg	milligram
MHC	major histocompatibility complex
mosm	Milliosmol
MS	Multiple Sclerosis
MTD	Maximum Tolerated Dose
MTX	Methotrexate
NaCl	Natrium Chloride
NK cell	Natural Killer Cell
NOAEL	No Observed Adverse Effect Level

PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PD	Pharmacodynamics
pH	Hydrogen ion concentration
Ph. Eur.	European Pharmacopoeia
PK/TK	Pharmacokinetics/ Toxicokinetics
PVC	Poly-Vinyl-Chloride
RA	Rheumatoid Arthritis
RAHA	Rhesus-Anti-Human-Antibodies
s.c.	subcutaneous
SDS-Page	Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis
SOP	Standard Operating Procedure
T1/2 el	Elimination Half-Life
TCR	T-cell recepor
TGN1112	Humanized IgG1 isotype agonistic anti-CD28 mAb
TGN1412	Humanized IgG4 isotype agonistic anti-CD28 mAb
Tmax	Timepoint at which the maximal concentration was observed
TNF	Tumour Necrosis Factor
TPP	Target Product Profile
TRAIL	TNF-related Apoptosis-inducing Ligand
UV	Ultraviolet
Vcen	Volume of the central compartment
Vd	Volume of distribution
Vss	Volume of distribution at steady state

7 REFERENCES

Abramowicz D, Schandene L, Goldman M, Crusiaux A, Vereerstraeten P, De Pauw L, Wybran J, Kinnaert P, Dupont E, Toussaint C. 1989. Release of tumor necrosis factor, interleukin-2, and gamma-interferon in serum after injection of OKT3 monoclonal antibody in kidney transplant recipients. Transplantation 47: 606-608.

Beyersdorf, N., Gaupp, S, Balbach, K, Schmidt, J, Toyka, KV, Lin, CH, Hanke, T, Hünig, T, Kerkau, T and, Gold R (2005). Selective targeting of regulatory T cells with CD28 superagonists allows effective therapy of experimental autoimmune encephalomyelitis. J Exp Med. (in press)

Bluestone, J. A., and Abbas, A. K. (2003). Natural versus adaptive regulatory T cells. Nat Rev Immunol 3, 253-257.

Bluestone, J. A., and Tang, Q. (2004). Therapeutic vaccination using CD4+CD25+ antigen-specific regulatory T cells. Proc Natl Acad Sci U S A *101 Suppl* 2, 14622-14626.

Cheson, B. D. (1995). Infectious and immunosuppressive complications of purine analog therapy. J Clin Oncol *13*, 2431-2448.

de Totero D, M. M., Rosso O, Clavio M, Balleari E, Foa R, Gobbi M. (2004). Resistance to CD95-mediated apoptosis of CD40-activated chronic lymphocytic leukemia B cells is not related to lack of DISC molecules expression. Hematol J 5, 152-160.

Dennehy, K. M., Kerstan, A., Bischof, A., Park, J. H., Na, S. Y., and Hunig, T. (2003). Mitogenic signals through CD28 activate the protein kinase Ctheta-NF-kappaB pathway in primary peripheral T cells. Int Immunol *15*, 655-663.

Dicker, F., Kater, A. P., Fukuda, T. and Kipps, T. J. (2005). Fas-ligand (CD178) and TRAIL synergistically induce apoptosis of CD40-activated chronic lymphocytic leukemia B cells. Blood *105*, 3193-3198.

Draize JH, Kelley EA. 1959. The urinary excretion of boric acid preparations following oral administration and topical applications to intact and damaged skin of rabbits. Toxicology 1: 267-276.

Dumont, F. J. (2004). Technology evaluation: abatacept, Bristol-Myers Squibb. Curr Opin Mol Ther *6*, 318-330.

Elflein, K., Rodriguez-Palmero, M., Kerkau, T., and Huenig, T. (2003). Rapid recovery from T lymphopenia by CD28 superagonist therapy. Blood.

Evans, E. J., Esnouf, R. M., Manso-Sancho, R., Gilbert, R. J., James, J. R., Yu, C., Fennelly, J. A., Vowles, C., Hanke, T., Walse, B., *et al.* (2005). Crystal structure of a soluble CD28-Fab complex. Nat Immunol *6*, 271-279.

Genestier, L, Paillot R, Fournel S, Ferraro C, Miossec P, Revillard JP. 1998. Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. J. Clin. Invest. 102:322-328.

Guinan, E. C., Boussiotis, V. A., Neuberg, D., Brennan, L. L., Hirano, N., Nadler, L. M., and Gribben, J. G. (1999). Transplantation of anergic histoincompatible bone marrow allografts. N Engl J Med *340*, 1704-1714.

Hara T, F. S., Hansen JA. (1985). Human T cell activation. II. A new activation pathway used by a major T cell population via a disulfide-bonded dimer of a 44 kilodalton polypeptide (9.3 antigen). J Exp Med *161*, 1513-1524.

Hodge, J. W., Abrams, S., Schlom, J., and Kantor, J. A. (1994). Induction of antitumour immunity by recombinant vaccinia viruses expressing B7-1 or B7-2 costimulatory molecules. Cancer Res *54*, 5552-5555.

Hodi, F. S., Mihm, M. C., Soiffer, R. J., Haluska, F. G., Butler, M., Seiden, M. V., Davis, T., Henry-Spires, R., MacRae, S., Willman, A., *et al.* (2003). Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci U S A *100*, 4712-4717.

Hsu DH, Shi JD, Homola M, Rowell TJ, Moran J, Levitt D, Druilhet B, Chinn J, Bullock C, Klingbeil C. 1999. A humanized anti-CD3 antibody, HuM291, with low mitogenic activity, mediates complete and reversible T-cell depletion in chimpanzees. Transplantation 68: 545-554.

Jego, G., Robillard, N., Puthier, D., Amiot, M., Accard, F., Pineau, D., Harousseau, J. L., Bataille, R., and Pellat-Deceunynck, C. (1999). Reactive plasmacytoses are expansions of plasmablasts retaining the capacity to differentiate into plasma cells. Blood *94*, 701-712.

Kalil, N., and Cheson, B. D. (1999). Chronic lymphocytic leukemia. Oncologist *4*, 352-369.

Keating, M. J. (1990). Fludarabine phosphate in the treatment of chronic lymphocytic leukemia. Semin Oncol *17*, 49-62.

Kerstan, A., and Hunig, T. (2004). Cutting edge: distinct TCR- and CD28-derived signals regulate CD95L, Bcl-xL, and the survival of primary T cells. J Immunol *172*, 1341-1345.

Kimby, E., Brandt, L., Nygren, P., and Glimelius, B. (2001). A systematic overview of chemotherapy effects in B-cell chronic lymphocytic leukaemia. Acta Oncol *40*, 224-230.

Klasen, S., Pages, F., Peyron, J. F., Cantrell, D. A., and Olive, D. (1998). Two distinct regions of the CD28 intracytoplasmic domain are involved in the tyrosine phosphorylation of Vav and GTPase activating protein-associated p62 protein. Int Immunol *10*, 481-489.

Kremer, J. M., Westhovens, R., Leon, M., Di Giorgio, E., Alten, R., Steinfeld, S., Russell, A., Dougados, M., Emery, P., Nuamah, I. F., *et al.* (2003). Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. N Engl J Med *349*, 1907-1915.

Kronenberg, M., and Rudensky, A. (2005). Regulation of immunity by self-reactive T cells. Nature *435*, 598-604.

Laport, G. G., Levine, B. L., Stadtmauer, E. A., Schuster, S. J., Luger, S. M., Grupp, S., Bunin, N., Strobl, F. J., Cotte, J., Zheng, Z., *et al.* (2003). Adoptive transfer of costimulated T cells induces lymphocytosis in patients with relapsed/refractory non-Hodgkin lymphoma following CD34+-selected hematopoietic cell transplantation. Blood *102*, 2004-2013.

LeBlanc, R., Hideshima, T., Catley, L. P., Shringarpure, R., Burger, R., Mitsiades, N., Mitsiades, C., Cheema, P., Chauhan, D., Richardson, P. G., *et al.* (2004). Immunomodulatory drug costimulates T cells via the B7-CD28 pathway. Blood *103*, 1787-1790.

Lin, C. H., and Hunig, T. (2003). Efficient expansion of regulatory T cells in vitro and in vivo with a CD28 superagonist. Eur J Immunol *33*, 626-638.

Liu, N. S., and O'Brien, S. (2004). Monoclonal antibodies in the treatment of chronic lymphocytic leukemia. Med Oncol *21*, 297-304.

Luhder, F., Huang, Y., Dennehy, K. M., Guntermann, C., Muller, I., Winkler, E., Kerkau, T., Ikemizu, S., Davis, S. J., Hanke, T., and Hunig, T. (2003). Topological requirements and signaling properties of T cell-activating, anti-CD28 antibody superagonists. J Exp Med *197*, 955-966.

Montserrat, E., Gomis, F., Vallespi, T., Rios, A., Romero, A., Soler, J., Alcala, A., Morey, M., Ferran, C., Diaz-Mediavilla, J., and et al. (1991). Presenting features and prognosis of chronic lymphocytic leukemia in younger adults. Blood *78*, 1545-1551.

Moreland, L. W., Alten, R., Van den Bosch, F., Appelboom, T., Leon, M., Emery, P., Cohen, S., Luggen, M., Shergy, W., Nuamah, I., and Becker, J. C. (2002). Costimulatory blockade in patients with rheumatoid arthritis: a pilot, dose-finding, double-blind, placebo-controlled clinical trial evaluating CTLA-4Ig and LEA29Y eighty-five days after the first infusion. Arthritis Rheum *46*, 1470-1479.

Moreton, P., and Hillmen, P. (2003). Alemtuzumab therapy in B-cell lymphoproliferative disorders. Semin Oncol *30*, 493-501.

Morra, E., Nosari, A., and Montillo, M. (1999). Infectious complications in chronic lymphocytic leukaemia. Hematol Cell Ther *41*, 145-151.

Morrison, V. A. (1998). The infectious complications of chronic lymphocytic leukemia. Semin Oncol *25*, 98-106.

O'Brien, S. (1999). Infectious Complications of Nucleoside Analogs (American Society of Hematology).

Paillot, R, Genestier L, Fournel S, Ferraro C, Miossec P, Revillard JP. 1998. Activation-dependent lymphocyte apoptosis induced by methotrexate. Transplant. Proc. 30: 2348-2350.

Phan, G. Q., Yang, J. C., Sherry, R. M., Hwu, P., Topalian, S. L., Schwartzentruber, D. J., Restifo, N. P., Haworth, L. R., Seipp, C. A., Freezer, L. J., *et al.* (2003). Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci U S A *100*, 8372-8377.

Redaelli, A., Laskin, B. L., Stephens, J. M., Botteman, M. F., and Pashos, C. L. (2004). The clinical and epidemiological burden of chronic lymphocytic leukaemia. Eur J Cancer Care (Engl) *13*, 279-287.

Riley, J. L., and June, C. H. (2005). The CD28 family: a T-cell rheostat for therapeutic control of T-cell activation. Blood *105*, 13-21.

Rodriguez-Palmero, M., Hara, T., Thumbs, A., and Hunig, T. (1999). Triggering of T cell proliferation through CD28 induces GATA-3 and promotes T helper type 2 differentiation in vitro and in vivo. Eur J Immunol *29*, 3914-3924.

Samonis, G., and Kontoyiannis, D. P. (2001). Infectious complications of purine analog therapy. Curr Opin Infect Dis *14*, 409-413.

Schmidt, J., Elflein, K., Stienekemeier, M., Rodriguez-Palmero, M., Schneider, C., Toyka, K. V., Gold, R., and Hunig, T. (2003). Treatment and prevention of experimental autoimmune neuritis with superagonistic CD28-specific monoclonal antibodies. J Neuroimmunol *140*, 143-152.

Shapiro, V. S., Mollenauer, M. N., and Weiss, A. (2001). Endogenous CD28 expressed on myeloma cells up-regulates interleukin-8 production: implications for multiple myeloma progression. Blood *98*, 187-193.

Sharpe, A. H., and Freeman, G. J. (2002). The B7-CD28 superfamily. Nat Rev Immunol 2, 116-126.

Tacke, M., Hanke, G., Hanke, T., and Hunig, T. (1997). CD28-mediated induction of proliferation in resting T cells in vitro and in vivo without engagement of the T cell receptor: evidence for functionally distinct forms of CD28. Eur J Immunol 27. 239-247.

studies marked as * on previous page 57 & rest of page 60 withheld under section 43 (2) of the FOIA as it relates to unpublished research. In the MHRA's view disclosure would, or would be likely to prejudice the commercial interests of TeGenero or associated third parties. In the Agency's view the public interest in disclosure is not outweighed by the public interest in maintaining confidence.

Thompson, J. A., Figlin, R. A., Sifri-Steele, C., Berenson, R. J., and Frohlich, M. W. (2003). A phase I trial of CD3/CD28-activated T cells (Xcellerated T cells) and interleukin-2 in patients with metastatic renal cell carcinoma. Clin Cancer Res 9, 3562-3570.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22, 4673-4680.