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(54) Titre : ADMINISTRATION D'ANTICORPS ANTI-CD3 DANS LE TRAITEMENT DE MALADIES AUTO-IMMUNES
(54) Title: ADMINISTRATION OF ANTI-CD3 ANTIBODIES IN THE TREATMENT OF AUTOIMMUNE DISEASES

(57) Abrégé/Abstract:
A method of treating an autoimmune disease, such as, diabetes or psoriasis, by administering an anti-CD3 antibody, or anti-CD3 antibody fragment. The anti-CD3 antibody, or anti-CD3 antibody fragment, is administered over a course of treatment wherein, during the course of treatment, the anti-CD3 antibody, or anti-CD3 antibody fragment is administered in a total amount which does not exceed 300 μg/kg when administered intravenously or, when administered other than intravenously, is administered in a total amount that does not exceed a total amount bioequivalent to 300μg/kg of intravenous administration thereof.

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(54) Title: ADMINISTRATION OF ANTI-CD3 ANTIBODIES IN THE TREATMENT OF AUTOIMMUNE DISEASES

(57) Abstract: A method of treating an autoimmune disease, such as, diabetes or psoriasis, by administering an anti-CD3 antibody, or anti-CD3 antibody fragment. The anti-CD3 antibody, or anti-CD3 antibody fragment, is administered over a course of treatment wherein, during the course of treatment, the anti-CD3 antibody, or anti-CD3 antibody fragment is administered in a total amount which does not exceed 300 μg/kg when administered intravenously or, when administered other than intravenously, is administered in a total amount that does not exceed a total amount bioequivalent to 300μg/kg of intravenous administration thereof.

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ADMINISTRATION OFANTI-CD3 ANTIBODIES IN THE TREATMENT OF AUTOIMMUNE DISEASES

This application claims the priority of U.S. Provisional Patent Application Serial No. _____, filed June 6, 2006, which Provisional Patent Application is converted U.S. Utility Patent Application No. 11/447,628 filed on June 6, 2006, which Utility Patent Application was converted to such Provisional Patent Application by *Request Under 37 CFR 1.53(c)(2)*, filed April 16, 2007, the disclosures of which application(s) are hereby incorporated by reference in their entireties.

This invention relates to the administration of anti-CD3 antibodies to treat an autoimmune disease, such as diabetes or psoriasis. In one embodiment, this invention relates to the administration of anti-CD3 antibodies or fragments thereof, to treat an autoimmune disease, such as diabetes or psoriasis, at reduced dosage levels.

Anti-CD3 antibodies, or fragments thereof, have been employed in the treatment of autoimmune diseases, including diabetes. For example, U.S. Patent No. 7,041,289 and published Canadian Patent Application No. 2,224,256 teach the treatment of autoimmune diseases, including diabetes, by administering an anti-CD3 antibody, or fragment thereof, in an amount of 5 to 20 mg per dose. In one embodiment, the anti-CD3 antibody is administered in an amount of 5 to 10 mg/day for 10 to 14 days.

Herold, et al., disclose a clinical study in which patients with Type I diabetes were given a monoclonal anti-CD3 antibody, hOKT3γ1 (Ala-Ala). In the study, the patients were given a total dose of 500 μg/kg of the antibody over a period of 14 days. The dosages given over the 14-day course of treatment were as follows:

Day 1 - 1.42 μg/kg

Day 2 - 5.67 μg/kg

Day 3 - 11.3 μg/kg

Day 4 - 22.6 μg/kg

Day 5-14 - 45.4 μg/kg

After the first cohort of subjects were treated, the total dose was increased to 620 μg/kg. (Herold, et al., Immunologic Research, Vol. 28, No. 2, pgs. 141-150 (2003); Herold, et al., New Engl. J. Med., Vol. 346, No. 22, pgs. 1692-1698 (May 30, 2002); Herold, et al., J. Clin. Invest., Vol. 111, No. 3, pgs. 409-418 (February 2003); Herold, et al., J. Clin. Invest., Vol. 113, No. 3, pgs. 346-349 (February 2004); Herold, et al., Diabetes, Vol. 54, pgs. 1763-1769 (June 2005).) Peak serum levels of 0.25 μg/ml were obtained, during days 5 to 14 of the treatment. (Herold, et al., 2002; Herold, et al., Immunologic Research, 2003.)

In accordance with an aspect of the present invention, there is provided a method of treating an autoimmune disease in an animal. In one embodiment, the disease is an established, spontaneous, and ongoing autoimmune disease. The method comprises administering to an animal an anti-CD3 antibody or anti-CD3 antibody fragment selected from the group consisting of anti-CD3 antibodies and fragments thereof which do not bind or have reduced binding to the Fc (gamma) receptors. The anti-CD3 antibody, or anti-CD3 antibody fragment thereof, is administered in a therapeutically effective amount over a course of treatment wherein during the course of treatment, the total amount of anti-CD3 antibody or anti-CD3 antibody fragment, when intravenously administered is administered in an amount which does not exceed 300 μg/kg.

The terms "anti-CD3 antibody" and "anti-CD3 antibody fragment", as used herein, mean antibodies or antibody fragments which recognize or bind to CD3.

As is known in the art, the term "μg/kg" means the amount of antibody and/or fragment thereof delivered, in micrograms, per kilogram of body weight of the animal being treated.

In one non-limiting embodiment, the autoimmune disease is diabetes. In another non-limiting embodiment, the autoimmune disease is psoriasis.

If administered other than intravenously, then such anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount such that the resulting area under the curve (AUC) from such administration is from about 80% to about 120% of the AUC produced by intravenous administration of such anti-CD3 antibody or anti-CD3 antibody fragment. Thereafter, such amounts are referred to as being an amount bioequivalent to intravenous administration.

The terms "area under the curve" and "AUC" are terms well known in the pharmaceutical arts. An AUC value is calculated by plotting a graph with data from systemic concentration of a therapeutic agent as a function of time, with the X-axis generally representing time and the Y-axis generally representing concentration of therapeutic agent. The area under the line formed by joining the various data points then is integrated into a numerical value. AUC values for the anti-CD3 antibody or anti-CD3 antibody fragment may be subject to inter-and intra-patient variation due to physiological and/or environmental factors present in individual patients during the administration of the anti-CD3 antibodies or anti-CD3 antibody fragments, in various formulations and/or compositions.

In one non-limiting embodiment, the anti-CD3 antibody, or anti-CD3 antibody fragment, is administered intravenously over a course of treatment in which the total amount of anti-CD3 antibody or anti-CD3 antibody fragment administered during the course of treatment does not exceed 175 μg/kg, and in another embodiment, such amount does not exceed 150 μg/kg, and in some embodiments, such amount does not exceed 50 μg/kg, or in the case of administration other than intravenous, in an amount bioequivalent to intravenous administration in such amounts.

In another non-limiting embodiment, the anti-CD3 antibody or anti-CD3 antibody fragment is administered intravenously over a course of treatment wherein the total amount of anti-CD3 antibody or anti-CD3 antibody fragment administered during the course of treatment is at least 8 μg/kg or in the case of administration other than intravenous, in an amount bioequivalent to intravenous administration in such amounts.

In another embodiment, the anti-CD3 antibody or anti-CD3 antibody fragment, on each day of treatment is administered intravenously in an amount which generally is not less than 0.1 μg/kg per day, or in the case of administration other than intravenously, in an amount bioequivalent to intravenous administration in such amount.

Over the course of treatment, the same dosage of anti-CD3 antibody or anti-CD3 antibody fragment may be given each day over the course of treatment, or different doses of the anti-CD3 antibody or anti-CD3 antibody fragment may be given on each day of the course of treatment. For example, the dose of anti-CD3 antibody or anti-CD3 antibody fragment may be varied on each day of the course of treatment, provided the total dosage of anti-CD3 antibody or anti-CD3 antibody fragment does not exceed 300 μg/kg. In one embodiment, the amount of anti-CD3 antibody or anti-CD3 antibody fragment given intravenously on any one day of the course of treatment does not exceed 30 μg/kg/day. In another embodiment, the amount does not exceed 25 μg/kg/day. In yet another embodiment, the amount does not exceed 20 μg/kg/day. In still another embodiment, the amount does not exceed 15 μg/kg/day, and in yet another embodiment the amount does not exceed 10 μg/kg/day or, in the case of administration other than intravenous, in an amount which does not exceed an amount bioequivalent to intravenous administration in such amounts.

In one embodiment, the course of treatment with respect to the various embodiments hereinabove described does not exceed 10 days. In another embodiment, the course of treatment does not exceed 8 days. In yet another embodiment, the course of treatment does not exceed 6 days. In a further embodiment, the course of treatment does not exceed 4 days. In another embodiment, the course of treatment does not exceed 3 days. In another embodiment, the course of treatment does not exceed one day.

In one embodiment, the dose of anti-CD3 antibody or anti-CD3 antibody fragment which is administered is increased on each succeeding day of the treatment, with the greatest dosage of anti-CD3 antibody or anti-CD3 antibody fragment being administered on the last day of the treatment.

In another embodiment, during the course of the treatment, an initial dose of anti-CD3 antibody or anti-CD3 antibody fragment is administered on the first day of the treatment. The dose then is increased on each succeeding day of the treatment until a preselected maximum daily dosage is reached. Then such maximum daily dosage is administered on each succeeding day of the remaining days of the treatment. In each case, the total dosage over the course of treatment does not exceed 300 μg/kg.

In one embodiment, the anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount of about 4.3 μg/kg on Day 1 of the treatment, and in an amount of about 7.1 μg/kg on Day 2 of the treatment. From Day 3 to Day 8 or 9 of the treatment, the anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount of about 14.3 μg/kg on each of such days of the treatment.

In another embodiment, the anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount of about 1.4 μg/kg on Day 1 of the treatment, in an amount of about 2.8 μg/kg on Day 2 of the treatment, and in an amount of about 4.3 μg/kg on Day 3 of the treatment. From Day 4 to Day 8 of the treatment, the anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount of about 7.1 μg/kg on each of such days of the treatment.

In a further embodiment, the anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount of about 1.4μg/kg on Day 1 of the treatment, in an amount of about 2.8μg/kg on Day 2 of the treatment, and in an amount of about 4.3μg/kg on Day 3 of the treatment. From Day 4 to Day 8 of the treatment, the anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount of about 10.7μg/kg on each of such days of the treatment.

In yet another embodiment, the anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount of about 1.4μg/kg on Day 1 of the treatment, in an amount of about 2.8μg/kg on Day 2 of the treatment, and in an amount of about 4.3μg/kg on Day 3 of the treatment.

From Day 4 to Day 8 of the treatment, the anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount of about 14.3μg/kg on each of such days of the treatment.

The anti-CD3 antibody, or anti-CD3 antibody fragment, may be administered systemically, such as by intravenous, intra-arterial, intraperitoneal, intramuscular, or subcutaneous administration. In general, when the anti-CD3 antibody or anti-CD3 antibody fragment is administered by methods other than by intravenous administration, such as by intramuscular or subcutaneous administration, the area under the curve (AUC) resulting from such administration is from about 80% to 120% of the area under the curve resulting from intravenous administration. Thus, when administered by methods other than by intravenous administration, such as by intramuscular or subcutaneous administration, the anti-CD3 antibody, or anti-CD3 antibody fragment, may be administered in doses higher or lower than the hereinabove described maximum dose for intravenous administration, provided that such amount administered other than intravenously does not exceed an amount bioequivalent to that of a dose that is bioequivalent to the maximum intravenous dose.

The anti-CD3 antibody or anti-CD3 antibody fragment may be a human antibody, an animal antibody, such as a non-human mammalian antibody, such as a rodent antibody, including but not limited to mouse and rat antibodies, a chimeric antibody, or a humanized antibody. Alternatively, the anti-CD3 antibody or anti-CD3 antibody fragment may include a combination of human, animal, chimeric, and/or humanized portions.

The anti-CD3 antibody or anti-CD3 antibody fragment may be a monoclonal or polyclonal antibody or fragment thereof. The anti-CD3 antibody, or anti-CD3 antibody fragment, in one embodiment, is a monoclonal antibody or antibody fragment, such as an F $(ab')_2$ fragment.

In another embodiment, the anti-CD3 antibody, or anti-CD3 antibody fragment, has an Fc region which is removed or modified, whereby binding of the anti-CD3 antibody or anti-CD3 antibody fragment to the Fc (gamma) receptors is reduced or eliminated. In one embodiment, the Fc region is aglycosylated, whereby binding to the Fc (gamma) receptors is reduced or eliminated.

For example, human Fc regions of IgG antibodies are known to be glycosylated at the asparagine residue at position 297, which makes up part of the N-glycosylation motif Asn^{297}-X^{298}-Ser^{299} or Thr^{299}, wherein X is any amino acid residue except proline. The anti-CD3 antibody may be aglycosylated by the replacement of Asn^{297} in the Fc region with another amino acid which cannot be glycosylated. Any other amino acid may be used. In one embodiment, Asn^{297} is replaced with Ala^{297}. Alternatively, glycosylation at Asn^{297} can be prevented by altering one of the other amino acid residues of the motif, such as, for example, by replacing amino acid residue 298 with proline, or amino acid residue 299 with any amino acid other than serine or threonine. Techniques for effecting such aglycosylation are well known to those skilled in the art, such as, for example, by site-directed mutagenesis. Examples of monoclonal anti-CD3 antibodies which are aglycosylated at amino acid residue 297 of the Fc region are described in U.S. Patent Nos. 5,585,097; 5,968,509; and 6,706,265, the contents of which are incorporated herein by reference.

In one embodiment, the anti-CD3 antibody includes a humanized heavy chain in which the complementarity determining regions, or CDRs, of the variable region of the heavy chain are rat CDRs and the remainder of the heavy chain is human, and a chimeric light chain in which the variable region is a rat variable region and the constant region is a human constant region, except that amino acid residues 1, 2, 3, 4, and 7 of the rat variable region have been mutated. In addition, the asparagine residue at position 297 of the Fc region has been replaced with alanine, whereby the Fc region has become aglycosylated. Such antibody sometimes is hereinafter referred to as "TRX4 antibody." The amino acid sequences of the light and heavy chains of and the nucleotide sequences encoding the light and heavy chains of the TRX4 antibody are shown in Figure 1.

In another embodiment, the Fc region is glycosylated; however, amino acid residues other than Asn^{297} of the Fc region have been deleted and/or mutated such that binding of the anti-CD3 antibody or fragment to the Fc (gamma) receptors has been reduced or eliminated.

For example, in one embodiment, each of the amino acid residues Leu^{234} and Leu^{235} of the Fc region have been changed to Ala^{234} and Ala^{235}, whereby binding to the Fc (gamma) receptors is eliminated. An example of such an antibody is the hOKT3γ1 (Ala-Ala) antibody, which is a humanized anti-CD3 monoclonal antibody that contains the binding region of the murine monoclonal antibody OKT3 (U.S. Patent No. 4,658,019) on a human IgG1, and wherein amino acid residues Leu^{234} Leu^{235} are replaced with alanine residues. Such antibody is described further in Herold, et al., Immunologic Research, Vol. 28, No. 2, pgs. 141-150 (2003), and Xu, et al., Cell Immunol., Vol. 200, No. 1, pgs. 16-26 (2000). Such modifications may be used in a humanized or chimeric antibody.

In another embodiment, the anti-CD3 antibody or anti-CD3 antibody fragment is of an isotype which does not bind to the Fc (gamma) receptors, such as IgA or IgD.

In one embodiment, the anti-CD3 antibodies or anti-CD3 antibody fragments employed in accordance with the present invention have a mitogenicity in humans which has been reduced or eliminated, i.e., such anti-CD3 antibodies or anti-CD3 antibody fragments do not induce mitosis or have a reduced ability to induce mitosis in the presence of human serum. The determination of whether an anti-CD3 antibody or anti-CD3 antibody fragment is non-mitogenic or has reduced mitogenicity may be made by techniques known to those skilled in the art, such as by testing such anti-CD3 antibodies or anti-CD3 antibody fragments in human serum in vitro.

The anti-CD3 antibody, or anti-CD3 antibody fragment, is administered to an animal in the amounts hereinabove described in order to treat an autoimmune disease, such as diabetes or psoriasis, in the animal. The animal may be a mammal, including human and non-human primates.

The anti-CD3 antibody, or anti-CD3 antibody fragment, may be administered in conjunction with an acceptable pharmaceutical carrier or diluent. Suitable pharmaceutical carriers or diluents include, but are not limited to, saline, dextrose, Ringer's lactate solution, or combinations thereof,

water, or any other physiological solution used for intravenous administration. The selection of an appropriate pharmaceutical carrier or diluent is within the scope of those skilled in the art.

In another embodiment, the anti-CD3 antibody or anti-CD3 antibody fragment is lyophilized. In such embodiment, the lyophilized anti-CD3 antibody or anti-CD3 antibody fragment is admixed with a carrier or diluent such as those hereinabove described at the time of administration.

In yet another embodiment, the anti-CD3 antibody or anti-CD3 antibody fragment is conjugated to a compound such as a polymer. In one embodiment, the polymer is a polyalkylene glycol. In one embodiment, the polyalkylene glycol is polyethylene glycol, or PEG.

The anti-CD3 antibody, or anti-CD3 antibody fragment, and an acceptable pharmaceutical carrier or diluent may be packaged in an appropriate container, such as a sterile vial. Such vial, in one embodiment, is packaged with instructions to administer the anti-CD3 antibody, or anti-CD3 antibody fragment, over a course of treatment wherein, during the course of treatment, the anti-CD3 antibody, or anti-CD3 antibody fragment, is administered intravenously over the course of treatment wherein during the course of treatment, the total amount of anti-CD3 antibody or anti-CD3 antibody fragment does not exceed 300 μg/kg/day, or with instructions to administer other than intravenously during a course of treatment wherein over the course of treatment, the total amount of anti-CD3 antibody or anti-CD3 antibody fragment administered does not exceed the bioequivalent to intravenous administration of the amounts hereinabove described.

When the anti-CD3 antibody or anti-CD3 antibody fragment is lyophilized, the lyophilized anti-CD3 antibody or anti-CD3 antibody fragment is packaged in a first sterile vial, and the pharmaceutical carrier or diluent is packaged in a second sterile vial. The two vials are packaged with instructions which, in addition to the instructions hereinabove described, also include instructions to add the pharmaceutical carrier or dilulent to the anti-CD3 antibody or anti-CD3 antibody fragment, followed by administration of the anti-CD3 antibody or anti-CD3 antibody fragment and pharmaceutical carrier or diluent to the patient as hereinabove described.

In accordance with another aspect of the present invention, there is provided a method of effecting T-cell receptor modulation in an animal. The method comprises administering to an animal an anti-CD3 antibody or anti-CD3 antibody fragment selected from the group consisting of anti-CD3 antibodies and fragments thereof which do not bind or have reduced binding to the Fc (gamma) receptors. The anti-CD3 antibody, or anti-CD3 antibody fragment thereof, is administered in an amount effective to effect T-cell receptor modulation in the animal. In one embodiment, the animal is selected from those hereinabove described.

In one embodiment, the anti-CD3 antibody or anti-CD3 antibody fragment is selected from those hereinabove described.

The term "T-cell receptor modulation" as used herein, means a decrease in the number of T-cell receptor (TCR) complex molecules or subunits on the surface of T-cells. In general, a T-cell receptor (TCR) includes alpha and beta (alpha/beta) subunits or gamma and delta (gamma/delta) subunits. The CD3 complex includes epsilon, gamma, and delta subunits. The CD3 subunits and zeta subunits form a complex with the subunits of the TCR. The decrease in the number of TCR complex molecules or subunits may result from factors including, but not limited to, the internalization of TCR complex molecules or subunits at a rate which exceeds re-expression of TCR complex molecules or subunits on the surface of T-cells. In one embodiment, the binding of the anti-CD3 antibody or anti-CD3 antibody fragment to the CD3/TCR complex provides for internalization of the alpha/beta or gamma/delta subunits of the TCR, and such internalization is effected at a rate which exceeds re-expression of such TCR alpha/beta or gamma/delta subunits on the surface of T-cells.

In one embodiment, the anti-CD3 antibody or anti-CD3 antibody fragment is administered intravenously in the amounts and/or courses of treatment hereinabove described, or when administered other than intravenously, in amounts bioequivalent to those of intravenous administration.

The anti-CD3 antibody, or anti-CD3 antibody fragment, and an acceptable pharmaceutical carrier (such as those hereinabove described) may be packaged as hereinabove described, with instructions to administer the anti-CD3 antibody or anti-CD3 antibody fragment intravenously in an amount effective to effect TCR modulation, or with instructions to administer other than intravenously in an amount bioequivalent to an effective intravenous amount for TCR modulation.

The invention now will be described with respect to the following drawings, wherein:

Figure 1 shows the amino acid sequences of the light and heavy chains of the TRX4 antibody, and the nucleic acid sequences encoding the light and heavy chains of the TRX4 antibody;

Figure 2 is a graph showing the mean absolute number of CD4+ T-cells in patients before and after such patients were given TRX4 antibody;

Figure 3 is a graph showing the mean absolute number of CD8+ T-cells in patients before and after such patients were given TRX4 antibody;

Figure 4 is a graph showing detection of cell-bound TRX4 antibody with anti-human IgG on CD4+ T-cells in patients before and after such patients were given TRX4 antibody;

Figure 5 is a graph showing modulation of T-cell receptor (TCR) αβ sites in patients as detected by staining CD4+ T-cells, before and after such patients were given TRX4 antibody, with a non-competing T-cell receptor antibody;

Figure 6 is a graph showing free CD3 sites on CD4+ T-cells in patients, before and after such patients were given TRX4 antibody, as detected with biotinylated TRX4;

Figure 7 is a graph showing the mean amount of (± standard deviation) amount of TRX4 antibody bound to CD4+ T cells in a cohort of patients who received 0.1 mg TRX4 on three successive days;

Figure 8 is a graph showing the mean (± standard deviation) absolute number of lymphocytes in four cohorts of patients who received TRX4. The first cohort received 0.1 mg TRX4 on three successive days. The second cohort received 0.5 mg TRX4 on three successive

days. The third cohort received 0.1 mg TRX4 on Day 1, 0.3 mg TRX4 on Day 2, and 0.5 mg TRX4 on Day 3. The fourth cohort received one injection of 0.3 mg TRX4;

Figure 9 is a graph showing the mean (± standard deviation) absolute number of CD4+ lymphocytes in the four cohorts mentioned with respect to Figure 8 hereinabove;

Figure 10 is a graph showing the mean (± standard deviation) absolute number of CD8+ lymphocytes in the four cohorts mentioned with respect to Figure 8 hereinabove;

Figure 11 is a graph showing the mean (± standard deviation) absolute number of T-cell receptor positive (TCR+) T lymphocytes (CD4+ and CD8+) in the four cohorts mentioned with respect to Figure 8 hereinabove;

Figure 12 is a graph showing the mean (± standard deviation) absolute number of CD2+ T lymphocytes (CD4+ and CD8+) in the four cohorts mentioned with respect to Figure 8 hereinabove;

Figure 13 is a graph showing the mean (± standard deviation) absolute number of CD4+ T cells pre-and post-TRX4 dosing in a cohort of patients who received 0.1 mg TRX4 on Day 1, 0.2 mg TRX4 on Day 2, 0.3 mg TRX4 on Day 3, and 0.5 mg TRX4 on Day 4;

Figure 14 is a graph showing the mean (± standard deviation) amount of TRX4 antibody bound to CD4+ T cells in the cohort mentioned with respect to Figure 13 hereinabove;

Figure 15 is a graph showing the modulation of TCR αβ sites measured in MESF units (± standard deviation) as detected by staining CD4+ T-cells pre- and post- TRX4 dosing, with a non-competing TCR antibody, in the cohort mentioned with respect to Figure 13 hereinabove;

Figure 16 is a graph showing the number (± standard deviation), measured in MESF units, of free CD3 sites, on CD4+ T cells pre- and post- TRX4 dosing, as detected with biotinylated TRX4, in the cohort mentioned with respect to Figure 13 hereinabove;

Figure 17 is a graph showing the modulation of TCR αβ sites, measured in MESF units (± standard deviation), as detected by staining CD4+ T-cells pre- and post- TRX4 dosing with a non-

competing TCR antibody in a cohort of patients that received 0.1 mg TRX4 on Day 1, 0.2 mg TRX4 on Day 2, 0.3 mg TRX4 on Day 3, and 0.75 mg TRX4 on Day 4;

Figure 18 is a graph showing the number (± standard deviation), measured in MESF units, of free CD3 sites on CD4+ T-cells, as detected with biotinylated TRX4, in the cohort mentioned with respect to Figure 17 hereinabove;

Figure 19 is a graph showing the mean (± standard deviation) absolute numbers of lymphocytes in four cohorts of patients who received TRX4. The first cohort (A ½) received 0.05 mg TRX4 on Day 1, 0.1 mg TRX4 on Day 2, 0.15 mg TRX4 on Day 3, and 0.25 mg TRX4 on Day 4. The second cohort received 0.1 mg TRX4 on Day 1, 0.2 mg TRX4 on Day 2, 0.3 TRX4 on Day 3, and 0.5 TRX4 on Day 4. The third cohort received 0.1 mg TRX4 on Day 1, 0.2 mg TRX4 on Day 2, 0.3 mg TRX4 on Day 3, and 0.75 TRX4 on Day 4. The fourth cohort received 0.1 mg TRX4 on Day 1, 0.2 mg TRX4 on Day 2, 0.3 mg TRX4 on Day 3, and 1.0 mg TRX4 on Day 4;

Figure 20 is a graph showing the mean (± standard deviation) absolute number of CD4+ lymphocytes in the four cohorts mentioned with respect to Figure 19 hereinabove;

Figure 21 is a graph showing the mean (± standard deviation) absolute number of CD8+ lymphocytes in the four cohorts mentioned with respect to Figure 19 hereinabove;

Figure 22 is a graph showing the mean (± standard deviation) absolute number of TCR+ T lymphocytes (CD4+ and CD8+) in the four cohorts mentioned with respect to Figure 19 hereinabove;

Figure 23 is a graph showing the mean (± standard deviation) absolute number of CD2+ T lymphocytes (CD4+ and CD8+) in the four cohorts mentioned with respect to Figure 19 hereinabove;

Figure 24 is a graph showing the mean (± standard deviation) amount, measured in MESF units, of TRX4 antibody bound to CD4+ T-cells, as detected by an anti-human IgG antibody on CD4+ T-cells in a cohort of patients who received 0.1 mg TRX4 on Day 1, 0.2 mg TRX4 on Day 2,

0.3 mg TRX4 on Day 3, and 0.5 mg TRX4 on each of Days 4 through 8, in a protocol entitled TRX4 Therapeutic Evaluation of Different Multi-Dose Regimens in Type 1 Diabetes Mellitus, or TTEDD;

Figure 25 is a graph showing the mean (± standard deviation) amount, measured in MESF units, of TRX4 antibody bound to CD8+ T-cells, as detected by an anti-human IgG antibody on CD8+ T-cells, in the cohort of patients mentioned with respect to Figure 24 hereinabove;

Figure 26 is a graph showing the number (± standard deviation), measured in MESF units, of free CD3 sites on CD4+ T-cells, as detected with biotinylated TRX4, in the cohort mentioned with respect to Figure 24 hereinabove;

Figure 27 is a graph showing the number (± standard deviation), measured in MESF units, of free CD3 sites on CD8+ T-cells, as detected with biotinylated TRX4, in the cohort mentioned with respect to Figure 24 hereinabove;

Figure 28 is a graph showing the number (± standard deviation) of TCR sites, expressed as percent of baseline, detected on CD4+ T-cells with a non-competing antibody, in the cohort mentioned with respect to Figure 24 hereinabove;

Figure 29 is a graph showing the number (± standard deviation) of TCR sites, expressed as percent of baseline, detected on CD8+ T-cells with a non-competing antibody, in the cohort mentioned with respect to Figure 24 hereinabove;

Figure 30 is a graph showing the mean (± standard deviation) absolute number of CD4+ T-cells in the cohort of patients mentioned with respect to Figure 24 hereinabove;

Figure 31 is a graph showing the mean (± standard deviation) absolute number of CD8+ T-cells in the cohort of patients mentioned with respect to Figure 24 hereinabove;

Figure 32 is a graph showing the mean (± standard deviation) absolute number of CD19+ B-cells in the cohort of patients mentioned with respect to Figure 24 hereinabove;

Figure 33 is a graph showing the mean (± standard deviation) absolute number of lymphocytes in four cohorts of patients who received an anti-CD3 antibody or a placebo. The first

cohort (EU) received 8 mg of the anti-CD3 antibody TRX4 each day for six consecutive days in a Phase II trial in Europe. The second cohort received a placebo. The third cohort (TTEDD) received 0.1 mg TRX4 on Day 1, 0.2 mg TRX4 on Day 2, 0.3 mg TRX4 on Day 3, and 0.5 mg TRX4 on each of Days 4 through 8. The fourth cohort (PSO) was a cohort of psoriasis patients that received a single intravenous infusion of 1 mg TRX4;

Figure 34 is a graph showing the mean (± standard deviation) absolute number of CD2+ T lymphocytes in the first, second, and third cohorts mentioned with respect to Figure 33 hereinabove;

Figure 35 is a graph showing the mean (± standard deviation) absolute number of CD8+ T lymphocytes in the four cohorts mentioned with respect to Figure 33 hereinabove;

Figure 36 is a graph showing the mean (± standard deviation) absolute number of CD4+ T lymphocytes in the four cohorts mentioned with respect to Figure 33 hereinabove;

Figure 37 is a graph showing the mean (± standard deviation) absolute number of TCR+ T lymphocytes (CD4+ and CD8+) in the four cohorts mentioned with respect to Figure 33 hereinabove; and

Figure 38 is a graph showing the mean cytokine release of the cytokines TNF-α and IL-6 in the first (EU) and third (TTEDD) cohorts mentioned with respect to Figure 33 hereinabove.

In each of Figures 2 through 38, the term "EOI" used therein means "end of infusion."

EXAMPLES

The invention now will be described with respect to the following examples; however, it is to be understood that the scope of the present invention is not intended to be limited thereby.

In the following examples, when dosages are given in terms of μg/kg, such dosages were calculated based on the average weight of a patient as being 70 kg.

EXAMPLE 1

TRX4 antibody, which is an anti-CD3 antibody having a humanized heavy chain and a chimeric light chain, and has an aglycosylated Fc region, in which Asn^{297} has been mutated to

Ala^{297}, was administered intravenously at a dose of 0.5mg, or about 7.1μg/kg, for three consecutive days to each of three patients diagnosed with Type I diabetes. The amino acid sequences of the light and heavy chains, and the nucleic acid sequences encoding such light and heavy chains of TRX4 are shown in Figure 1. CD4+ and CD8+ T-lymphocytes were counted before, during, and after dosing. The absolute number of lymphocytes was determined by multiplying the total white cell count from the complete blood count by the percentage of lymphocytes as determined by a differential cell determination by flow cytometry. Absolute counts for each of CD4+ and CD8+ T-lymphocytes were calculated by multiplying the absolute number of lymphocytes in the lymphocyte gate by the percent of lymphocytes in the lymphocyte gate (based on forward and side light scatter parameters), bearing CD4 or CD8. As shown in Figures 2 and 3, a transient decrease of both CD4+ and CD8+ T-lymphocytes in peripheral blood (lymphopenia) was observed during dosing. The number of lymphocytes recovered to baseline levels within 2 weeks.

Cell-bound TRX4 was detected on CD4+ and CD8+ T-cells using a fluorescent labeled anti-human IgG antibody reagent. TRX4 was detected on both CD4+ and CD8+ cells 1 hour after administration of each dose of TRX4. Figure 4 depicts detection of cell-bound TRX4 with anti-human IgG as measured in MESF (molecules of equivalent soluble fluorophores) units on CD4+ cells pre- and post-TRX4 dosing. Cell-bound TRX4 decreased after dosing and the values returned to levels close to baseline 24 hours after each dose.

Modulation of the T-cell receptor (TCR) complex was observed on CD4+ and CD8+ T-cells after each dose of TRX4. Figure 5 shows modulation of TCRαβ sites as detected by staining CD4+ T-cells pre- and post-TRX4 dosing with a non-competing TCR antibody labeled with fluorescein isothiocyanate (FITC). The partial modulation returned to approximately 80% of baseline before the second and third doses of TRX4. By three weeks, the levels were approximately 80% of baseline.

A decrease in the number of free CD3 sites was observed after each dose of TRX4. Figure 6 is a graph of free CD3 sites in CD4+ T-cells as measured in MESF units. The number of free CD3 sites returned to levels close to baseline before the second and third doses of TRX4. The number of free CD3 sites returned to levels close to baseline shortly after the third dose of TRX4 (Day 5).

The above results show that a number of changes in pharmacodynamic parameters were observed. Transient lymphopenia of both CD4+ and CD8+ T-cells occurred after TRX4 administration. In addition, cell-bound antibody was detected, as well as partial modulation of the TCR/CD3 complex and saturation of CD3 after TRX4 administration.

EXAMPLE 2

A three-day dose escalation study or conditioning regimen with two cohorts of 6 to 8 subjects each is undertaken. Cohort A receives 0.1 mg (about 1.4 μg/kg) of TRX4 antibody on Day 1, 0.3 mg (about 4.3 μg/kg) of TRX4 antibody on Day 2, and 0.5 mg (about 7.1 μg/kg) of TRX4 antibody on Day 3. Cohort B receives 0.3 mg (about 4.3 μg/kg) on Day 1, 0.5 mg (about 7.1 μg/kg) on Day 2, and 1.0 mg (about 14.3 μg/kg) on Day 3.

After the above conditioning regimen is completed, a six- to eight-day dosing regimen is conducted in which each subject is subjected to the same three-day conditioning regimen described in Example 2 for Cohort B, coupled with additional treatment doses. Each subject receives 0.3 mg (4.3 μg/kg) of TRX4 on Day 1, 0.5 mg (7.1 μg/kg) of TRX4 on Day 2 and 1.0 mg (14.3 μg/kg) of TRX4 on each of Days 3 through 8.

While such cohort is being dosed, the doses given on Days 4 through 6 to 8 are escalated to determine the maximum safe dose. Once a maximum safe dose is identified, another dosing cohort in the pivotal trial is added.

The pivotal trial is a randomized, double blind, placebo controlled trial in which more than one dosing regimen arm is compared with the placebo. Follow-up testing for efficacy and safety is conducted at 6, 12, 18, and 24 months, and can be conducted up to 48 months if necessary. End-

points are mixed meal stimulated C-peptide AUC at 2, 3, and 4 hours post-ingestion, insulin usage, and hemoglobin A1c (HbA1c).

EXAMPLE 3

PHARMACOKINETIC AND PHARMACODYNAMIC DATA FROM SUBJECTS DOSED WITH TRX4

In this example, pharmacokinetic (PK) and pharmacodynamic (PD) parameters of TRX4 were evaluated in subjects from four cohorts, which, with the exception of Cohort 4, received various sequences of 3 intravenous doses of TRX4 on consecutive days. In this example, Cohort 1 received 0.1mg TRX4 on each of Days 1, 2, and 3. Cohort 2 received 0.5mg TRX4 on each of Days 1, 2, and 3. Cohort 3 received 0.1mg TRX4 on Day 1, 0.3mg TRX4 on Day 2, and 0.5mg TRX4 on Day 3, Cohort 4 received 0.3mg TRX4. As will be described further hereinbelow, T cell pharmacodynamic parameters included counts of T lymphocytes and lymphocyte subsets in the peripheral blood compartment as well as flow cytometric evaluation of bound TRX4, T cell receptor (CD3/TCR complex) modulation from the cell membrane, and saturation of the CD3/TCR complex by TRX4 on T cells in the peripheral pool. Detectable quantities (≥0.02 μg/mL) of TRX4 most often were observed in samples obtained from subjects given multiple intravenous TRX4 doses of 0.5 mg. Similarly, consistent binding of TRX4 to T cells, modulation and saturation of the CD3/TCR complex, and transient decreases in absolute numbers of peripheral T cells in blood were observed most consistently in cohorts receiving at least one 0.5mg dose. In those cohorts, consistent detectable binding of TRX4 to the T cell membrane and modulation of the CD3/TCR complex and partial saturation persisted for more than 24 hours after the last dose. Taken together, a dosing regimen containing a multiplicity of ≥0.5 mg doses would be predicted to result in TRX4 serum concentrations that are known to inhibit T cell function in vitro while evoking TRX4 binding, partial or complete saturation of the CD3/TCR complex, partial or complete modulation of the CD3/TCR

complex, and transient lymphopenia in vivo, all relevant PD parameters thought to be associated with TRX4 function.

Pharmacodynamic Data Summary

All subjects who received TRX4 in intravenous doses >0.3 mg had rapid, transient decreases in the numbers of lymphocytes (both CD4+ and CD8+ T cells) in the blood, probably caused by redistribution or re-trafficking of T cells in the periphery. For most dosing regimens, CD4+ and CD8+ T cell counts were generally within the normal range (0.53-1.76 x 10^9/L for CD4+ T cells and 0.30-1.03 x 10^9/L for CD8+ T cells) with the exception of transient decreases below the normal range in many subjects after doses of 0.3 mg and higher. The decrease in peripheral lymphocytes most likely was a redistribution phenomenon, because in all cohorts lymphopenia was transient, cell numbers had largely returned to at least 90% of baseline by day 14, and no clinical evidence of cell lysis (increased LDH, K+, etc.) was observed. No significant changes were observed in CD19+ B cell counts. The values observed for B cells were within the normal range (0.06-0.66 x 10^9/L) for all subjects. The PD changes observed during and immediately after TRX4 infusions were consistent with what was observed in the Phase II study conducted in the EU (8 mg x 6) (Keymeulen, et al. N. Engl. J. Med, Vol. 352, pgs 2598-2608 (June 23, 2005)), although the degree of lymphopenia was less pronounced in this example and was of a shorter duration, consistent with the lower doses of TRX4 being administered than in the EU study. (Keymeulen, 2005).

Cell-bound TRX4 was detected in all cohorts in a dose-dependent manner. Cell-bound TRX4 was detected for 48 hours after doses of 0.5 mg. Partial CD3/TCR modulation was evident in all cohorts, with the exception of subjects who received 3 x 0.1 mg, after TRX4 administration. CD3/TCR modulation largely was dose dependent. Modulation of 80% or greater was observed after doses of 0.5 mg. Partial modulation also persisted for 24 hours after these doses. Modulation was measured indirectly in the EU study; the data presented here from this example show a lower

degree of CD3/TCR modulation and a shorter duration of CD3/TCR modulation than in the EU study, consistent with the lower doses of TRX4.

A decrease in free binding sites (indicating CD3 saturation by TRX4) was evident and coincident with the detection of TRX4 bound to T cells. The extent and duration of CD3 saturation were largely dose dependent. Incomplete saturation was present until Day 5 in the cohorts that received 3 doses of TRX4 (with the exception of 0.1 mg x 3).

PHARMACOKINETIC DATA

Determination of TRX4 Serum Levels

Serum levels of TRX4 were determined by an ELISA assay conducted under good laboratory practices, or GLP. Blood samples were collected before infusion of TRX4, at the end of each infusion, and 2 hours after the end of infusion. The ELISA assay uses two anti-TRX4 monoclonal antibodies, one as the capture antibody and the second as the bridging antibody. The limit of quantitation (LOQ) of this assay is 0.0199 μg/mL. The results are shown in Table 1 below.

Serum concentrations of TRX4 for the majority of samples from all cohorts were either below or just above the LOQ. Because of the limited number of samples with TRX4 concentrations above the LOQ, the limited number of subjects assessed, and intersubject variability, a pharmacokinetic analysis and definitive conclusions concerning product disposition could not be made. Nevertheless, the greatest number of samples containing detectable levels of TRX4 were from Cohort 2, the only cohort in which subjects were given a multiplicity of the 0.5 mg dose (Cohort 2). In this cohort, maximum serum concentrations at the end of infusion approached 0.10 μg/mL in some subjects, but this finding was not consistent in each subject nor observed after each dose. Nadir serum concentrations prior to the third 0.5 mg dose were 0.02 - 0.03 μg/mL in two of three subjects, while the third subject had undetectable levels. Given the limited data from the other cohorts, meaningful comparative observations could not be drawn.

Table 1. TRX4 Serum concentration detected using an ELISA assay (3 Dose Cohorts)

Unbound TRX4 Detected in Serum By ELISA														
Cohort 1 (0.1, 0.1, 0.1 mg)	Patent ID	Baseline	Day 1-Pre	Day 1 1st Dose E0I	Day 1 1st Dose 1.5 hr E0I	Day 1 1st Dose 2 hr E0I	Day 1 1st Dose 3 hr E0I	Day 1 1st Dose 8 hr E0I	Day 2-Pre	Day 2 2nd Dose E0I	Day 3-Pre	Day 3 3rd Dose 2 hr E0I	Day 3 3rd Dose 8 hr E0I	Day 4
	230001	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	NSA	0.0199	0.0199	0.0199	0.0199	0.0199
	350011	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	NSA	NSA	NSA	NSA	0.0199
	160001	0 0199	0.0199	0.0199	0.0199	0.0199	**0.0201**	NSA	0 0199	**0.0239**	0.0199	0.0199	**0.0225**	**0.0205**
	160002	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	NSA	0.0199	0.0199	0.0199	0.0199	NSA	0.0199
	160008	0 0199	NSA	NSA	NSA	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199
Cohort 2 (0.5, 0.5, 0.5 mg)	Patent ID	Baseline	Day 1-Pre	Day 1 1st Dose E0I	Day 1 1st Dose 1.5 hr E0I	Day 1 1st Dose 2 hr E0I	Day 1 1st Dose 3 hr E0I	Day 1 1st Dose 8 hr E0I	Day 2-Pre	Day 2 2nd Dose E0I	Day 3-Pre	Day 3 3rd Dose 2 hr E0I	Day 3 3rd Dose 8 hr E0I	Day 4
	310001	0.0199	NSA	**0.0909**	**0.0212**	0.0199	0.0199	0.0199	0.0199	**0.1022**	**0.0312**	**0.0374**	**0.034**	**0.0282**
	160006	0.0199	0.0199	**0.0412**	0.0199	0.0199	**0.023**	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199
	160014	0.0199	0.0199	**0.0771**	0.0199	0.0199	0.0199	**0.0243**	**0.023**	**0.0299**	**0.0247**	NSA	NSA _	0.0199
Cohort 3 (0.1 0 3, 0 5 mg)	Patent ID	Baseline	Day 1-Pre	Day 1 1st Dose E0I	Day 1 1st Dose 1.5 hr E0I	Day 1 1st Dose 2 hr E0I	Day 1 1st Dose 3 hr E0I	Day 1 1st Dose 8 hr E0I	Day 2-Pre	Day 2 2nd Dose E0I	Day 3-Pre	Day 3 3rd Dose 2 hr E0I	Day 3 3rd Dose 8 hr E0I	Day 4
	230004	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	**0.0248**	0.0199	0.0199	0.0199	0.0199
	230005	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	**0.0257**	0.0199	**0.0207**	0.0199	0.0199
	170001	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0 0199
	160016	0.0199	0.0199	0.0199	0.0199	0.0199	0 0199	0.0199	0.0199	0.0199	0.0199	0.0199	0 0199	0.0199
Cohort 4 (0.3mg)	Patent ID	Baseline	Day 1-Pre	Day 1 1st Dose E0I	Day 1 1st Dose 1.5 hr E0I	Day 1 1st Dose 2 hr E0I	Day 1 1st Dose 3 hr E0I	Day 1 1st Dose 8 hr E0I	Day 2-Pre	Day 2 2nd Dose E0I	Day 3-Pre	Day 3 3rd Dose 2 hr E0I	Day 3 3rd Dose 8 hr E0I	Day 4
	350001	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	NSA	0.0199	NSA	NSA	0.0199

Lower Limit of Quantitation (LLQ) = 0.0199 µg/mL NSA: No Sample Available NR Not Reported Samples reported at < or = LLQ are designated as 0.0199

Pharmacodynamic assessment in subjects dosed with TRX4

Flow Cytometry Analysis of Lymphocyte Subsets, Cell-bound TRX4, and CD3/T cell Receptor Complex Saturation and Modulation

Assessment of Circulating Lymphocyte Phenotype and Number

Flow cytometry immunophenotyping was used to monitor changes in peripheral blood lymphocytes and subsets of total T cells, CD4+ T cells and CD8+ T cells. CD19+ B cells also were monitored as B cells are a target of Epstein Barr virus (EBV), and EBV reactivation was seen in the Phase II study conducted in the EU. (Keymeulen, 2005). Natural Killer (NK) cells were monitored in the 3 dose cohorts; no significant changes in NK levels were observed. Activated T cells, defined as T cells expressing CD25, CD38, CD69, or HLA-DR were monitored in the 3 dose cohorts; no increase in activated T cells was observed in any subject after TRX4 administration.

Method of Calculation of Absolute Lymphocyte Counts

Absolute counts for each lymphocyte subset per liter were calculated based on CD markers by multiplying the absolute number of lymphocytes per liter by the percentage of lymphocytes in the lymphocyte flow cytometry gate (as determined using forward and side light scatter parameters) bearing the CD marker of interest. To facilitate accurate enumeration of lymphocyte populations that occur at low frequencies, 50,000 events were collected by flow cytometry. The absolute number of lymphocytes was determined by multiplying the total white blood cell (WBC) count (from a hematology sample taken at the same time as the flow cytometry sample) by the percentage of lymphocytes as determined by the WBC differential cell count. Absolute counts and percentages were calculated for each parameter, and changes from baseline were determined for each post-baseline assessment.

Detection of TRX4 Bound to CD4+ and CD8+ T cells

Cell-bound TRX4 was detected on CD4+ and CD8+ T cells using an anti-human IgG antibody reagent. Fluorescence intensity was quantified by using standard units known as

Molecules of Equivalent Soluble Fluorochrome (MESF). MESF units were determined by comparing the fluorescence intensity signal from a microbead standard to the signal from the sample solution stained with the same fluorochrome. There is a direct relationship between the MESF value of a cell population and the number of binding antibodies. Use of MESF standardizes data collected on different days. The MESF of the anti-human IgG was used to quantify the amount of cell-bound TRX4.

CD3/T Cell Receptor (TCR) Complex Analysis: CD3/TCR Modulation and Saturation

CD3 is one of the components of the TCR complex. CD4+ T cells express approximately twice the number of CD3/TCR complexes as CD8+ T cells. To evaluate the level of surface expression of the CD3/TCR complex and its modulation, αß TCR expression was determined for both CD4+ and CD8+ T cells using the antibody BMA031. (Abcam, Inc., Cambridge, MA; Borst, et al., Hum. Immunol., Vol. 29, pgs. 175-188 (1990)). Binding of this antibody is not blocked by TRX4 bound to the CD3 surface molecule when TRX4 serum levels are below 1 µg/mL. Serum levels greater than 1 µg/mL were not detected in any of subjects described in this example. The MESF of the anti-TCR αß antibody was used to quantify the number of CD3/TCR complexes present on T cells.

Free TRX4 binding sites (unoccupied by previously administered TRX4) were detected by staining with biotinylated TRX4. The MESF of bound biotinylated TRX4 is directly proportional to the availability of free TRX4 binding sites.

Cohort Summaries:

Unless noted, the changes in PD parameters observed were similar in CD4+ and CD8+ T cells.

Cohort One (0.1 mg x 3) (n=4):

Lymphocyte Numbers

Circulating T cells and B cells did not change significantly after TRX4 dosing in this cohort.

Detection of TRX4 Bound to CD4+ T cells

Maximum amounts of TRX4 bound to CD4+ T cells were detected at the end of the TRX4 infusion (Figure 7). Peak amounts occurred after the second dose of TRX4 (300,000 MESF units), although bound TRX4 was appreciably lower than what was observed in cohorts with higher doses. Bound TRX4 decreased on all T cells after Day 3, and no bound TRX4 was detected on the surface of T cells by Day 14.

T cell Receptor Analysis

No significant modulation of the CD3/TCR receptor complex (that is, no decrease in MESF of TCR αß) was observed on CD4+ T cells after dosing with TRX4.

A slight and transient decrease in free CD3 sites was evident in CD4+ T cells at the end of the first infusion of TRX4; free CD3 sites returned to baseline levels 2 hours after the end of infusion. Approximately 37% saturation of CD3 was observed at the end of infusion with recovery to baseline levels by 2 hours after the end of infusion. No significant decrease in free CD3 sites occurred after the second or third dose of TRX4. Free CD3 sites remained at baseline levels for the remainder of the study.

Cohort Two (0.5 mg x 3) (n=3; 2 subjects received 3 doses, 1 subject received 2 doses):

Lymphocyte Numbers

Circulating T cell counts were reduced in all subjects after the first infusion of TRX4 and remained below baseline levels during the 3 days of dosing. Lymphocyte counts began to increase at Day 4 and were close to baseline levels by Day 5. No significant changes were observed in circulating B cells.

Detection of TRX4 Bound to CD4+ T cells

Maximum TRX4 binding was detected on CD4+ T cells at the end of the TRX4 infusion on each day of dosing. The amount of binding (approximately 600,000 MESF units after each dose) was greater than what was observed in Cohort 1, consistent with a higher dose of TRX4. Cell-bound TRX4 was still present (400,000 MESF units) 2 hours after the end of dosing. TRX4 binding returned to levels close to baseline and was generally not detected prior to the second and third doses; however, TRX4 binding was detected on the surface of CD4+ T cells after the third dose until Day 5.

T Cell Receptor Analysis

Significant CD3/TCR modulation was observed on CD4+ T cells at the end of each TRX4 infusion. Approximately 60% modulation was observed after the first dose, approximately 70% modulation after the second dose and 80% modulation after the third dose. Approximately 40% modulation was observed 2 hours after the end of infusion at all 3 doses. No modulation was evident before the second dose. Approximately 20% modulation was evident prior to the third dose. After the third dose, modulation was evident until Day 14.

A decrease in free CD3 sites was evident on CD4+ T cells at the end of each infusion of TRX4. Approximately 90% saturation was observed after the first dose, approximately 80% saturation after the second dose and 95% saturation after the third dose. Approximately 55% saturation was observed 2 hours after the end of infusion at all 3 doses. Significant saturation was not evident prior to the second and third doses. After the third dose, partial saturation was observed until Day 5.

Cohort Three (0.1 mg, 0.3 mg, 0.5 mg) (n=4):

Lymphocyte Numbers

Circulating T cells were modestly reduced in all subjects after the 0.3 mg and 0.5 mg doses of TRX4. Lymphocyte counts began to increase at Day 4 and were close to baseline levels by Day 5. No significant changes were observed in circulating B cells.

Detection of TRX4 Bound to CD4+ T cells.

Cell-bound TRX4 was detected on CD4+ T cells at the end of the TRX4 infusion on each day of dosing. TRX4 binding returned to levels close to baseline between doses and generally was not detected prior to the second or third dose. The increase in cell-bound TRX4 occurred in a dose dependent manner with the greatest amount of TRX4 detected after the 0.5 mg dose (100,000 MESF units after 0.1 mg, 300,000 MESF units after 0.3 mg and 800,000 MESF units after 0.5 mg). TRX4 was no longer detected on the surface of T cells by Day 4.

T Cell Receptor Analysis

CD3/TCR modulation was observed on CD4+ T cells. Modulation was dose dependent with 15% modulation observed after 0.1 mg, 50% modulation after 0.3 mg and 80% modulation after 0.5 mg. CD3/TCR expression recovered to levels close to baseline between dosing. TCR modulation was not detectable after Day 5.

There were dose dependent decreases in free CD3 sites on CD4+ T cells at the end of each infusion of TRX4. Transient partial saturation was observed after 0.1 mg (45%) and 0.3 mg (70%). Free CD3 sites returned to close to baseline levels 2 hours after the end of the first 2 infusions. After 0.5 mg, 90% saturation was observed at the end of infusion, and 45% saturation was still evident 2 hours after the last infusion. No significant decrease in free CD3 sites was observed after Day 5.

Lymphocyte Counts

The mean absolute lymphocyte count, CD4+ lymphocyte count, CD8+ lymphocyte count, TCR+ (CD4+CD8+) lymphocyte count, and CD2+ lymphocyte count were measured for up to 14 weeks after the start of the treatment for each of the cohorts. Figure 8 shows the median total number of all lymphocytes for each of the four cohorts up to 14 weeks after treatment. Figure 9 shows the median total number of CD4+ T-lymphocytes for each of the four cohorts up to 14 weeks after treatment. Figure 10 shows the median total number of CD8+ T-lymphocytes for each of the four cohorts up to 14 weeks after treatment. Figure 11

shows the median total number of TCR+(CD4+ and CD8+) T-lymphocytes for each of the four cohorts up to 14 weeks after treatment. Figure 12 shows the median total number of CD2+ T-lymphocytes for each of the four cohorts up to 14 weeks after treatment.

The lymphocyte counts for all cohorts had returned to at or near baseline levels within approximately 5 days after the start of the treatment, and remained at or near such baseline levels.

EXAMPLE 4

In this example, pharmacokinetic and pharmacodynamic parameters of TRX4 were evaluated in subjects from four cohorts that received various sequences of 4 intravenous doses of TRX4 on consecutive days. Cohort A*, which included one patient, received 0.05mg TRX4 on Day 1, 0.1mg TRX4 on Day 2, 0.15mg on Day 3, and 0.25mg on Day 4. Cohort A, which included 4 patients, received 0.1mg TRX4 on Day 1, 0.2mg TRX4 on Day 2, 0.3mg on Day 3, and 0.5mg on Day 4. Cohort B, which included 4 patients, received 0.1mg TRX4 on Day 1, 0.2mg TRX4 on Day 2, 0.3mg on Day 3, and 0.75mg on Day 4. Cohort C, which included one patient, received 0.1mg TRX4 on Day 1, 0.2mg TRX4 on Day 2, 0.3mg on Day 3, and 1.0mg on Day 4.

As in Example 3, T-cell pharmacodynamic parameters included counts of T-lymphocytes and lymphocyte subsets in the peripheral blood compartment as well as flow cytometric evaluation of bound TRX4, T cell receptor (CD3/TCR complex) modulation from the cell membrane, and saturation of the CD3/TCR complex by TRX4 on T cells in the peripheral pool. As in Example 3, consistent binding of TRX4 to T cells, modulation and saturation of the CD3/TCR, and transient decreases in absolute numbers of peripheral T cells in blood were observed most consistently in cohorts receiving at least one 0.5mg dose. In those cohorts, consistent binding of TRX4 to the T cell membrane and modulation of the CD3/TCR and partial saturation persisted for more than 24 hours after the last dose.

Also, all subjects who received TRX4 in intravenous doses >0.3mg had rapid, transient decreases in the numbers of lymphocytes (both CD4+ and CD8+ cells) in the blood. As in Example 3, for most dosing regimens, CD4+ and CD8+ T cell counts generally were within the normal range (0.53-1.76X 10^9/l for CD4+ T cells and 0.30-1.03X 10^9/l for CD8+ T cells) with the exception of transient decreases below the normal range in many subjects after doses of 0.3 mg and higher. The decrease in peripheral blood lymphocytes, as in Example 3, most likely was a redistribution phenomenon, because in all cohorts lymphopenia was transient, cell numbers largely had returned to at least 90% of baseline by Day 14, and no clinical evidence of cell lysis (increased LDH,K+ etc.) was observed. Also, no significant changes were observed in CD19+ B cell counts. The values observed for B cells were within the normal range (0.06-0.66 X 10^9/l) for all subjects. The pharmacodynamic changes observed during and immediately after TRX4 infusions were consistent with what was observed in the Phase II study conducted in Europe (8mg/day for 6 days) (Keymeulen, 2005), although the degree of lymphopenia was less pronounced in this example and was of a shorter duration, which is consistent with the lower doses of TRX4 being administered than in the European study (Keymeulen, 2005).

As in Example 3, cell-bound TRX4 was detected in all cohorts in a dose-dependent manner. Cell-bound TRX4 was detected for 48 hours after doses of 0.5 mg and higher. Partial CD3/TCR modulation was evident in all cohort after TRX4 administration. CD3/TCR modulation was largely dose dependent. Modulation of 80% or greater was observed after doses of 0.5 mg and higher. Partial modulation also persisted for 24 hours after these doses. Modulation was measured indirectly in the EU study (Keymeulen, 2005); the data presented here in this example show a lower degree of CD3/TCR modulation and a shorter duration of CD3/TCR modulation than in the EU study, consistent with the lower doses of TRX4.

A decrease in free TRX4 binding sites (indicating CD3 saturation by TRX4) was evident and coincident with the detection of TRX4 bound to T cells. The extent and duration

of CD3 saturation were largely dose dependent. Incomplete saturation was present until Day 5 in the lowest 4-dose cohort (0.05 mg, 0.1 mg, 0.15 mg and 0.25 mg). In the other 4-dose cohorts, saturation of 90% or greater was observed after doses of 0.5 mg and higher and partial saturation (approximately 40%) was present at 24 hours after the infusion. Incomplete saturation was present until Week 3 in subjects who received 4 doses of TRX4 (0.1 mg, 0.2 mg, 0.3 mg and 0.5 mg). Saturation was present until Day 7 in subjects who received 4 doses of TRX4 with a fourth dose of either 0.75 mg or 1 mg.

Pharmacokinetic Data

Determination of TRX4 Serum Levels

Serum levels of TRX4 were determined using the ELISA assay described in Example 3. As in Example 3, the limit of quantitation (LOQ) of the assay was 0.0199μg/ml. The results are shown in Table 2 below.

Table 2. TRX4 Serum concentration detected using an ELISA assay (4 Dose Cohorts)

Cohort A (0.1, 0.2, 0.3, 0.5 mg)	Patient ID	Baseline	Day 1 - Pre	Day 1 First Dose- EOI	Day 1 First Dose- 1 5 hr EOI	Day 1 Ent Dose- 2 hr EOI	Day 1 First Dose- 3 hr EOI	Day 1 First Dose- 8 hr EOI	Day 2- Pre	Day 2 Second Dose- EOI	Day 3- Pre	Day 3 Third Dose- 2 hr EOI	Day 3 Third Dose- 8 hr EOI	Day 4-Pre	Day 4 Fourth Dose- 2 hr EOI	Day 4 Fourth Dose- 8 hr EOI	Day
	310002	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	**0.02**	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199
	390001	0.0199	0.0199	0.0199	NSA	0.0199	0.0199	0.0199	0.0199	NR	NSA	NR	0.0199	0.0199	0.0199	0.0199	0.0199
	350013	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	NSA	**0.0203**	NSA	0.0199	0.0199	0.0199	NSA
	350012	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	NSA	0.0199	NSA	0.0199	0.0199	0.0199	0.0199
	310003*	0.0199	NSA	NSA	NSA	NSA	NSA	0.0199	0.0199	**0.0208**	0.0199	0.0199	0 0199	0.0199	0.0199	0.0199	0.0199
• Subject received three doses of TRX4 (0.1, 0.2, 0.3mg)																	

Cohort A* (0.05, 0 1, 0.15, 0.25 mg)	Patient ID	Baseline	Day 1 - Pre	Day 1 First Dose- EOI	Day 1 First Dose- 1 5 hr EOI	Day 1 Ent Dose- 2 hr EOI	Day 1 First Dose- 3 hr EOI	Day 1 First Dose- 8 hr EOI	Day 2- Pre	Day 2 Second Dose- EOI	Day 3- Pre	Day 3 Third Dose- 2 hr EOI	Day 3 Third Dose- 8 hr EOI	Day 4-Pre	Day 4 Fourth Dose- 2 hr EOI	Day 4 Fourth Dose- 8 hr EOI	Day
	160020	0.0199	0.0199	0.0199	0.0199	0.0199	NSA	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199

Cohort B (0 1, 0 2, 0 3, 0 75 mg)	Patient ID	Baseline	Day 1 - Pre	Day 1 First Dose- EOI	Day 1 First Dose- 1 5 hr EOI	Day 1 Ent Dose- 2 hr EOI	Day 1 First Dose- 3 hr EOI	Day 1 First Dose- 8 hr EOI	Day 2- Pre	Day 2 Second Dose- EOI	Day 3 Third Dose- EOI	Day 4-Pre	Day 4 Fourth Dose- 2 hr EOI	Day 4 Fourth Dose- 8 hr EOI	Day 5
	350021	0.0199	0.0199	0.0199	0.0199	0.0199	NSA	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199
	380001	NSA	0.0199	0.0199	0.0199	0.0199	NSA	NSA	0.0199	0.0199	NSA	NSA	NSA	NSA	NSA
	390005	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	NR	0.0199	0.0199	0.0199	0.0199
	350015	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199

Cohort RT-4A* (0.05, 0.1, 0.15, 0.25 mg)	Patient ID	Baseline	Day 1 - Pre	Day 1 First Dose- EOI	Day 1 First Dose- 1 5 hr EOI	Day 1 Ent Dose- 2 hr EOI	Day 1 First Dose- 3 hr EOI	Day 1 First Dose- 8 hr EOI	Day 2- Pre	Day 2 Second Dose- EOI	Day 3- Pre	Day 3 Third Dose- 2 hr EOI	Day 3 Third Dose- 8 hr EOI	Day 4-Pre	Day 4 Fourth Dose- 2 hr EOI	Day 4 Fourth Dose- 8 hr EOI	Day 5
	160020	0.0199	0.0199	0.0199	0.0199	0.0199	NSA	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199

Cohort C (0.1, 0.2, 0.3, 1.0 mg)	Patient ID	Baseline	Day 1 - Pre	Day 1 First Dose- EOI	Day 1 First Dose- 1 5 hr EOI	Day 1 Ent Dose- 2 hr EOI	Day 1 First Dose- 3 hr EOI	Day 1 First Dose- 8 hr EOI	Day 2- Pre	Day 2 Second Dose- EOI	Day 3 Third Dose- EOI	Day 4-Pre	Day 4 Fourth Dose- 2 hr EOI	Day 4 Fourth Dose- 8 hr EOI	Day 5
	390010	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	0.0199	NR	NR	0.0199	0.0199	0.0199	0.0199

Lower Limit of Quantitation (LLQ) = 0.0199µg/mL

NSA: No Sample Available

NR: Not Reported

Samples reported at< or = LLQ are designated as 0.0199

Pharmacodynamic Assessment in Subjects Dosed with TRX4: Flow Cytometry Analysis of Lymphocyte Subsets, Phenotype, and Numbers; Cell Bound TRX4; and CD3/T Cell Receptor Complex Saturation and Modulation

Flow cytometry immunophenotyping was used as in Example 3 to monitor changes in peripheral blood lymphocytes and subsets of total T-cells, CD4+ T-cells and CD8+ T-cells, and CD19+ B cells also were monitored.

Absolute counts for lymphocytes and for lymphocyte subsets were calculated based on CD markers as described in Example 3. Absolute counts and percentages were calculated for each parameter, and changes from baseline were determined for each post-baseline assessment.

Cell-bound TRX4 was detected on CD4+ and CD8+ T-cells using an anti-human IgG antibody reagent, and fluorescence intensity was quantified using standard MESF units as described in Example 3.

In order to evaluate the level of surface expression of the CD3/TCR complex and its modulation, αß TCR expression was determined for both CD4+ and CD8+ T-cells using the antibody BMA031, as described in Example 3. The MESF of the anti-TCR αß antibody was used to quantify the number of CD3/TCR complexes present on T-cells.

Free TRX4 binding sites (unoccupied by previously administered TRX4) were detected by staining with biotinylated TRX4 . The MESF of bound biotinylated TRX4 is directly proportional to the availability of free TRX4 binding sites.

Cohort A* (0.05 mg, 0.1 mg, 0.15 mg, 0.25 mg) (n=1):

Lymphocyte Numbers

Circulating T cell counts were reduced modestly after the first dose of TRX4. Lymphocyte counts returned to baseline levels at Day 14. A transient decrease in circulating B cells was observed on the second and third days of dosing.

Detection of TRX4 Bound to CD4+ T cells

Cell-bound TRX4 was detected on CD4+ T cells at the end of the TRX4 infusion on each day of dosing (300,000 MESF units after 0.05 mg, 700,000 MESF units after 0.1 mg, 800,000 MESF units after 0.15 mg and 80,000 MESF units after 0.25 mg). The greatest amounts of cell-bound TRX4 were detected after the second and third doses. Only a slight increase in cell-bound TRX4 was detected after the fourth dose which was likely due to the simultaneous occurrence of TCR modulation. TRX4 was no longer detected on the surface of T cells by Day 5 on either cell subset.

T cell Receptor Analysis

CD3/TCR modulation was observed on CD4+ T cells in a somewhat dose dependent manner with 45% modulation after 0.05 mg, 20% modulation after 0.1 mg, 25% modulation after 0.15 mg and 80% modulation after 0.25 mg. CD3/TCR modulation was no longer detectable by Day 5 and subsequent levels were close to baseline.

A decrease in free CD3 sites was evident on CD4+ T cells after each dose of TRX4 and this occurred in a dose dependent manner with 50% saturation after 0.05 mg, 70% saturation after 0.1 mg, 80% saturation after 0.15 mg, and 95% saturation after 0.25 mg. This returned to levels close to baseline levels 2 hours after the end of each infusion. No significant decrease in free CD3 sites was observed after Day 5.

Cohort A (0.1 mg, 0.2 mg, 0.3 mg, 0.5 mg) (n=4):

Lymphocyte Numbers

Circulating CD4+ T cells counts were reduced modestly after the first dose of TRX4 and remained so until Day 7 when they returned to baseline (Figure 13). No significant changes were observed in circulating B cells.

Detection of TRX4 Bound to CD4+ T cells

Cell-bound TRX4 was detected on CD4+ T cells at the end of the TRX4 infusion on each day of dosing (200,000 MESF units after 0.1 mg, 375,000 MESF units after 0.2 mg, 600, 000 MESF units after 0.3 mg and 450,000 MESF units after 0.5 mg) (Figure 14). The greatest amount of cell-bound TRX4 was detected after the third and fourth doses of TRX4. Cell-bound TRX4 was still present 2 hours after the end of the 0.5 mg infusion (400,000 MESF units) with detectable TRX4 still present approximately 24 hours later. TRX4 was no longer detected on the surface of T cells by Day 7.

T cell Receptor Analysis

CD3/TCR complex modulation was observed on CD4+ T cells after dosing (Figure 15). Modulation was dose dependent with 45% modulation after 0.1 mg, 40% modulation after 0.2 mg. 60% modulation after 0.3 mg and 80% modulation after 0.5 mg. The degree of modulation was most consistent between subjects at the 0.5 mg dose. CD3/TCR expression recovered to levels close to baseline between doses so that no modulation was evident prior to the second, third, and fourth doses; however, after the fourth dose of 0.5 mg, partial CD3/TCR modulation was evident until Week 3, after which CD3/TCR levels returned to baseline.

There were dose-dependent decreases in free CD3 sites on CD4+ T cells after each infusion with 40% saturation after 0.1 mg, 70% saturation after 0.2 mg, 85% saturation after 0.3 mg and 95% saturation after 0.5 mg (Figure 16). 60% saturation was evident 2 hours after the end of the last infusion and 35% saturation still was evident the next day.

Cohort B (0.1 mg, 0.2 mg, 0.3 mg, 0.75 mg) (n=4):

Lymphocyte Numbers

Circulating T cell counts were reduced modestly after the first dose and remained so until Day 7 when they returned to baseline. No significant changes were observed in circulating B cells.

Detection of TRX4 Bound to CD4+ T cells

Cell-bound TRX4 was detected on CD4+ T cells at the end of the TRX4 infusion on each day of dosing (2000,000 MESF units after 0.1mg, 400,000 MESF after 0.2mg,

420,000 MESF units after 0.3 mg and 480,000 MESF units after 0.75 mg). The greatest amount of cell-bound TRX4 was detected after the third and fourth doses. Cell-bound TRX4 was still present 2 hours after the end of the 0.75 mg infusion (550,000 MESF units) with detectable TRX4 still present approximately 24 hours later (200,000 MESF units). TRX4 was no longer detected on the surface of T cells by Day 7.

T Cell Receptor Analysis

CD3/TCR modulation was observed on CD4+ T cells after dosing (Figure 17). Modulation was dose dependent with 20% modulation after 0.1 mg, 30% modulation after 0.2 mg, 60% modulation after 0.3 mg and 85% modulation after 0.75 mg. Modulation recovered to levels close to baseline between doses so that no modulation was evident prior to the second, third and fourth doses, however, after the fourth dose of 0.75 mg, 50% modulation was still evident 2 hours after the end of the last infusion. Partial CD3/TCR modulation was evident until Week 2, after which CD3/TCR levels remained close to baseline.

There were dose-dependent decreases in free CD3 sites on CD4+ T cells at the end of each infusion. No significant saturation was detected after 0.1 mg, with 60% saturation after 0.2 mg,

80% saturation after 0.3 mg and 95% saturation after 0.75 mg (Figure 18). 70% saturation was evident 2 hours after the end of the last infusion, and 40% saturation was still evident the next day (Day 5). A full return to baseline levels occurred by Day 7.

Cohort C (0.1 mg, 0.2 mg, 0.3 mg, 1.0 mg) (n=1):

Lymphocyte Numbers

Circulating CD4+ T cells counts were reduced modestly at the end of each infusion of TRX4 but returned to levels close to baseline within 24 hours. No significant changes were observed in circulating B cells.

Detection of TRX4 Bound to CD4+ T cells

Cell-bound TRX4 was detected on CD4+ T cells at the end of the TRX4 infusion on each day of dosing (800,000 MESF units after 0.1 mg, 1,200,000 MESF units after 0.2 mg, 600,000 MESF units after 0.3 mg and 750,000 MESF units after 1.0 mg). The greatest amount of cell-bound TRX4 was detected after the second dose. Cell-bound TRX4 was still present 2 hours after the end of the 1.0 mg infusion (600,000 MESF units) with detectable TRX4 still present approximately 24 hours later (200, 000 MESF units). TRX4 was no longer detected on the surface of T cells by Day 7.

T cell Receptor Analysis

CD3/TCR modulation was observed on CD4+ T cells after dosing. Modulation was dose dependent with 25% modulation after 0.1 mg, 75% modulation after 0.2 mg, 85% modulation after 0.3 mg and 88% modulation after 1.0 mg. Modulation recovered to levels close to baseline between dosing so that no modulation was evident prior to the second, third and fourth doses. 50% modulation was still evident 2 hours after the end of the last infusion, and this was still evident on Day 5. CD3/TCR levels appeared to be returning to baseline by Day 7.

Decrease in free CD3 sites were evident on CD4+ T cells at the end of each infusion and occurred in a dose dependent manner with 50% saturation after 0.1 mg, 95% saturation after 0.2 mg, 99% saturation after 0.3 mg and 97% saturation after 1.0 mg. 50% saturation was evident 2 hours after the end of the last infusion, and 30% saturation was still evident the next day (Day 5). A full return to baseline levels occurred by Day 7.

Lymphocyte Counts

The mean absolute lymphocyte count, CD4+ lymphocyte count, CD8+ lymphocyte count, TCR + (CD4+CD8+) lymphocyte count, and CD2+ lymphocyte count were measured for up to 12 or 13 weeks after the start of the treatment for each of the cohorts. Figure 19 shows the median total number of all lymphocytes for each of the four cohorts up to 13 weeks after treatment. Figure 20 shows the median total number of CD4+ T-lymphocytes for each of the four cohorts up to 13 weeks after treatment. Figure 21 shows the median total number of CD8+ T-lymphocytes for each of the four cohorts up to 13 weeks after treatment. Figure 22 shows the median total number of TCR+ (CD4+ and CD8+) T-lymphocytes for each of the four cohorts up to 13 weeks after treatment. Figure 23 shows the median total number of CD2+ T-lymphocytes for each of the four cohorts up to 13 weeks after treatment.

EXAMPLE 5

Based upon the pharmacokinetic and pharmacodynamic data obtained in Examples 3 and 4, an 8-day TRX4 dosing regimen was developed. In this example, each patient in a cohort of six patients received intravenous doses of 0.1 mg TRX4 on Day 1, 0.2 mg TRX4 on Day 2, 0.3 mg TRX4 on Day 3, and 0.5 mg TRX4 on each of Days 4 through 8. This regimen or protocol was entitled TRX4 Therapeutic Evaluation of Different Multi-Dose Regimens in Type 1 Diabetes Mellitus, or TTEDD.

In this example, cell-bound TRX4 on CD4+ T cells and CD8+ T-cells was determined using anti-human IgG antibody reagents, and fluorescence intensity was quantified by using standard MESF units as described in Example 3. Results are shown graphically in Figures 24 and 25.

Free TRX4 CD3 binding sites (unoccupied by previously administered TRX4) on CD4+ T cells and CD8+ T-cells were detected by staining with biotinylated TRX4 as described in Example 3. The MESF of bound biotinylated TRX4 is directly proportional to the availability of free TRX4 binding sites. Results are shown graphically in Figures 26 and 27.

In order to evaluate the level of surface expression of the CD3/TCR complex and its modulation, αß TCR expression was determined for CD4+ T cells and CD8+ T-cells using the antibody BMA01 as described in Example 3. As noted hereinabove, binding of this antibody is not blocked by TRX4 bound to the CD3 surface molecule when TRX4 serum levels are below 1 µg/ml. The MESF of the anti-TCR αß antibody was used to quantify the number of CD3/TCR complexes present on T cells. Results are shown graphically in Figures 28 and 29 as percentages of the baseline value.

Absolute counts for lymphocytes and for each lymphocyte subset per liter were calculated based on CD markers as described in Example 3. Absolute counts and percentages were calculated for each parameter, and changes from baseline were determined for each post-baseline assessment.

Detection of TRX4 Bound to CD4+ T cells and CD8+ T cells

The amounts of TRX4 bound to CD4+ T cells (Figure 24) and bound to CD8+ T cells (Figure 25) increased significantly above baseline within 2 hours after each infusion, and then decreased before the next day's infusion. The amounts of TRX4 bound to CD4+ T cells and CD8+ T cells prior to the infusions on Days 2 through 8, however, were at or above baseline. The amount of binding was greatest after the infusions on Days 4 and 6. When the infusions of TRX4 were stopped after Day 8, TRX4 binding levels had returned to baseline between Week 2 and Week 3.

T Cell Receptor Analysis

The number of free CD3 sites on CD4+ T cells (Figure 26) and on CD8+ T cells (Figure 27) decreased after each infusion of TRX4. Almost complete saturation was achieved after the infusion on Day 7. When the infusions were stopped after Day 8, free CD3 sites on CD4+ T-cells returned to near baseline levels at about Week 5 (Figure 26) and free CD3 sites on CD8+ T cells returned to near baseline levels at about Week 4 (Figure 27).

CD3/TCR modulation on CD4+ T cells (Figure 28) and on CD8+ T cells (Figure 29) was observed at the end of each TRX4 infusion. Greater than 80% modulation on CD4+ T cells was observed after the infusions on each of Days 5 through 8 (Figure 28). Approximately 80% modulation on CD8+ T cells was observed after the infusions on each of Days 5 through 8 (Figure 29). When the infusions were stopped after Day 8, modulation of CD4+ T cells was observed until about Week 3 (Figure 28), and modulation of CD8+ T cells also was observed until about Week 3. (Figure 29).

Lymphocyte Numbers

The CD4+ T cell count was reduced after the first infusion of TRX4 and remained below baseline levels during the 8 days of dosing (Figure 30). Levels were at about 20% of baseline after the TRX4 infusion on Day 7. When the infusions were stopped after Day 8, the CD4+ T cell count increased gradually, but did not return to baseline until approximately Week 10.

CD8+ T cell counts also were reduced after the first infusion of TRX4 and remained significantly below baseline levels throughout the 8 days of dosing (Figure 31). When the infusions were stopped after Day 8, the CD8+ T cell count increased, but did not reach baseline levels until about Week 6.

CD19+ B cell counts remained at or near baseline levels throughout the 8 day dosing regimen, and up to and through Week 12. (Figure 32).

The pharmacodynamic parameters observed in the 8 day TRX4 dosing regimen described herein were compared with other TRX4 dosing regimens, namely (i) the European Union Phase II trial in which 35 new onset Type 1 Diabetes patients were given infusions of 8 mg of TRX4 on six consecutive days (Keymeulen, 2005), and (ii) a study in which each patient in a cohort of four patients suffering from psoriasis was given a single intravenous infusion of 1 mg of TRX4. This study is described further in Example 6 hereinbelow. A placebo group of 40 patients who received 0.9% saline solution was used as a control.

Figure 33 shows the average total number of lymphocytes in the six patients treated in this example, as compared to the patients treated with the humanized aglycosylated anti-CD3 antibody in the European Union Phase II trial (EU) (Keymeulen, 2005), and the four psoriasis patients given 1 mg TRX4 (PSO). As shown in Figure 33, the total numbers of lymphocytes in the six patients treated in this example and the psoriasis patients approximated each other over a

period of 10 weeks, and approximated the total number of lymphocytes in the patients of the European trial for approximately 3 weeks. The patients in the European trial then had a significant rise in the number of lymphocytes, which was due to Epstein-Barr Virus (EBV) - associated CD8+ T cell lymphocytosis.

Figure 34 shows the number of CD2+ T lymphocytes in the six patients treated in this example, as well as those in the Type 1 diabetes patients treated in the European study. The numbers of CD2+ T lymphocytes in these groups approximated each other until Week 3, after which occurred a significant rise in the number of CD2+ T lymphocytes in the patients treated in the European study. This again was due to EBV-associated CD8+ T cell lymphocytosis, because the CD2+ T lymphocytes also are CD8+.

Figure 35 shows the total number of CD8+ T lymphocytes in the six patients treated in this example, as well as the Type 1 diabetes patients treated in the European Phase II trial, and the four psoriasis patients who were given 1 mg TRX4. The numbers of CD8+ T lymphocytes in the six patients treated in this example and the psoriasis patients approximated each other over a period of 10 weeks, while the numbers of CD8+ T lymphocytes of the six patients treated in this example and the Type 1 diabetes patients treated in the European Phase II trial approximated each other for a period of about 2 weeks, after which there was a significant rise in the number of CD8+ T lymphocytes in the Type 1 diabetes patients treated in the European Phase II trial. This rise in the number of CD8+ T lymphocytes was due to EBV-associated CD8+ T cell lymphocytosis.

Figure 36 shows the CD4+ T lymphocyte counts for the six patients treated in this example, as well as the Type 1 diabetes patients treated in the European Phase II trial, and the four psoriasis patients who received 1 mg TRX4. The six patients treated in this example, the

type 1 diabetes patients treated in the European Phase II trial, and the four psoriasis patients who received 1 mg TRX4 had similar decreases and increases in the number of CD4+ T lymphocytes over a period of 10 weeks.

Figure 37 shows the TCR+ T lymphocyte counts for the six patients treated in this example, the Type 1 diabetes patients treated in the European Phase II trial, and the four psoriasis patients who received 1 mg of TRX4. Such TCR+ T lymphocytes are CD4+ and CD8+. The numbers of TCR+ T lymphocytes in the six patients treated in this example and the four psoriasis patients approximated each other over a period of 10 weeks. Similar changes in the numbers of TCR+ T lymphocytes were observed in the six patients treated in this study and the Type 1 diabetes patients treated in the European Phase II trial over a period of about 2 weeks, after which there was a significant rise in the number of TCR+ T lymphocytes in the Type 1 diabetes patients treated in the European Phase II trial. This was due to EBV-associated CD8+ and CD4+T cell lymphocytosis.

As shown in Figures 33 through 37, the pharmacodynamic parameters, as measured in terms of various lymphocyte counts, of the six patients treated in this example, were similar to those of the Type 1 diabetes patients treated in the Phase II European trial (Keymeulen, 2005), and the four psoriasis patients who received 1 mg of TRX4.

As has been noted previously (Keymeulen, 2005), the Type 1 diabetes patients in the European Phase II trial who received the humanized aglycosylated anti-CD3 antibody TRX4 had better maintenance of residual beta cell function than those in the placebo group. Insulin doses also increased in the placebo group but not in the patients treated with the CD3 antibody.

As will be explained in further detail in Example 6 hereinbelow, the four psoriasis patients who received an intravenous infusion of 1 mg of TRX4 showed a reduction in their psoriasis area severity index (PASI) scores at 8 weeks post-infusion.

Thus, the treatment regimen employing TRX4 as described in this example has pharmacodynamic parameters similar to other treatment regimens employing anti-CD3 antibodies, which have been effective in treating diabetes or psoriasis.

In addition, release of the cytokines TNF-α and Interleukin-6 (IL-6) was measured by an ELISA assay (R and D Systems, Minneapolis, MN) at one hour after the end of infusion on each of the eight days of the treatment regimen, and the mean cytokine release for each of TNF-α and IL-6 in pg/ml was determined. The mean cytokine release concentrations for each of Days 1 through 8 were compared to the mean cytokine release concentrations for the patients in the Phase II European trial (Keymeulen, 2005) at one hour after the end of infusion of their first dose of 8 mg of TRX4. The results are shown graphically in Figure 38. As shown in Figure 38, the dosing regimen of 0.1 mg TRX4 on Day 1, 0.2 mg TRX4 on Day 2, 0.3 mg TRX4 on Day 3, and 0.5 mg TRX4 on Days 4 through 8 provided a significant reduction in the release of the cytokines TNF-α and IL-6, as compared to the Phase II European trial.

EXAMPLE 6

Four human patients, referred to herein as Cohort 1, each had received a physician's assessment of at least moderate psoriasis with at least 10% of the body surface area affected, and were eligible for or had systemic therapy. Each patient of Cohort 1 was given 1 mg of TRX4 by intravenous infusion over a period of one hour. None of the patients of Cohort 1 had received any systemic agents for psoriasis treatment or any potent immunosuppressive agents within four

weeks prior to receiving TRX4. During the eight week period following administration of TRX4, none of the patients of Cohort 1 received any topical or systemic treatment for psoriasis.

Immediately after the administration of TRX4, it was observed that the absolute lymphocyte counts for all patients of Cohort 1 had decreased. The decrease was observed just after the completion of the one-hour infusion of TRX4, and was most prominent between Days 1 and 2, and an increase of the lymphocyte counts began on Day 3. The lymphocyte counts returned to baseline by Day 6.

Modulation of the CD3/TCR complex, as detected by staining with an antibody to the TCR, was observed most profoundly immediately after the administration of TRX4, with full recovery of the TCR complex on the cell surface within 6 days after dosing. Serum levels of TRX4 were detected in the blood immediately after completion of the administration of TRX4, and for up to one hour after administration.

Baseline PASI scores were measured for each of the patients in Cohort 1 at one day (Day-1) before receiving TRX4. PASI scores also were measured for each of the patients in Cohort 1 during Week 8 after receiving TRX4.

The PASI scores for each of the four patients of Cohort 1, who received a 1 mg intravenous infusion of TRX4, at baseline (Day-1) and during Week 8 after administration of TRX4, are shown in Table 3 hereinbelow.

Table 3

	PASI Score	
Patient	Day-1	Week 8
1	12.2	6.6
2	27.0	16.9
3	15.8	7.8
4	16.4	8.5

In addition, five human patients, hereinafter referred to as "Cohort 2", were given 2 mg of TRX4 antibody, and seven human patients, hereinafter referred to as "Cohort 3", were given 4 mg of TRX4 antibody.

As with Cohort 1, each of the patients of Cohort 2 and Cohort 3 was given TRX4 by intravenous infusion over a period of one hour.

As with Cohort 1, each of the patients of Cohort 2 and Cohort 3 had received a physician's assessment of at least moderate psoriasis with at least 10% of body surface area affected, and were eligible for or had systemic therapy.

None of the patients of Cohort 2 or Cohort 3 had received any systemic agents for psoriasis treatment or any potent immunosuppressive agents within four weeks prior to receiving TRX4.

Baseline PASI scores were measured for each of the patients in Cohort 2 and Cohort 3 at one day (Day-1) before receiving TRX4. PASI scores also were measured for each of the patients in Cohort 2 and Cohort 3 during Week 8 after receiving TRX4.

The PASI scores of Patients 1 through 5 of Cohort 2 are given in Table 4 hereinbelow. Patient 5 of Cohort 2, at 7 weeks after receiving TRX4, started to receive a daily topical application of a topical corticosteroid cream including 0.05% halobetasol propionate, an agent used for the treatment of psoriasis. The remaining patients of Cohort 2 did not receive any topical or systemic treatment for psoriasis in the 8 weeks after receiving TRX4.

The PASI scores for each of the five patients of Cohort 2, who received a 2 mg intravenous infusion of TRX4, at baseline (Day-1) and during Week 8 after administration of TRX4, were as follows:

Table 4

	PASI Score	
Patient	Day-1	Week 8
1	13.4	10.8
2	13.8	14.6
3	12.8	11.1
4	12.5	4.9
5	10.7	8.9

The PASI scores of Patients 1 through 7 of Cohort 3 are given in Table 5 hereinbelow. Except for Patient 7, none of the patients of Cohort 3 received any topical or systemic treatment for psoriasis in the 8 weeks after receiving TRX4. Patient 7 began Ultraviolet B light therapy for psoriasis one week after receiving TRX4.

Table 5

	PASI Score	
Patient	Day-1	Week 8
1	14.2	10.8
2	17.6	14.7
3	14.5	9.4
4	12.9	15.0
5	20.1	17.8
6	23.1	17.6
7	25.8	17.8

The disclosures of all patents, publications (including published patent applications), depository accession numbers, and database accession numbers are incorporated herein by reference to the same extent as if each patent, publication, depository accession number, and database accession number were specifically and individually incorporated by reference.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

WHAT IS CLAIMED IS:

1. A method of treating an autoimmune disease, comprising:

administering to an animal an anti-CD3 antibody or anti-CD3 antibody fragment selected from the group consisting of anti-CD3 antibodies and fragments thereof which do not bind or have reduced binding to the Fc (gamma) receptors, over a course of treatment wherein over the course of treatment, said anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount wherein the total amount does not exceed 300 μg/kg when administered intravenously, and when administered other than intravenously the total amount administered does not exceed the bioequivalent of intravenous administration of 300 μg/kg.

2. The method of Claim 1 wherein said autoimmune disease is diabetes.

3. The method of Claim 1 wherein said autoimmune disease is psoriasis.

4. The method of Claim 1 wherein said antibody or fragment is administered in a therapeutically effective amount in which the total amount does not exceed 175 μg/kg when administered intravenously and when administered other than intravenously the total amount does not exceed the amount bioequivalent to intravenous administration of 175 μg/kg.

5. The method of Claim 4 wherein said antibody or fragment is administered in a total amount which does not exceed 150 μg/kg when administered intravenously and when administered other than intravenously the total amount does not exceed the amount bioequivalent to intravenous administration of 150 μg/kg.

6. The method of Claim 5 wherein said antibody or fragment thereof is administered in a total amount which does not exceed 50 μg/kg when administered intravenously and when administered other than intravenously the total amount does not exceed the amount bioequivalent to intravenous administration of 50 μg/kg.

7. The method of Claim 6 wherein said antibody or fragment is administered in a total amount which is at least 8 μg/kg when administered intravenously and when administered other than intravenously the total amount is at least an amount bioequivalent to intravenous administration of 8 μg/kg.

8. The method of Claim 1 wherein said anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount which is not less than 0.1 μg/kg per day when administered intravenously and when administered other than intravenously the amount is not less than the amount bioequivalent to intravenous administration thereof of 0.1 μg/kg per day.

9. The method of Claim 1 wherein said anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount which does not exceed 30 μg/kg per day when administered intravenously and when administered other than intravenously the amount does not exceed the amount bioequivalent to intravenous administration thereof of 30 μg/kg per day.

10. The method of Claim 9 wherein said anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount which does not exceed 25 μg/kg per day when administered intravenously and when administered other than intravenously the amount does not exceed the amount bioequivalent to intravenous administration thereof of 25 μg/kg per day.

11. The method of Claim 10 wherein said anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount which does not exceed 20 μg/kg per day when administered intravenously and when administered other than intravenously the amount does not exceed the amount bioequivalent to intravenous administration thereof of 20 μg/kg per day.

12. The method of Claim 11 wherein said anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount which does not exceed 15 μg/kg per day when

administered intravenously and when administered other than intravenously the amount does not exceed the amount bioequivalent to intravenous administration thereof of 15 µg/kg per day.

13. The method of Claim 12 wherein said anti-CD3 antibody or anti-CD3 antibody fragment is administered in an amount which does not exceed 10 µg/kg per day when administered intravenously and when administered other than intravenously the amount does not exceed the amount bioequivalent to intravenous administration thereof of 10 µg/kg per day.

14. The method of Claim 1 wherein said course of treatment does not exceed 10 days.

15. The method of Claim 14 wherein said course of treatment does not exceed 8 days.

16. The method of Claim 15 wherein said course of treatment does not exceed 6 days.

17. The method of Claim 1 wherein said antibody or fragment is administered intravenously.

18. The method of Claim 1 wherein said animal is a mammal.

19. The method of Claim 18 wherein said mammal is a primate.

20. The method of Claim 19 wherein said primate is a human.

21. A method of effecting T-cell receptor modulation in an animal comprising:

administering to an animal an anti-CD3 antibody or anti-CD3 antibody fragment selected from the group consisting of anti-CD3 antibodies and fragments thereof which do not bind or have reduced binding to the Fc (gamma) receptors, said anti-CD3 antibody or anti-CD3 antibody fragment being administered in an amount effective to effect T-cell receptor modulation in the animal.

22. The method of Claim 21 wherein said antibody or fragment is administered intravenously.

23. The method of Claim 21 wherein said animal is a mammal.

24. The method of Claim 23 wherein said mammal is a primate.

25. The method of Claim 24 wherein said primate is a human.

26. A product, comprising:

an anti-CD3 antibody or anti-CD3 antibody fragment which does not bind or has reduced binding to the Fc (gamma) receptors;

an acceptable pharmaceutical carrier; and

instructions to administer said antibody, or fragment, for the treatment of diabetes over a course of treatment wherein, during said course of treatment, the total amount of said antibody, or fragment, is administered in an amount which does not exceed 300 μg/kg when administered intravenously.

Light Chain Coding Region Sequence

```
ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC GAC     60
 M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H   S   D

ATC CAG CTG ACT CAG CCC AAC TCT GTG TCT ACG TCT CTA GGA AGC ACA GTC AAG CTG TCT    120
 I   Q   L   T   Q   P   N   S   V   S   T   S   L   G   S   T   V   K   L   S

TGC ACA CTC AGC TCT GGT AAC ATA GAA AAC AAC TAT GTG CAC TGG TAC CAG CTA TAT GAG    180
 C   T   L   S   S   G   N   I   E   N   N   Y   V   H   W   Y   Q   L   Y   E

GGA AGA TCT CCC ACC ACT ATG ATT TAT GAT GAT GAT AAG AGA CCG GAT GGT GTC CCT GAC    240
 G   R   S   P   T   T   M   I   Y   D   D   D   K   R   P   D   G   V   P   D

AGG TTC TCT GGC TCC ATT GAC AGG TCT TCC AAC TCA GCC TTC CTG ACA ATC CAT AAT GTG    300
 R   F   S   G   S   I   D   R   S   S   N   S   A   F   L   T   I   H   N   V

GCA ATT GAA GAT GAA GCT ATC TAC TTC TGT CAT TCT TAT GTT AGT AGT TTT AAT GTT TTC    360
 A   I   E   D   E   A   I   Y   F   C   H   S   Y   V   S   S   F   N   V   F

GGC GGT GGA ACA AAG CTC ACT GTC CTT CGA CAG CCC AAG GCT GCC CCC TCG GTC ACT CTG    420
 G   G   G   T   K   L   T   V   L   R   Q   P   K   A   A   P   S   V   T   L

TTC CCG CCC TCC TCT GAG GAG CTT CAA GCC AAC AAG GCC ACA CTG GTG TGT CTC ATA AGT    480
 F   P   P   S   S   E   E   L   Q   A   N   K   A   T   L   V   C   L   I   S

GAC TTC TAC CCG GGA GCC GTG ACA GTG GCC TGG AAG GCA GAT AGC AGC CCC GTC AAG GCG    540
 D   F   Y   P   G   A   V   T   V   A   W   K   A   D   S   S   P   V   K   A

GGA GTG GAG ACC ACC ACA CCC TCC AAA CAA AGC AAC AAC AAG TAC GCG GCC AGC AGC TAC    600
 G   V   E   T   T   T   P   S   K   Q   S   N   N   K   Y   A   A   S   S   Y

CTG AGC CTG ACG CCT GAG CAG TGG AAG TCC CAC AGA AGC TAC AGC TGC CAG GTC ACG CAT    660
 L   S   L   T   P   E   Q   W   K   S   H   R   S   Y   S   C   Q   V   T   H

GAA GGG AGC ACC GTG GAG AAG ACA GTG GCC CCT ACA GAA TGT TCA TAG                    708
 E   G   S   T   V   E   K   T   V   A   P   T   E   C   S   *
```

FIG..1

(4 Heavy Chain Coding Region Sequence

```
ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC GAG     60
 M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H   S   E

GTC CAA CTG CTG GAG TCT GGG GGC GGT TTA GTG CAG CCT GGA GGG TCC CTG AGA CTC TCC    120
 V   Q   L   L   E   S   G   G   G   L   V   Q   P   G   G   S   L   R   L   S

TGT GCA GCC TCA GGA TTC ACT TTC AGT AGC TTT CCA ATG GCC TGG GTC CGC CAG GCT CCA    180
 C   A   A   S   G   F   T   F   S   S   F   P   M   A   W   V   R   Q   A   P

GGG AAG GGT CTG GAG TGG GTC TCA ACC ATT AGT ACC AGT GGT GGT AGA ACT TAC TAT CGA    240
 G   K   G   L   E   W   V   S   T   I   S   T   S   G   G   R   T   Y   Y   R

GAC TCC GTG AAG GGC CGA TTC ACT ATC TCC AGA GAT AAT AGC AAA AAT ACC CTA TAC CTG    300
 D   S   V   K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y   L

CAA ATG AAT AGT CTG AGG GCT GAG GAC ACG GCC GTC TAT TAC TGT GCA AAA TTT CGG CAG    360
 Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C   A   K   F   R   Q

TAC AGT GGT GGC TTT GAT TAC TGG GGC CAA GGG ACC CTG GTC ACC GTC TCC TCA GCC TCC    420
 Y   S   G   G   F   D   Y   W   G   Q   G   T   L   V   T   V   S   S   A   S

ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA    480
 T   K   G   P   S   V   F   P   L   A   P   S   S   K   S   T   S   G   G   T

GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC    540
 A   A   L   G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N

TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC    600
 S   G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G   L

TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG ACC TAC ATC    660
 Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q   T   Y   I

TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GTT GAG CCC AAA TCT    720
 C   N   V   N   H   K   P   S   N   T   K   V   D   K   K   V   E   P   K   S

TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA    780
 C   D   K   T   H   T   C   P   P   C   P   A   P   E   L   L   G   G   P   S
```

FIG..1

```
GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC   840
 V   F   L   F   P   P   K   P   K   D   T   L   M   I   S   R   T   P   E   V

ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG   900
 T   C   V   V   V   D   V   S   H   E   D   P   E   V   K   F   N   W   Y   V

GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC GCC AGC ACG   960
 D   G   V   E   V   H   N   A   K   T   K   P   R   E   E   Q   Y   A   S   T

TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC  1020
 Y   R   V   V   S   V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y

AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC  1080
 K   C   K   V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A

AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC  1140
 K   G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D   E   L   T

AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG  1200
 K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D   I   A   V

GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC  1260
 E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T   P   P   V   L   D

TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG  1320
 S   D   G   S   F   F   L   Y   S   K   L   T   V   D   K   S   R   W   Q   Q

GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG  1380
 G   N   V   F   S   C   S   V   M   H   E   A   L   H   N   H   Y   T   Q   K

AGC CTC TCC CTG TCT CCG GGT AAA TGA                                             1407
 S   L   S   L   S   P   G   K   *
```

FIG..1

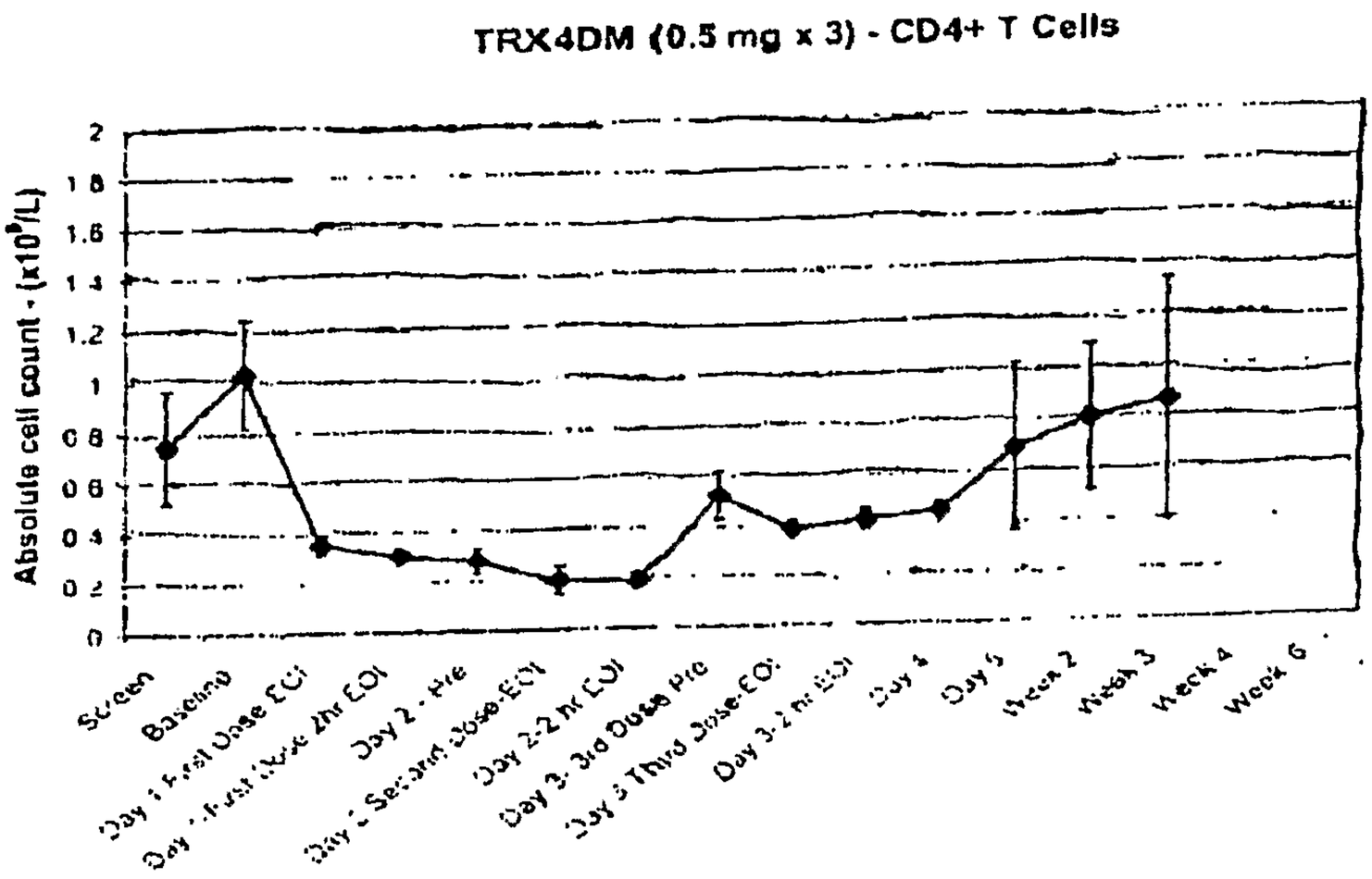
TRX4DM (0.5 mg x 3) - CD4+ T Cells
Absolute cell count - (x10⁹/L)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Screen
Baseline
Day 1 First Dose EOI
Day 1 First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose EOI
Day 2 2 hr EOI
Day 3 3rd Dose Pre
Day 3 Third Dose EOI
Day 3 2 hr EOI
Day 4
Day 5
Week 2
Week 3
Week 4
Week 6

FIG..2

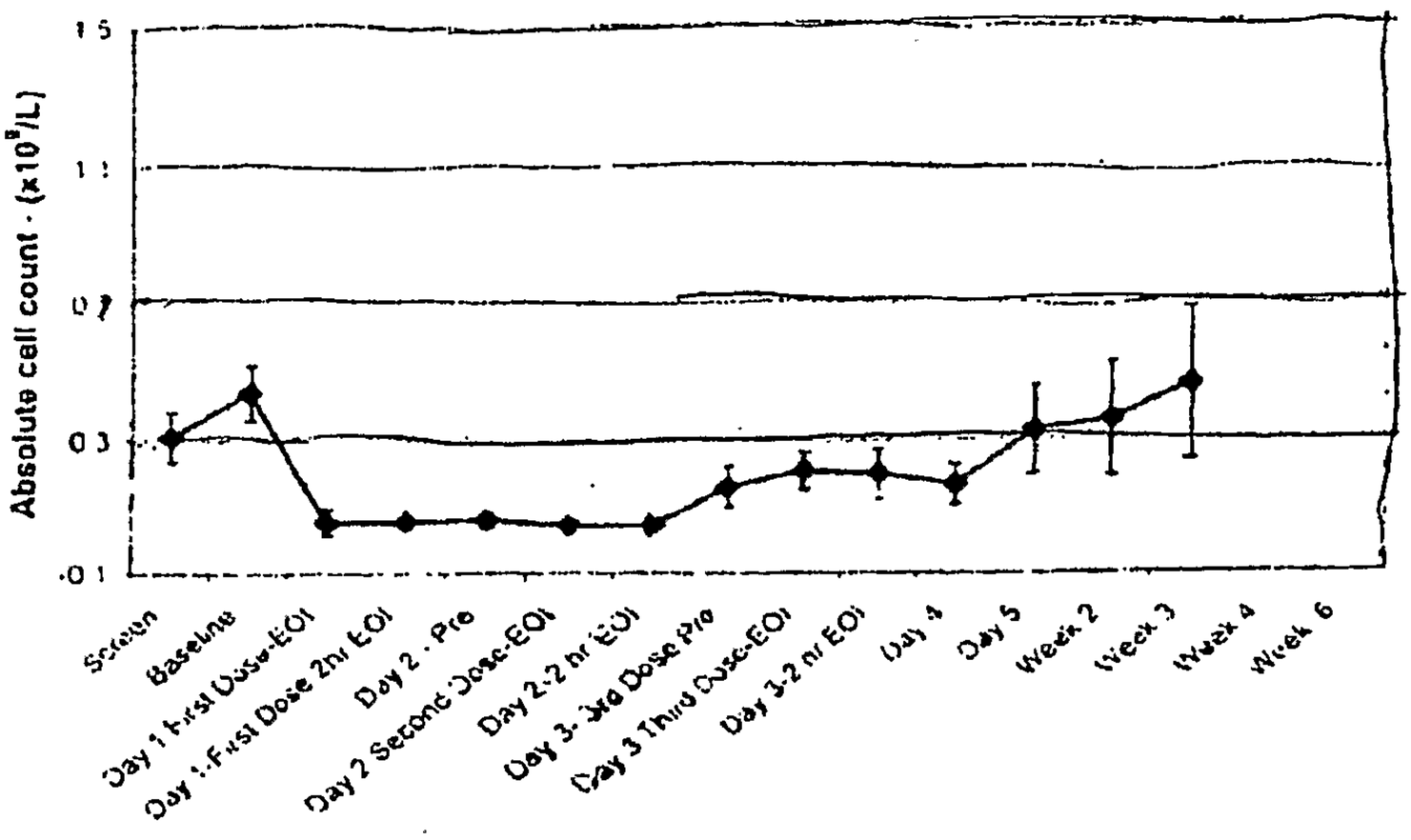
TRX4DM (0.5 mg x 3) - CD8+ T Cells
Absolute cell count - (x10⁹/L)
1 5
1 1
0 7
0 3
0 1
Screen
Baseline
Day 1 First Dose-EOI
Day 1 First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2 2 hr EOI
Day 3 - 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3 2 hr EOI
Day 4
Day 5
Week 2
Week 3
Week 4
Week 6

FIG..3

TRX4DM (0.5 mg x 3) - Cell Bound TRX4 detected by an anti-human IgG on CD4+ T Cells

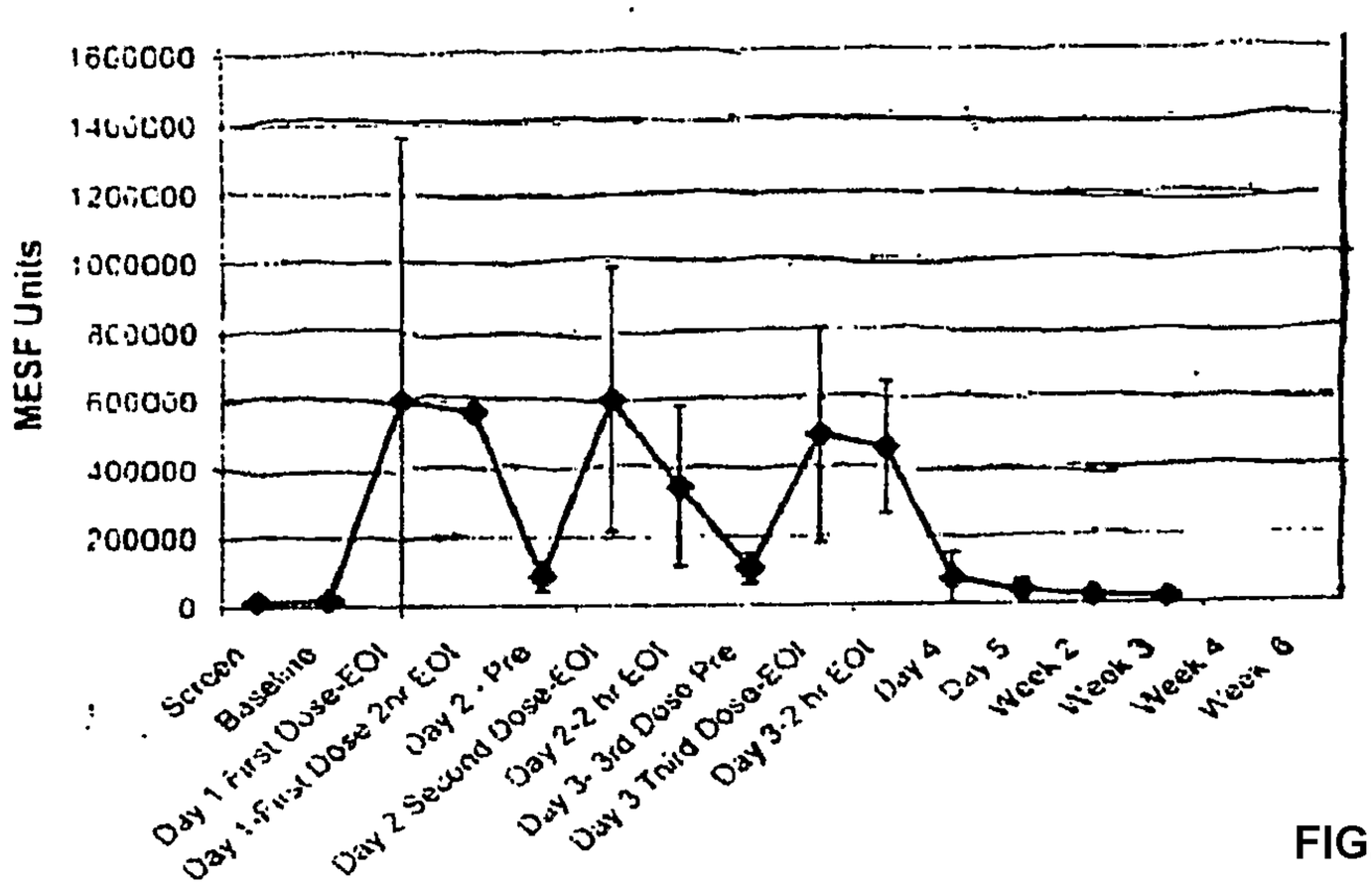

FIG..4

TRX4DM (0.5 mg x 3) - TCR sites detected on CD4+ T cells with a non-competing Antibody

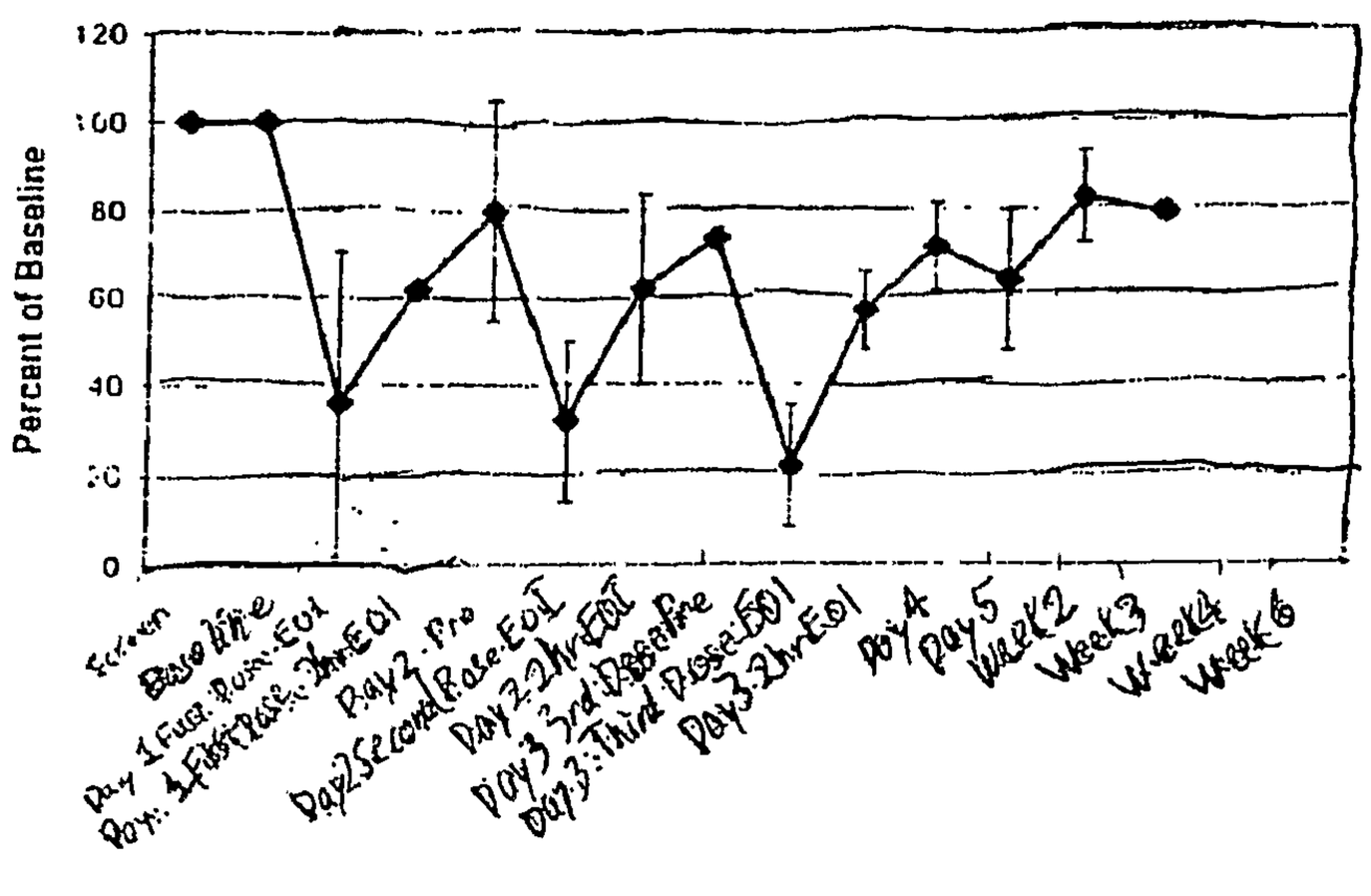

FIG..5

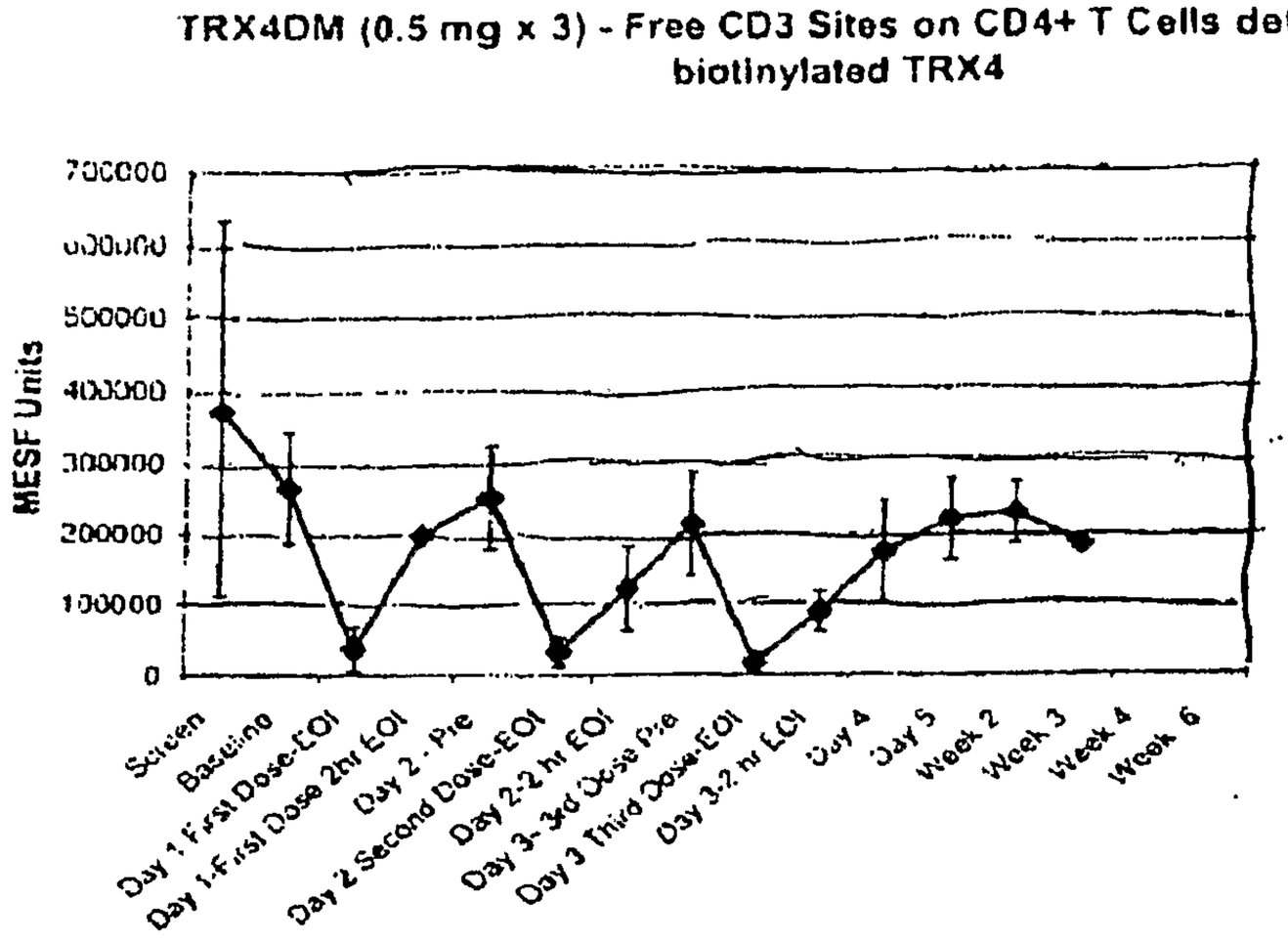
TRX4DM (0.5 mg x 3) - Free CD3 Sites on CD4+ T Cells detected with biotinylated TRX4
MESF Units
700000
600000
500000
400000
300000
200000
100000
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1-First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3-2 hr EOI
Day 4
Day 5
Week 2
Week 3
Week 4
Week 6

FIG..6

TRX4 (0.1mg x3) - Cell-Bound TRX4 Detected By an Anti-Human Ig on CD4+ T Cells

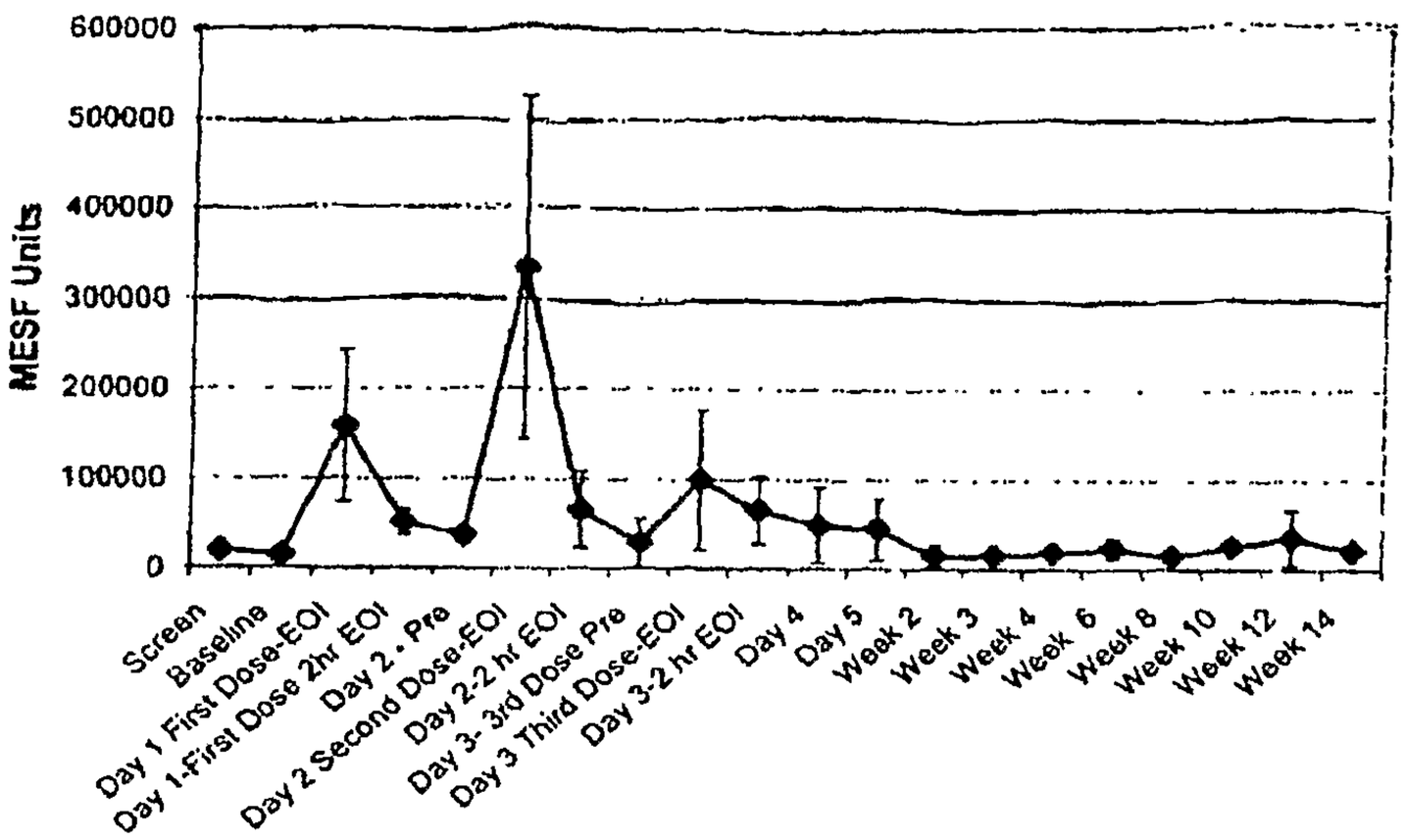

FIG..7

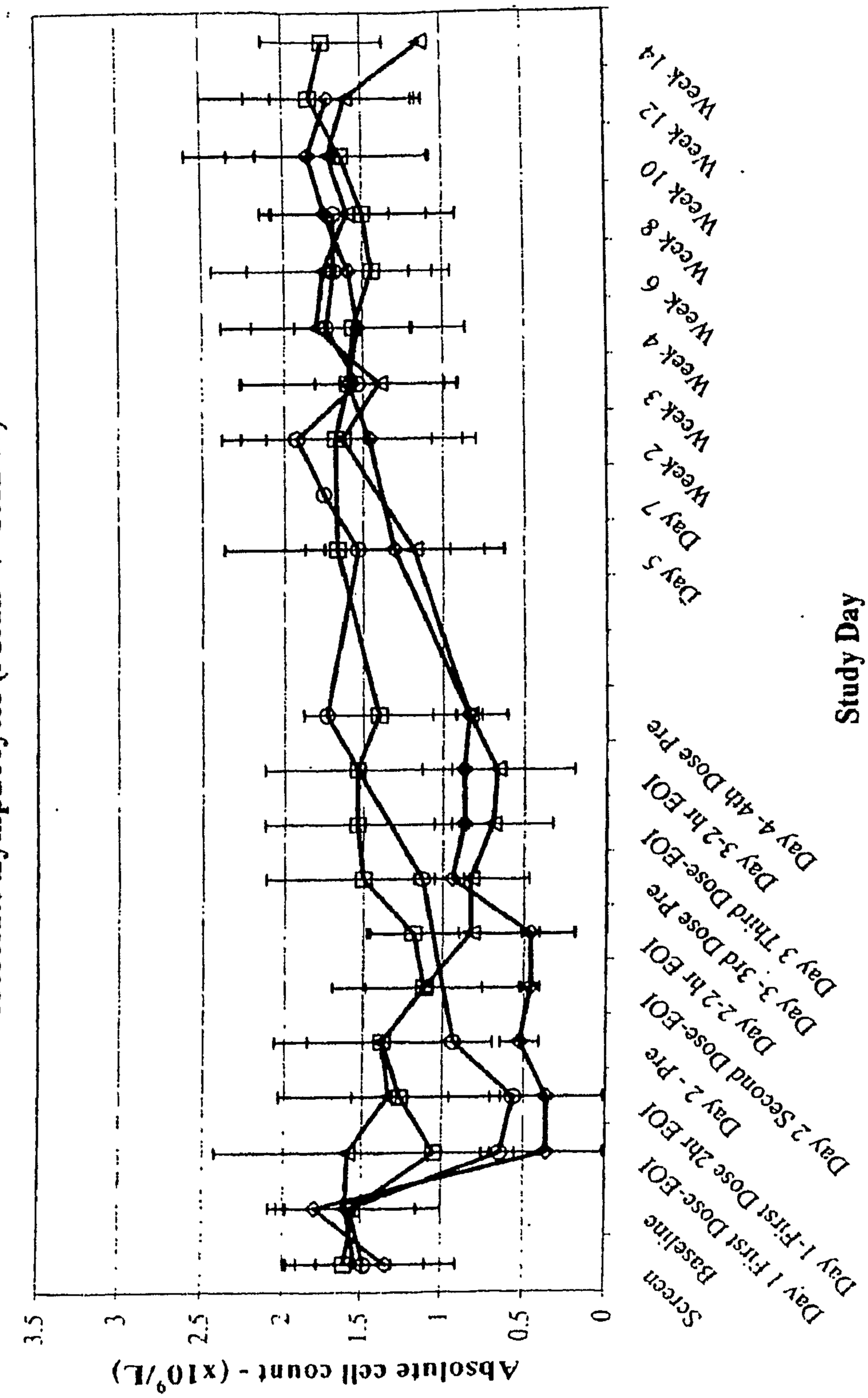
Absolute Lymphocytes (Mean +/- StdDev)
Absolute cell count - (x10⁹/L)
3.5
3
2.5
2
1.5
1
0.5
0
Study Day
Screen
Baseline
Day 1 First Dose-EOI
Day 1-First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre
Day 5
Day 7
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12
Week 14
0.1, 0.1, 0.1
0.5, 0.5, 0.5
0.1, 0.3, 0.5
0.3

FIG..8

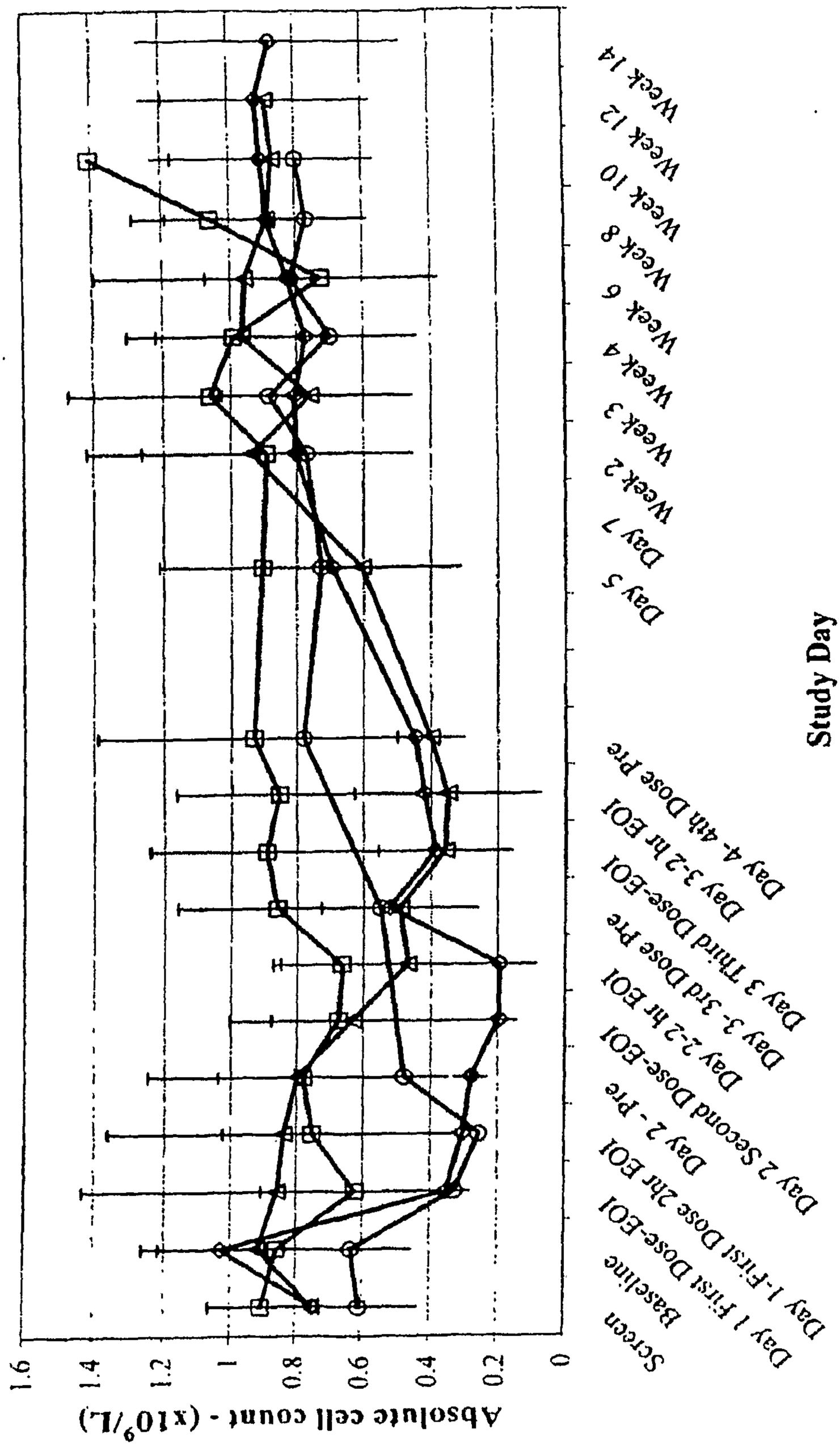
CD4+ Lymphocytes (Mean +/- StdDev)
Absolute cell count - (x10^9/L)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1-First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre
Day 5
Day 7
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12
Week 14
Study Day
0.1, 0.1, 0.1
0.5, 0.5, 0.5
0.1, 0.3, 0.5
0.3

FIG..9

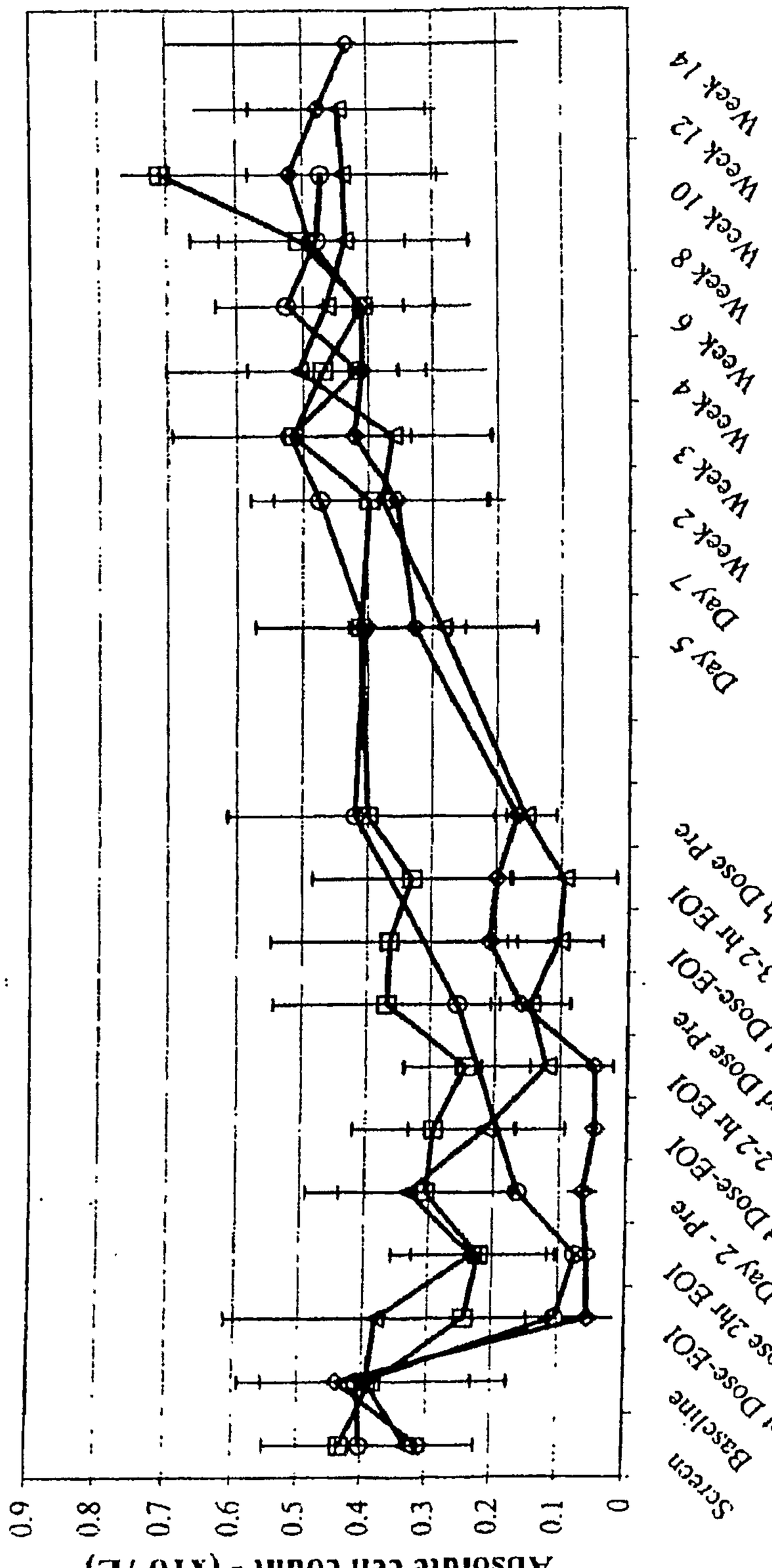
CD8+ Lymphocytes (Mean +/- StdDev)
Absolute cell count - (x10⁹/L)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1-First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre
Day 5
Day 7
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12
Week 14
Study Day
0.1, 0.1, 0.1
0.5, 0.5, 0.5
0.1, 0.3, 0.5
0.3

FIG..10

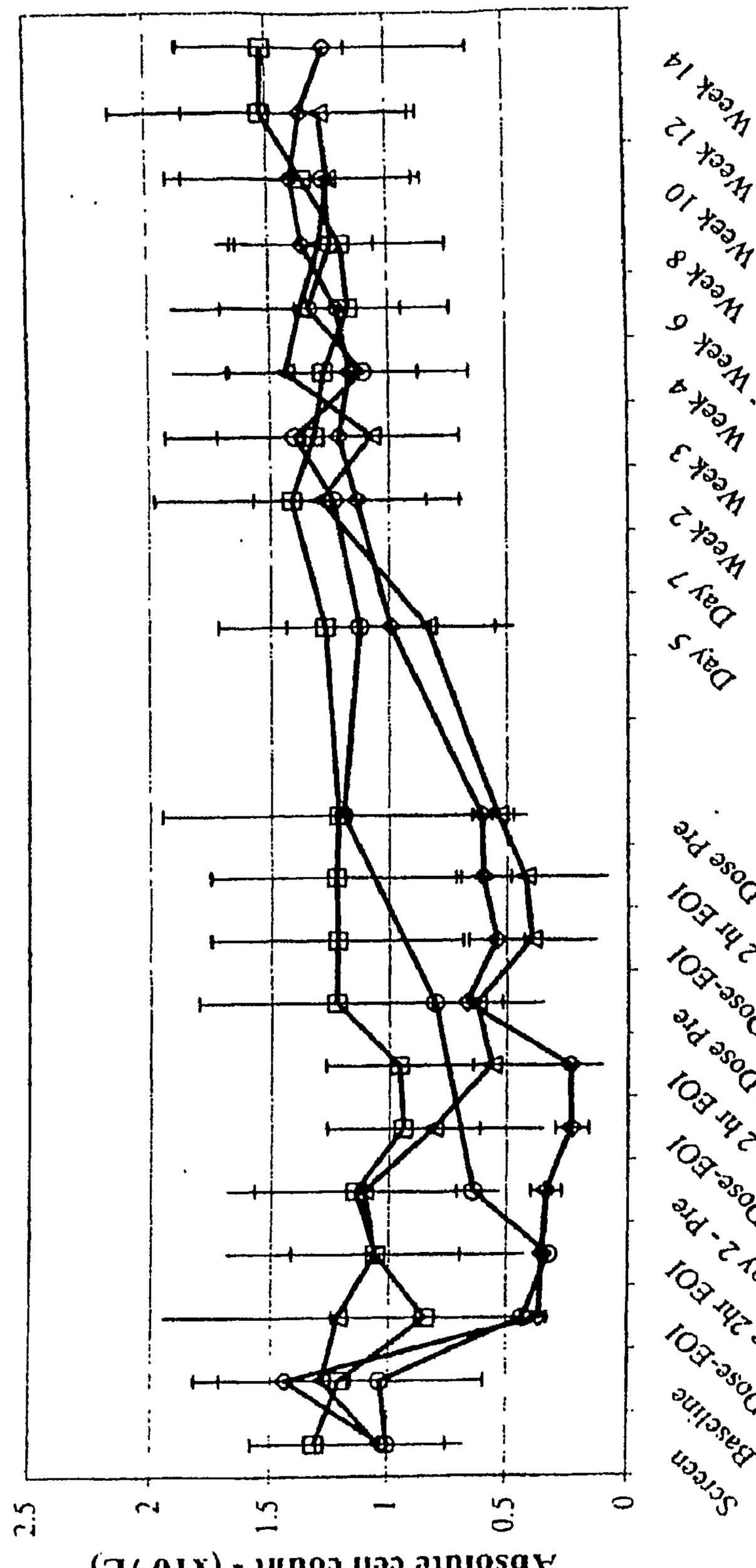
TCR+ T Lymphocytes (CD4+ and CD8+)
Mean +/- StdDev
Absolute cell count - (x10⁹/L)
2.5
2
1.5
1
0.5
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1-First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre
Day 5
Day 7
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12
Week 14
Study Day
0.1, 0.1, 0.1
0.5, 0.5, 0.5
0.1, 0.3, 0.5
0.3

FIG..11

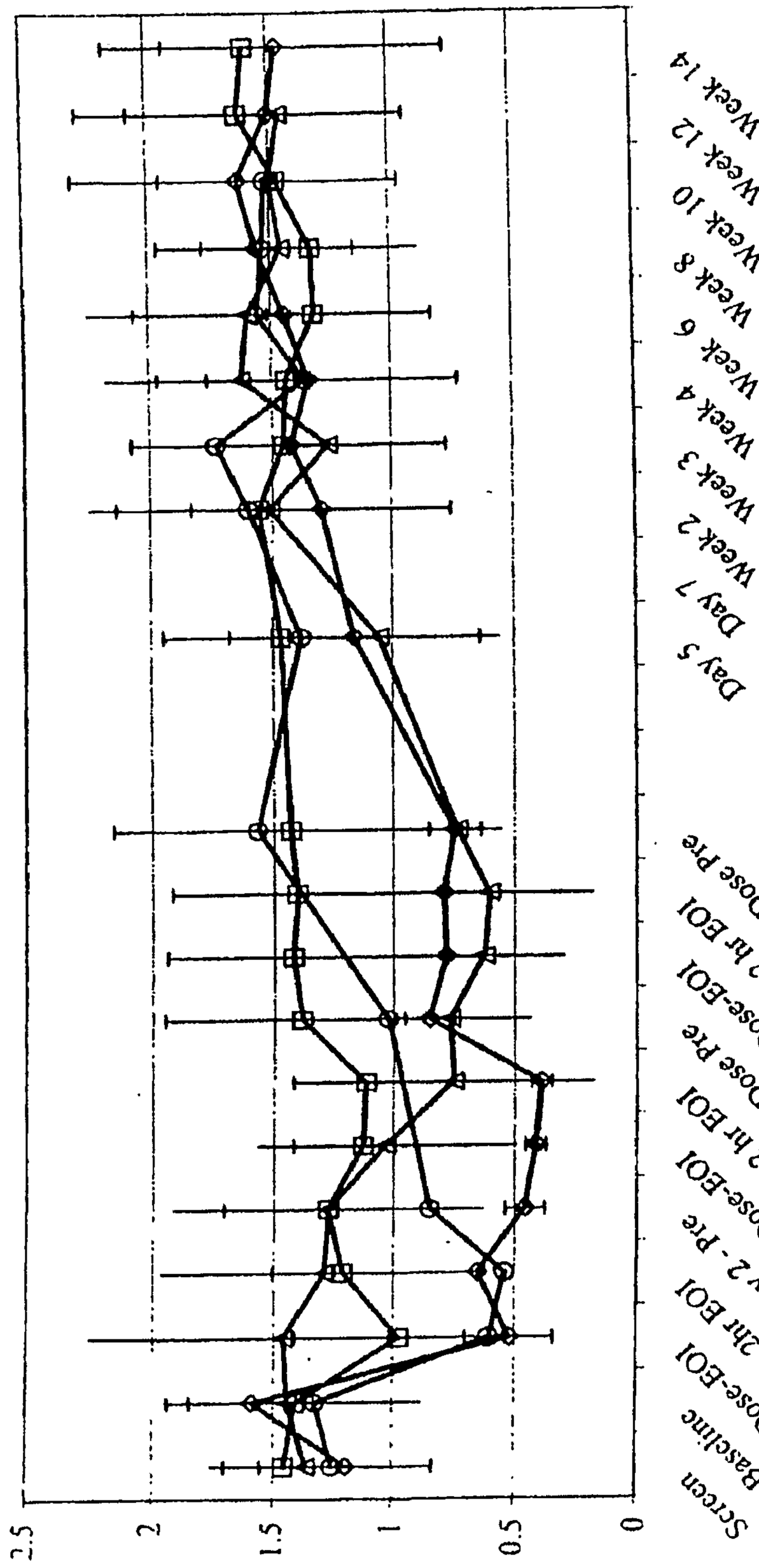
CD2+ T Lymphocytes (CD4+ and CD8+)
Mean +/- StdDev
Absolute cell count - (x10^9/L)
2.5
2
1.5
1
0.5
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1-First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre
Day 5
Day 7
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12
Week 14
Study Day
0.1, 0.1, 0.1
0.5, 0.5, 0.5
0.1, 0.3, 0.5
0.3

FIG..12

TRX4 (0.1mg, 0.2mg, 0.3mg, 0.5mg) – CD4+ TCells

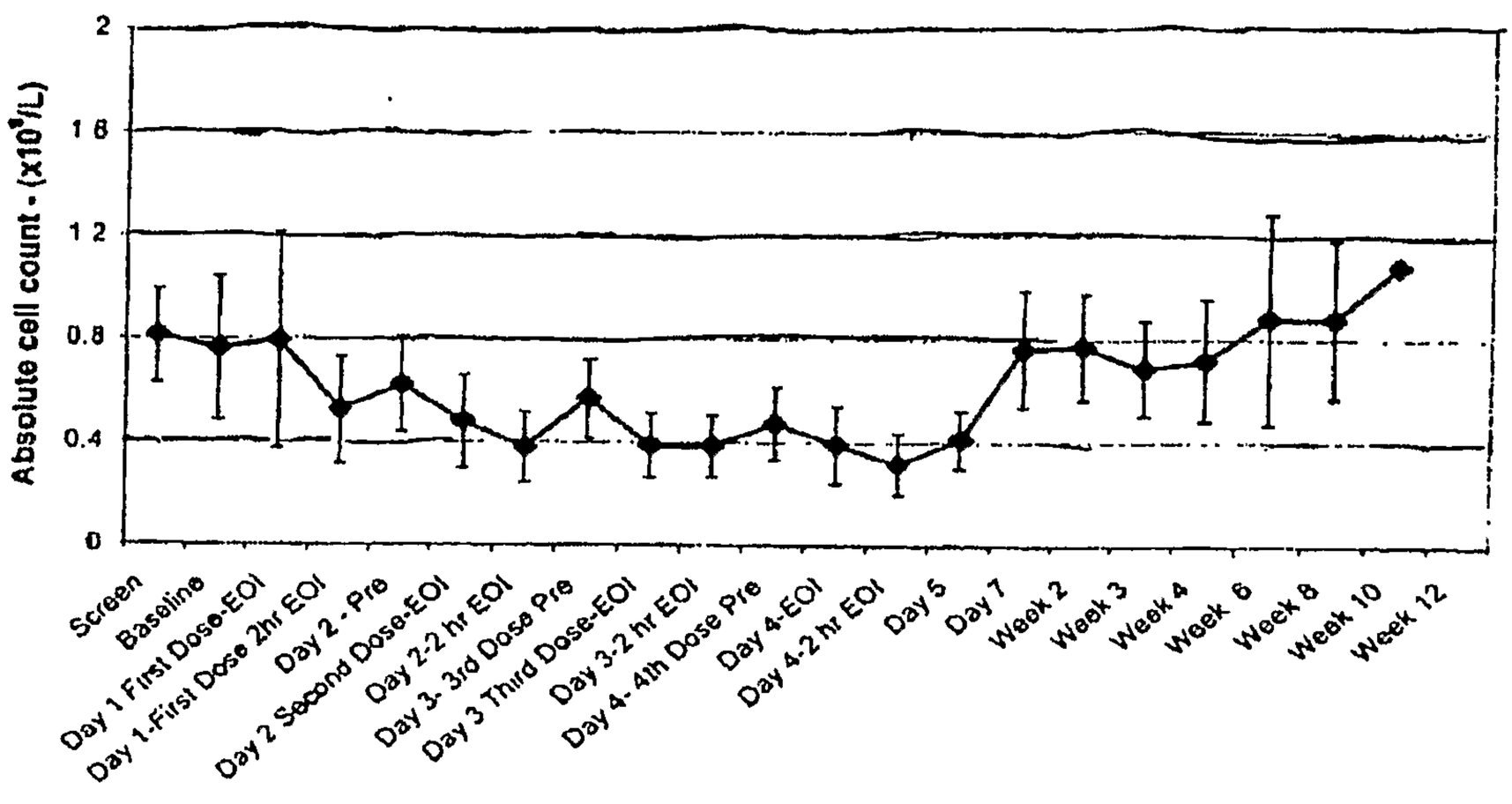

FIG..13

. TRX4 (0.1mg, 0.2mg, 0.3mg, 0.5mg) - Cell-Bound TRX4 Detected By an Anti-HumanIg On CD4+ TCells

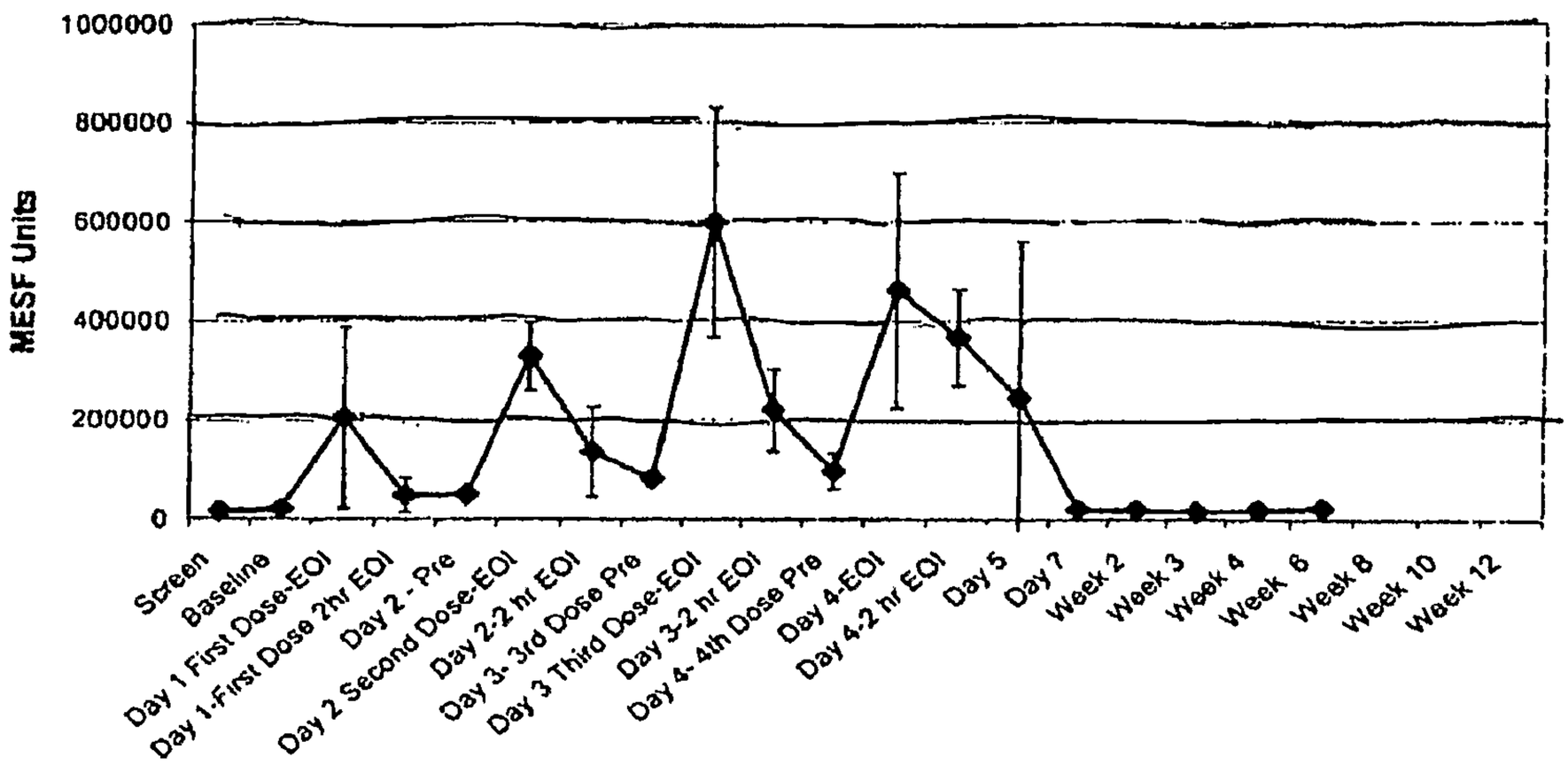

FIG..14

TRX4 (0.1mg, 0.2mg, 0.3mg, 0.5mg) TCR Sites Detected on CD4+ T Cells with a Non-Competing Antibody

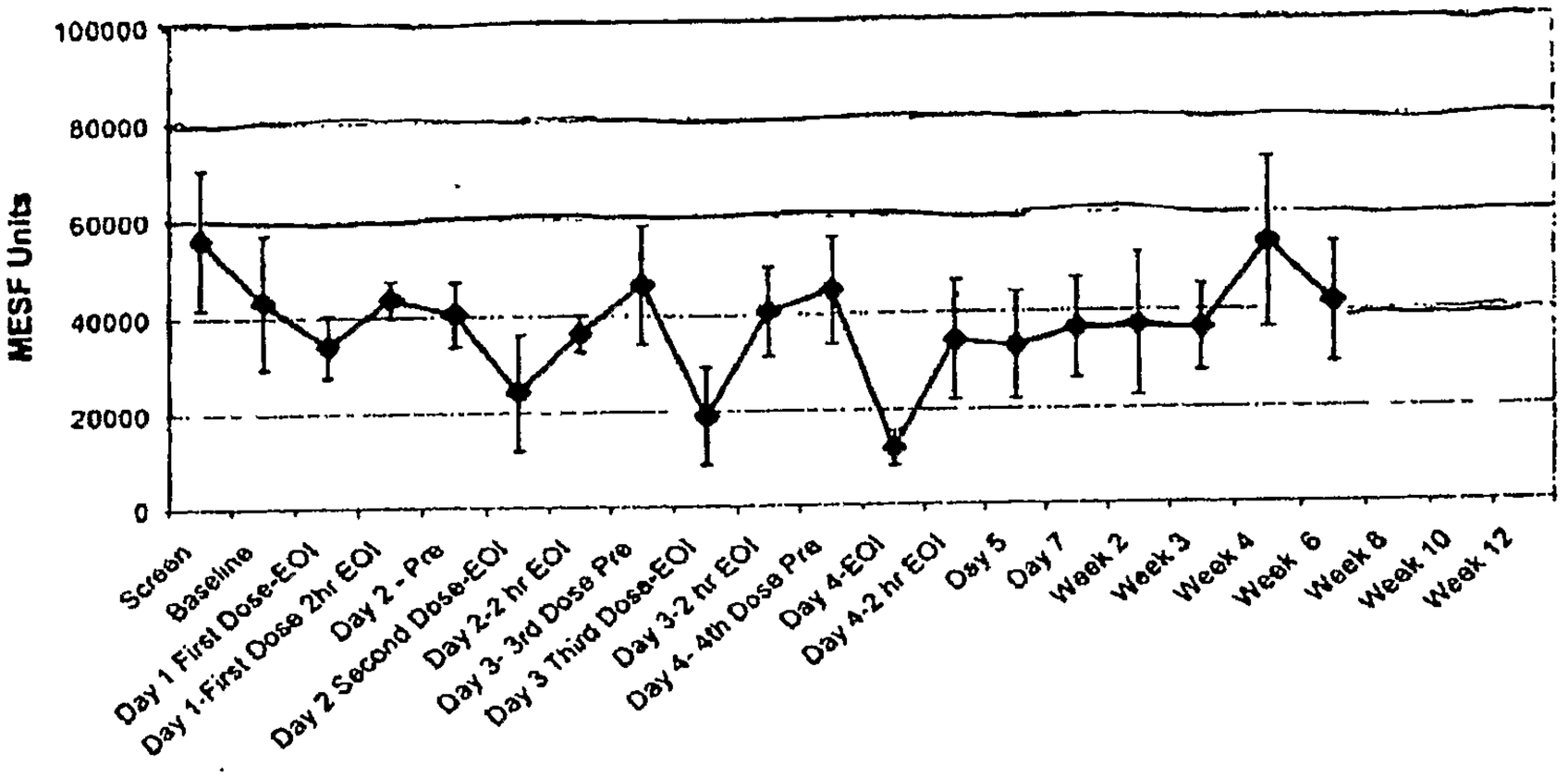

FIG..15

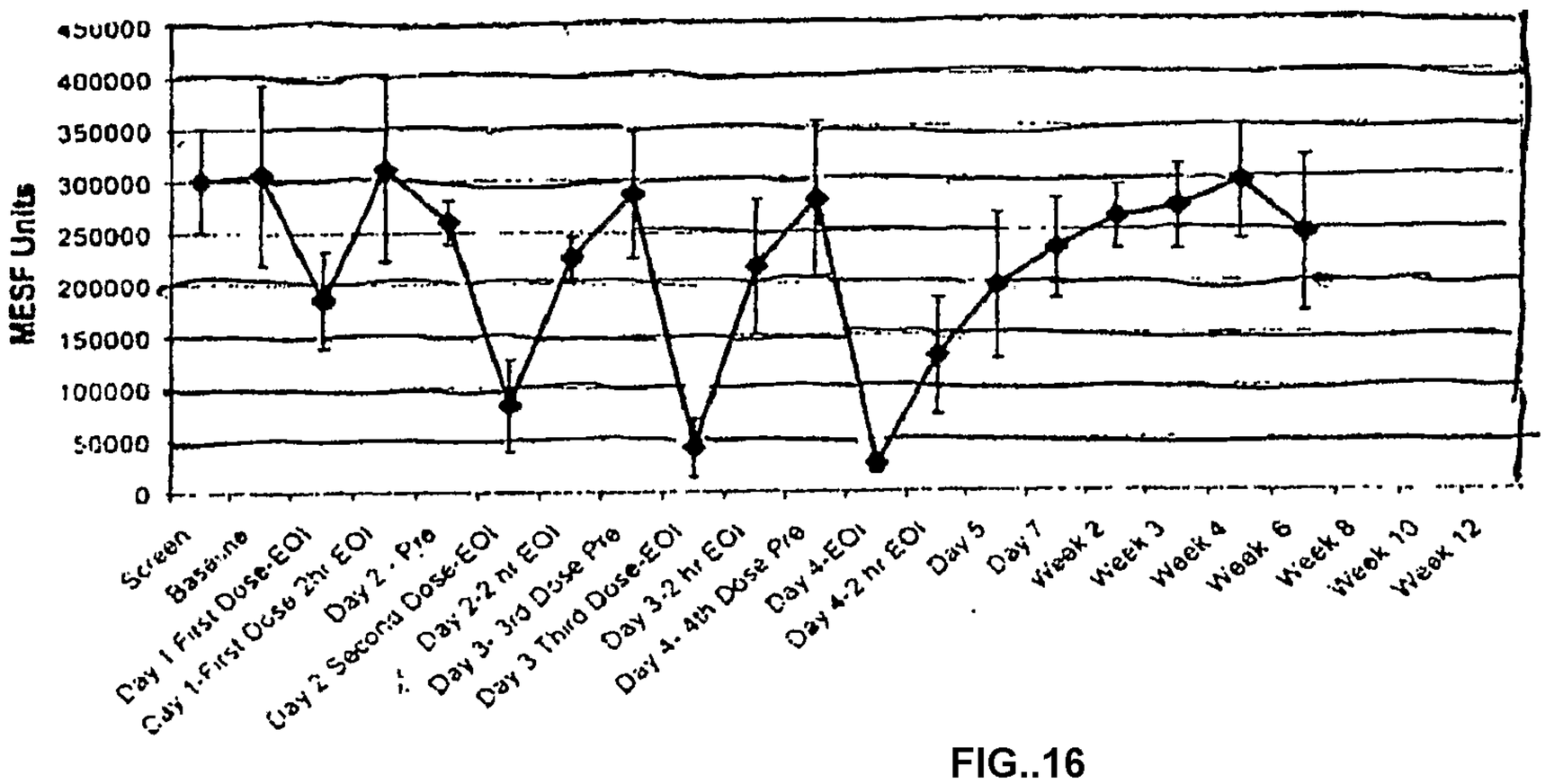

FIG..16

TRX4 (0.1mg, 0.2mg, 0.3mg, 0.5mg) - Free CD3 Sites on CD4+ Cells Detected with Biotinylated TRX4

TRX4 (0.1mg, 0.2mg, 0.3mg, 0.75mg) - TCR Sites
Detected on CD4+ T Cells with a
Non-Competing Antibody

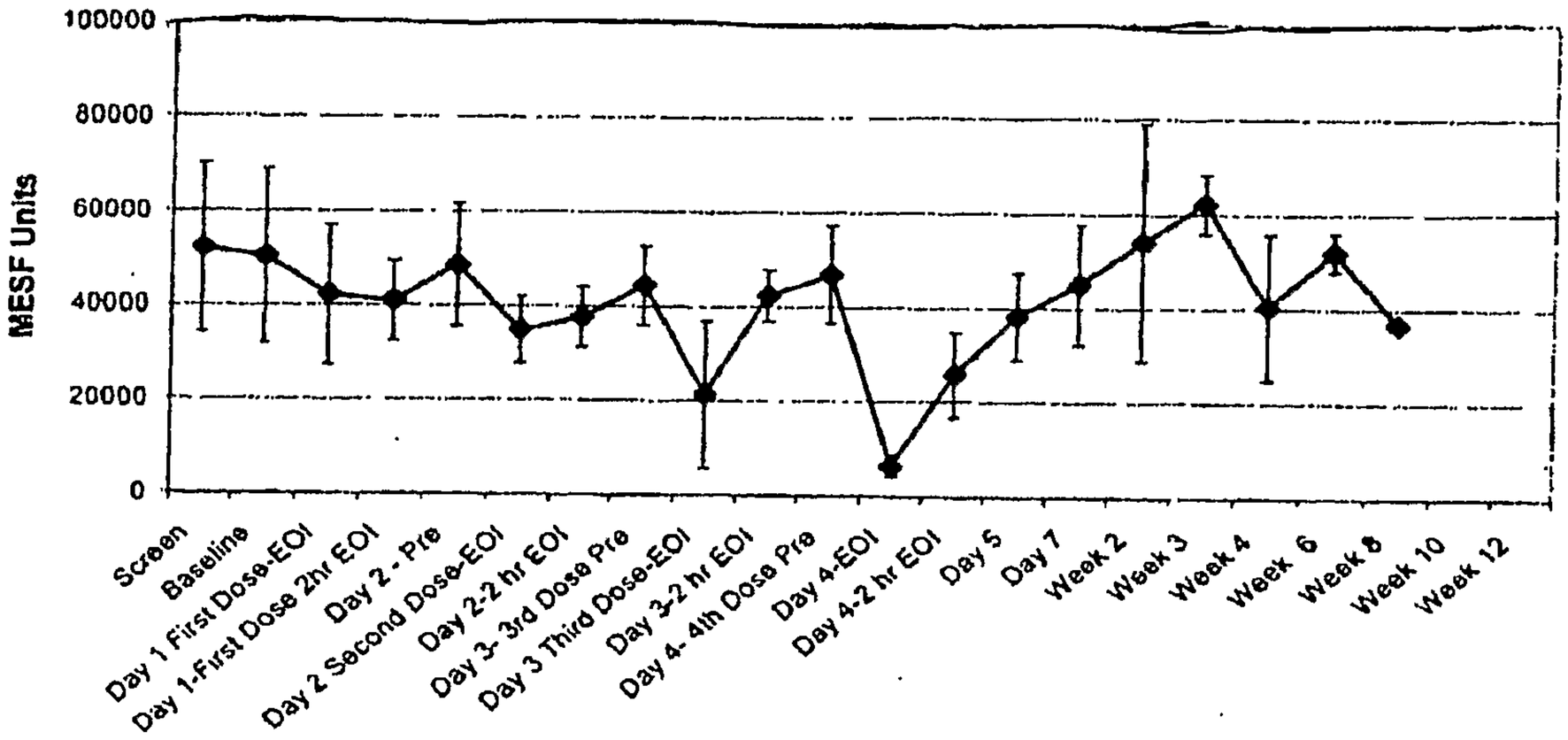

FIG..17

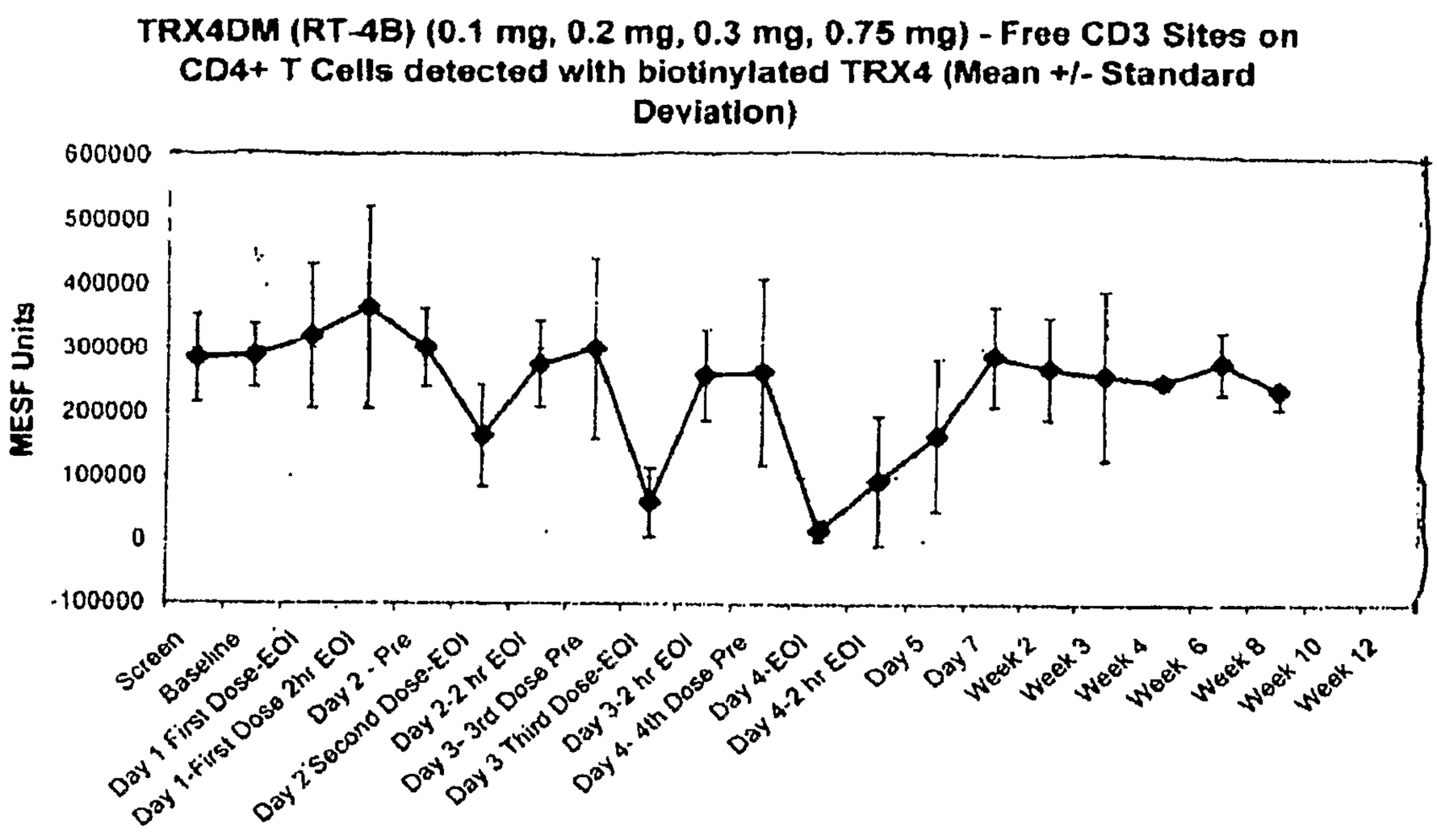
TRX4DM (RT-4B) (0.1 mg, 0.2 mg, 0.3 mg, 0.75 mg) - Free CD3 Sites on CD4+ T Cells detected with biotinylated TRX4 (Mean +/- Standard Deviation)
MESF Units
600000
500000
400000
300000
200000
100000
0
-100000
Screen
Baseline
Day 1 First Dose-EOI
Day 1-First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre
Day 4-EOI
Day 4-2 hr EOI
Day 5
Day 7
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12

FIG..18

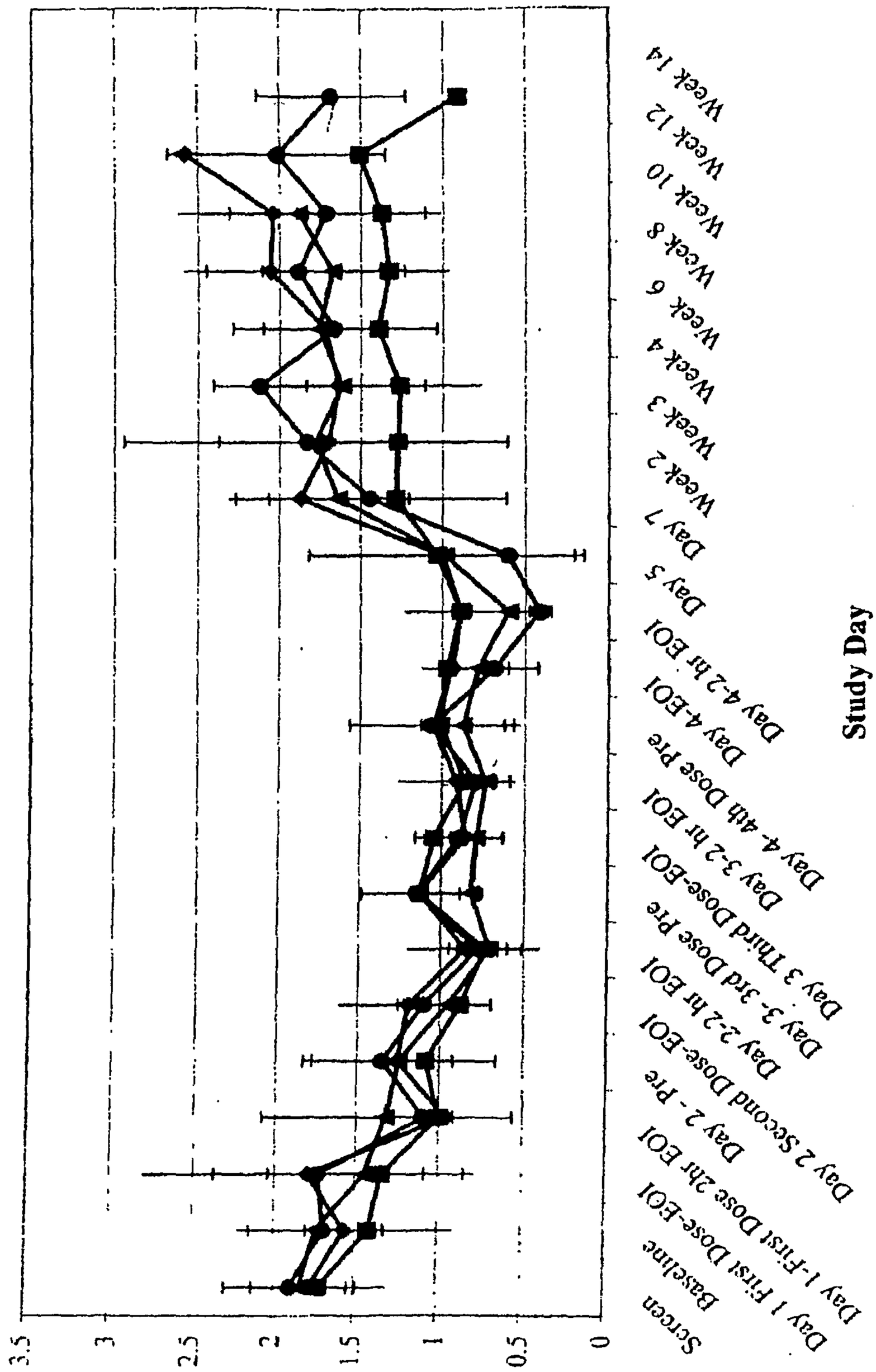
Absolute Lymphocytes (Mean +/- StdDev)
Absolute cell count - (x10⁹/L)
3.5
3
2.5
2
1.5
1
0.5
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1-First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre
Day 4-EOI
Day 4-2 hr EOI
Day 5
Day 7
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12
Week 14
Study Day
A 1/2
0.1, 0.2, 0.3, 0.5
0.1, 0.2, 0.3, 0.75
0.1, 0.2, 0.3, 1.0

FIG..19

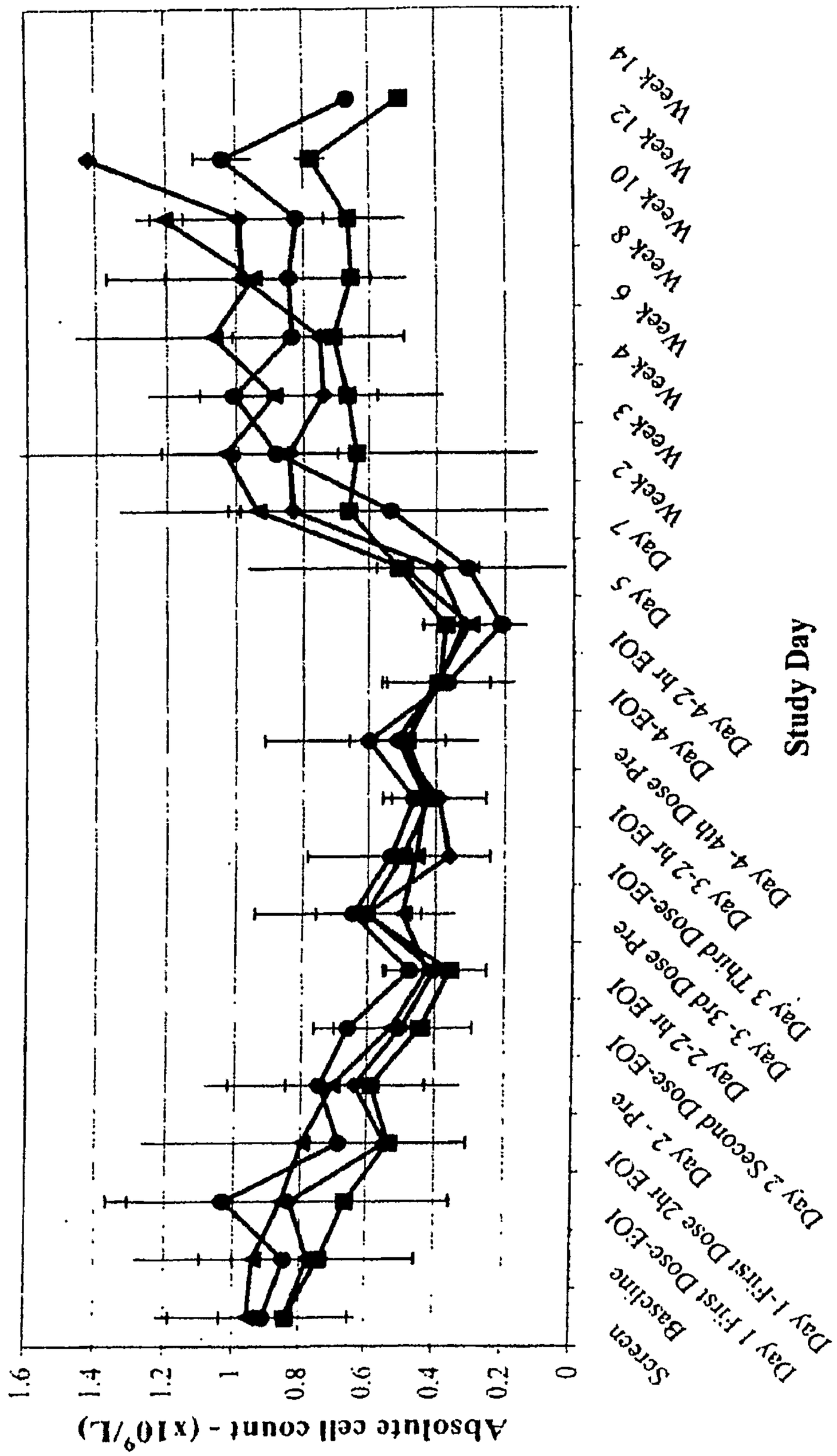
CD4+ Lymphocytes (Mean +/- StdDev)
Absolute cell count - (x10^9/L)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Screen
Baseline
Day 1 -First Dose-EOI
Day 1-First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre
Day 4-EOI
Day 4-2 hr EOI
Study Day
Week 1
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12
Week 14
A 1/2 0.1, 0.2, 0.3, 0.5 0.1, 0.2, 0.3, 0.75 0.1, 0.2, 0.3, 1.0

FIG..20

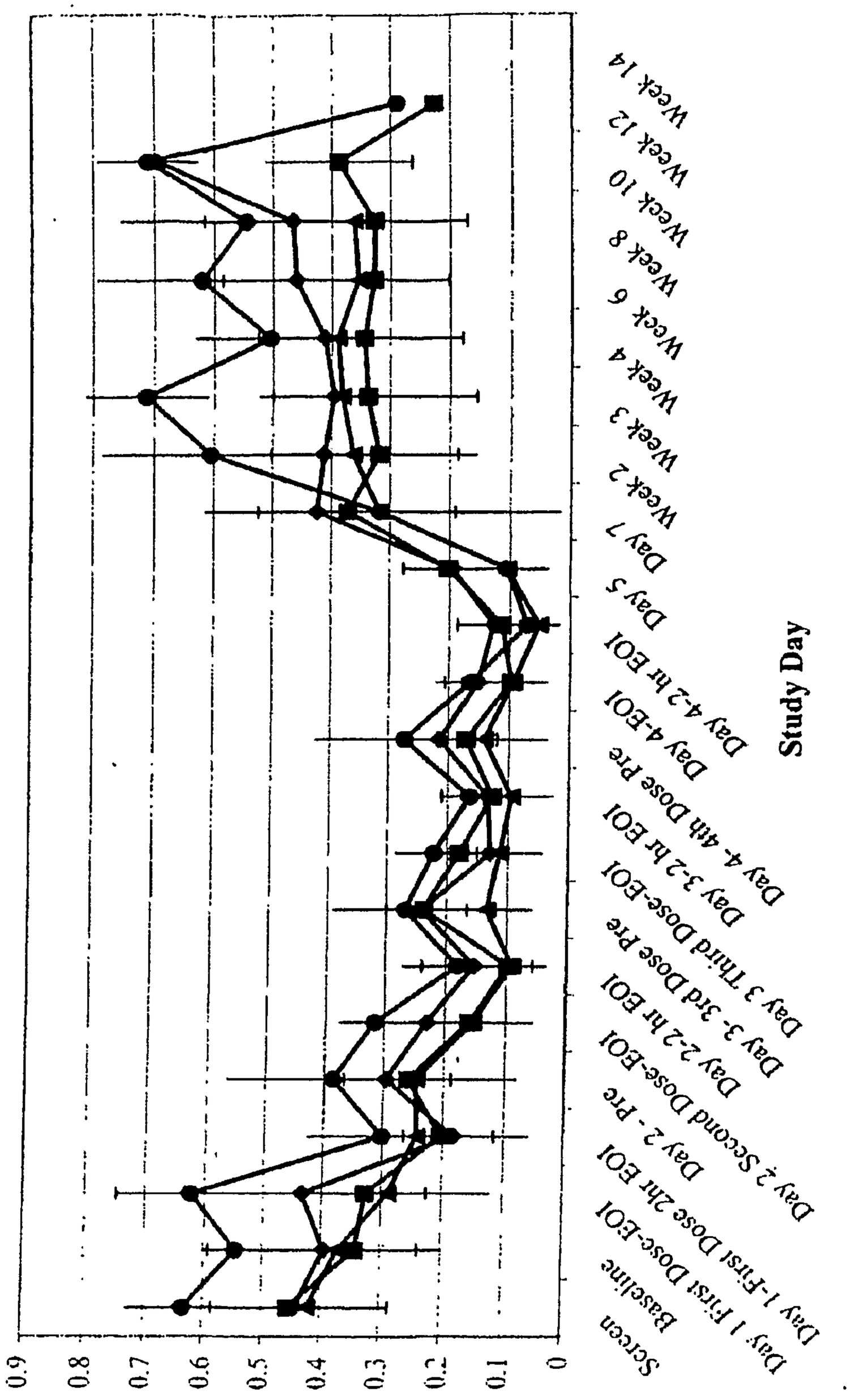
CD8+ Lymphocytes (Mean +/- StdDev)
Absolute cell count - (x10^9/L)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1-First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre
Day 4-EOI
Day 4-2 hr EOI
Day 5
Day 7
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12
Week 14
Study Day
A 1/2
0.1, 0.2, 0.3, 0.5
0.1, 0.2, 0.3, 0.75
0.1, 0.2, 0.3, 1.0

FIG..21

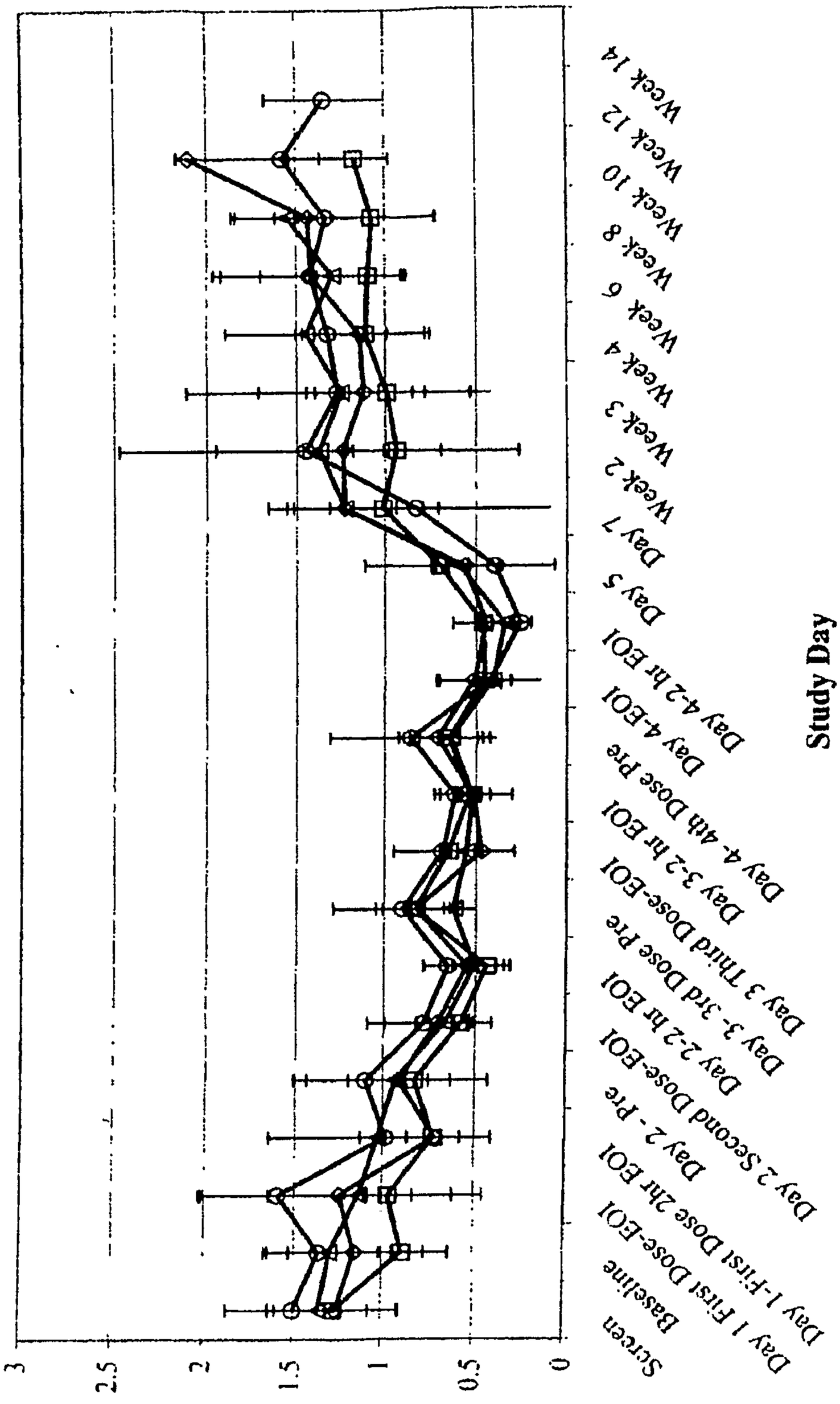
TCR+ T Lymphocytes (CD4+ and CD8+)
Mean +/- StdDev
Study Day
A 1/2 0.1, 0.2, 0.3, 0.5 0.1, 0.2, 0.3, 0.75 0.1, 0.2, 0.3, 1.0
Absolute cell count - (x)10⁹/L)
3
2.5
2
1.5
1
0.5
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1-First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre
Day 4-EOI
Day 4-2 hr EOI
Day 5
Day 7
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12
Week 14

FIG..22

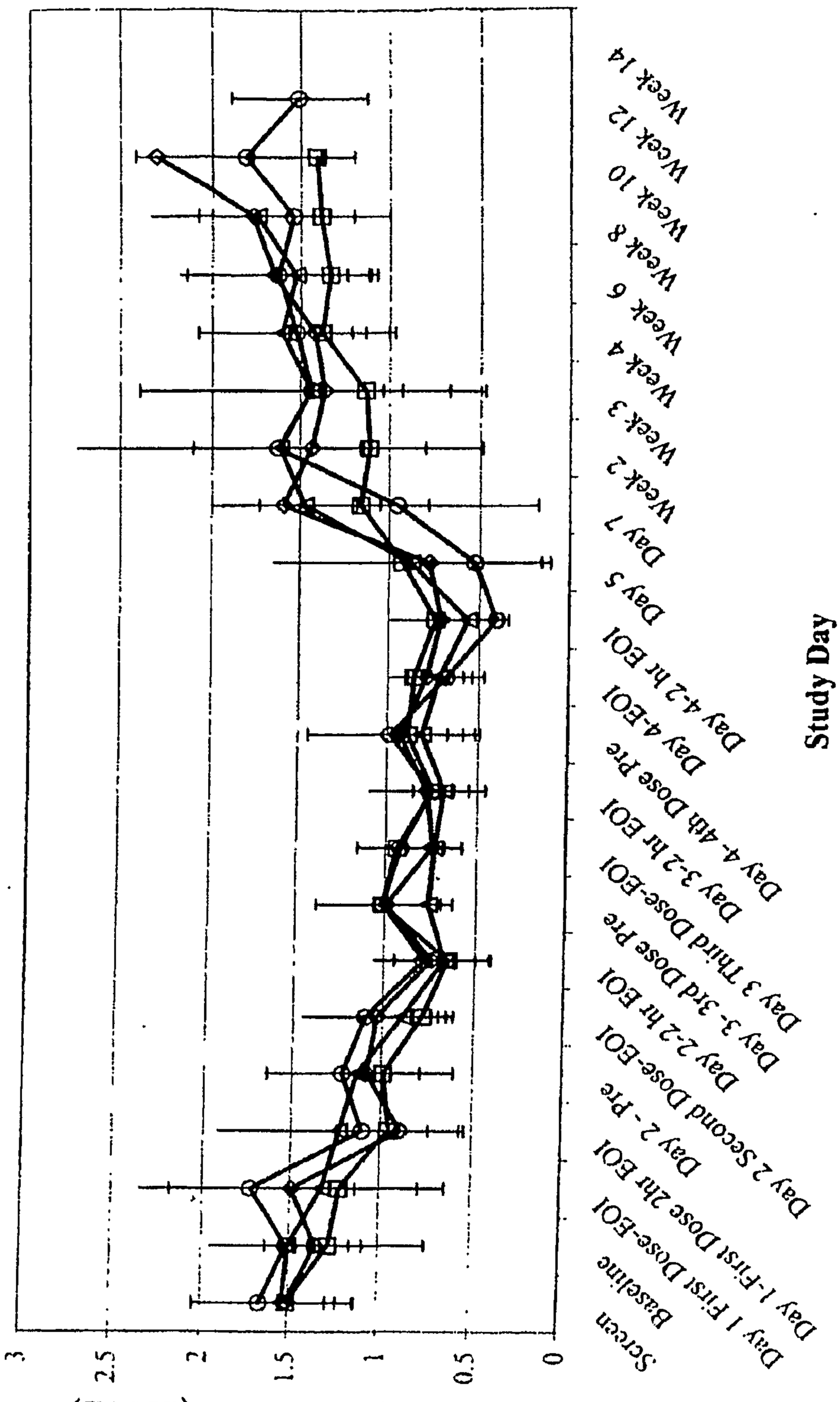
CD2+ T Lymphocytes (CD4+ and CD8+)
Mean +/- StdDev
Absolute cell count - (x10⁹/L)
3
2.5
2
1.5
1
0.5
0
A 1/2 0.1, 0.2, 0.3, 0.5 0.1, 0.2, 0.3, 0.75 0.1, 0.2, 0.3, 1.0
Study Day
Screen
Baseline
Day 1 First Dose-EOI
Day 1-First Dose 2hr EOI
Day 2 - Pre
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3 - 3rd Dose Pre
Day 3 Third Dose-EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre
Day 4-EOI
Day 4-2 hr EOI
Day 5
Day 7
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12
Week 14

FIG..23

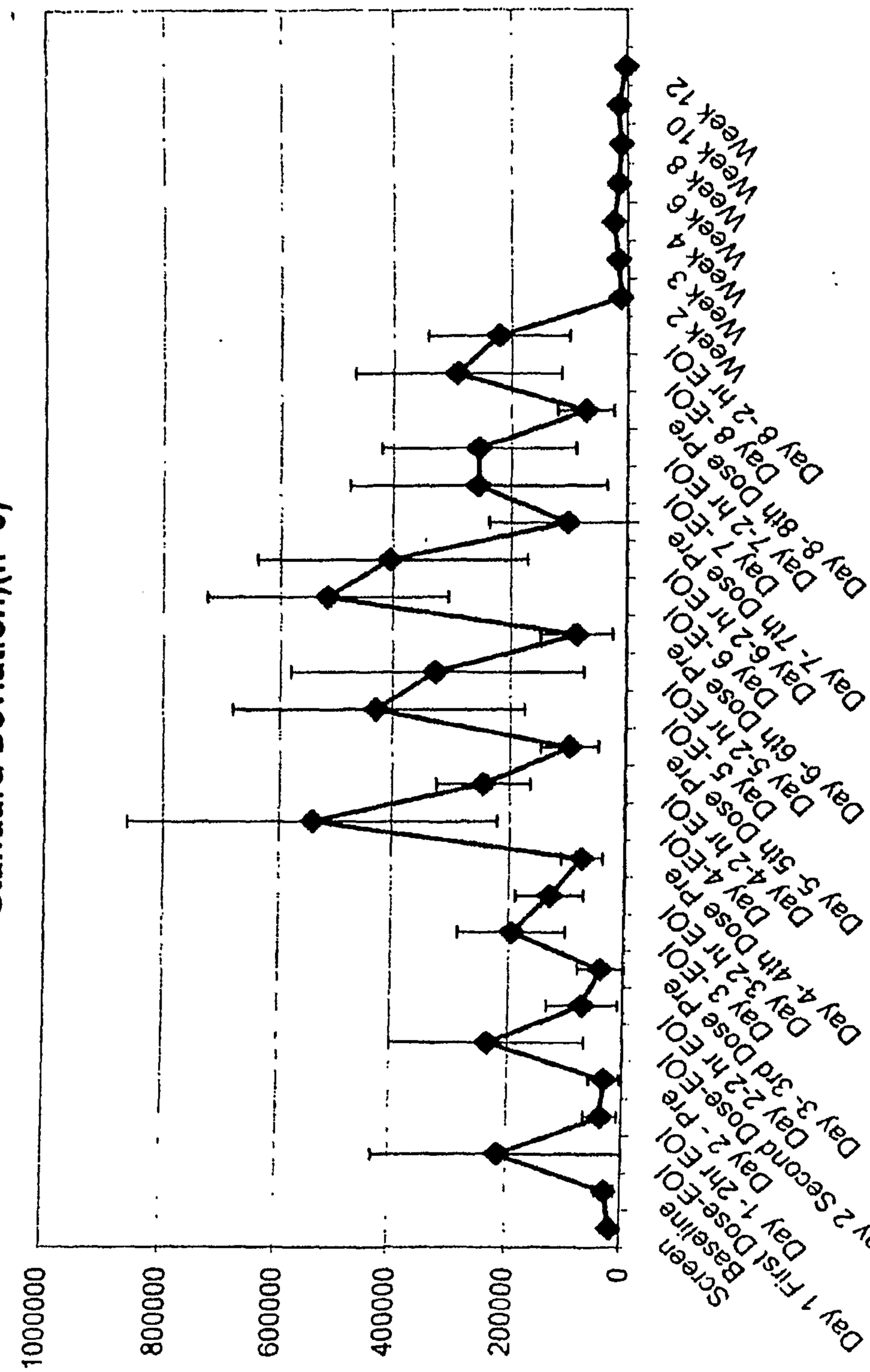
(0.1 mg, 0.2 mg, 0.3 mg, 0.5 mg x 5) - Cell Bound
TRX4 detected by an anti-human IgG on CD4+ T Cells (Mean +/-
Standard Deviation)(n=6)
MESF Units
100000
80000
60000
40000
20000
0
Screen
Baseline EOI
Day 1 First Dose-EOI
Day 1- 2hr EOI
Day 2 - Pre EOI
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre EOI
Day 4-2 hr EOI
Day 5- 5th Dose Pre EOI
Day 5-2 hr EOI
Day 6- 6th Dose Pre EOI
Day 6-2 hr EOI
Day 7- 7th Dose Pre EOI
Day 7-2 hr EOI
Day 8- 8th Dose Pre EOI
Day 8-2 hr EOI
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12

FIG..24

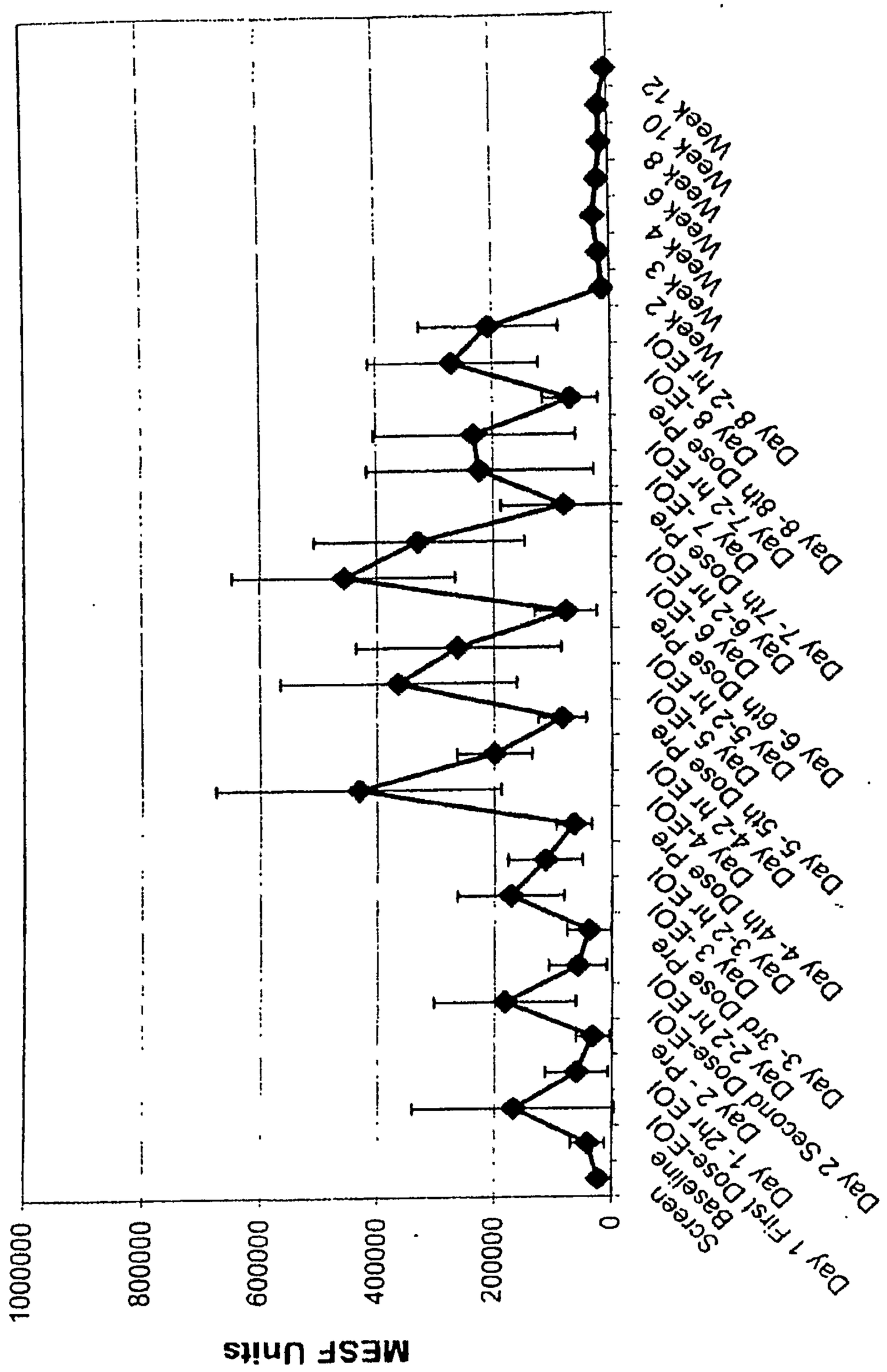
TRX4 detected by an anti-human IgG on CD8+ T Cells (Mean +/- Standard Deviation)(n=6)
(0.1 mg, 0.2 mg, 0.3 mg, 0.5 mg x 5) - Cell Bound
MESF Units
1000000
800000
600000
400000
200000
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1- 2hr EOI
Day 2 - Pre EOI
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3 - 3rd Dose Pre EOI
Day 3-2 hr EOI
Day 4 - 4th Dose Pre EOI
Day 4-2 hr EOI
Day 5 - 5th Dose Pre EOI
Day 5-2 hr EOI
Day 6 - 6th Dose Pre EOI
Day 6-2 hr EOI
Day 7 - 7th Dose Pre EOI
Day 7-2 hr EOI
Day 8 - 8th Dose Pre EOI
Day 8 -2 hr EOI
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12

FIG..25

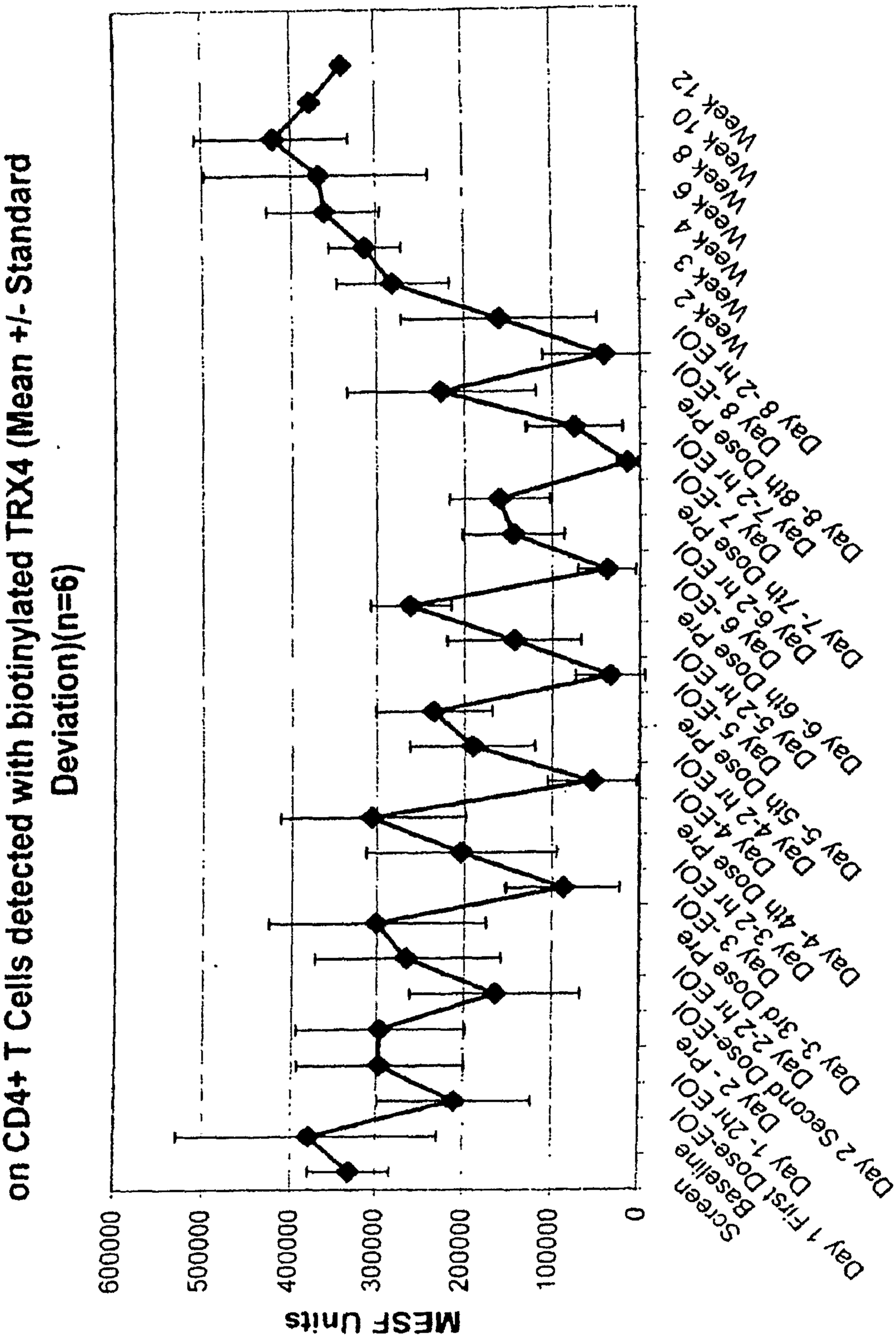
(0.1 mg, 0.2 mg, 0.3 mg, 0.5 mg x 5) - Free CD3 Sites on CD4+ T Cells detected with biotinylated TRX4 (Mean +/- Standard Deviation)(n=6)
MESF Units
600000
500000
400000
300000
200000
100000
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1- 2hr EOI
Day 2 - Pre EOI
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre EOI
Day 4-2 hr EOI
Day 5- 5th Dose Pre EOI
Day 5-2 hr EOI
Day 6- 6th Dose Pre EOI
Day 6-2 hr EOI
Day 7- 7th Dose Pre EOI
Day 7-2 hr EOI
Day 8- 8th Dose Pre EOI
Day 8 -2 hr EOI
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12

FIG..26

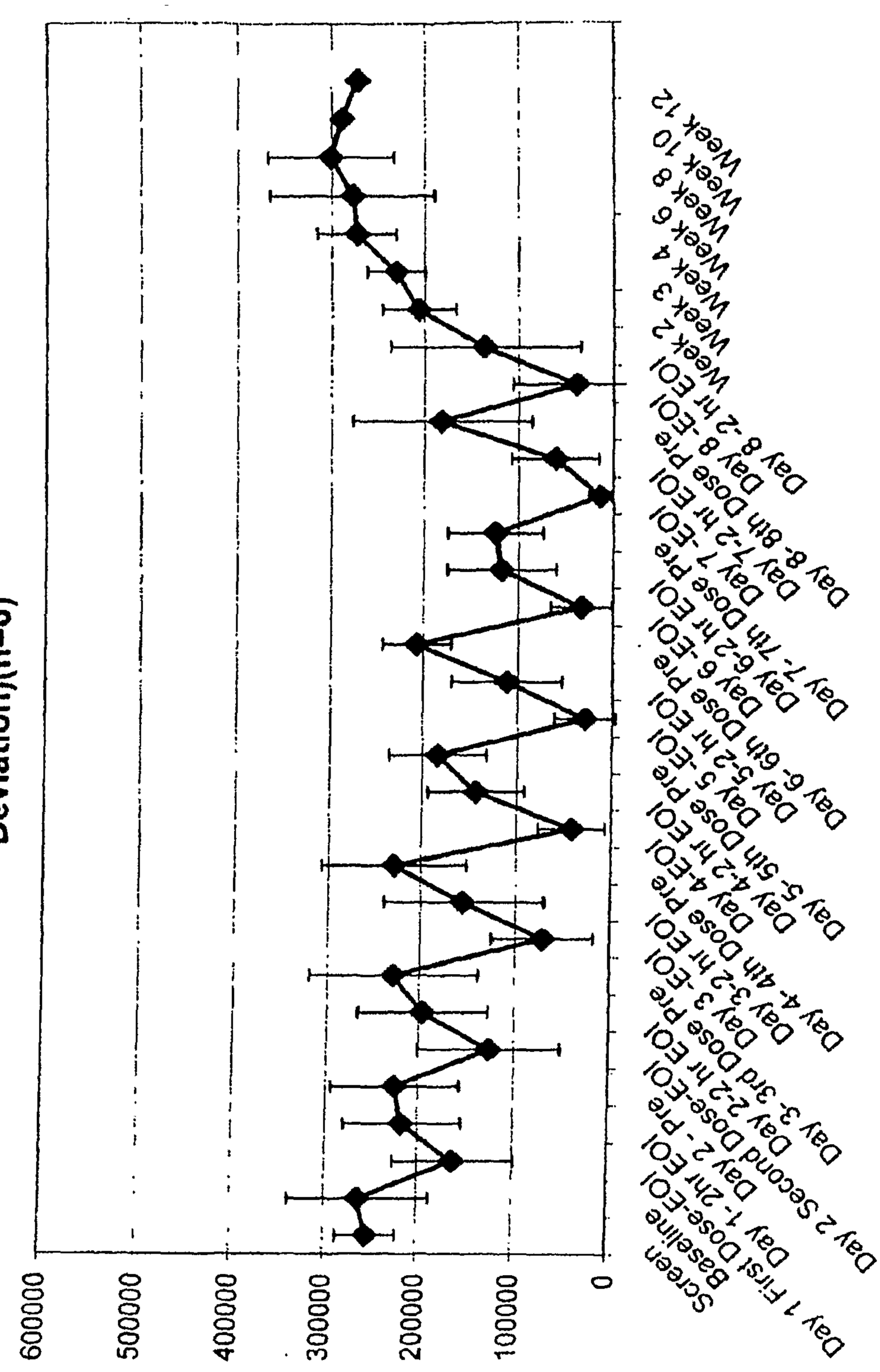
(0.1 mg, 0.2 mg, 0.3 mg, 0.5 mg x 5) - Free CD3 Sites
on CD8+ T Cells detected with biotinylated TRX4 (Mean +/- Standard Deviation)(n=6)
MESF Units
600000
500000
400000
300000
200000
100000
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1- 2hr EOI
Day 2 - Pre EOI
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre EOI
Day 4-2 hr EOI
Day 5- 5th Dose Pre EOI
Day 5-2 hr EOI
Day 6- 6th Dose Pre EOI
Day 6-2 hr EOI
Day 7- 7th Dose Pre EOI
Day 7-2 hr EOI
Day 8- 8th Dose Pre EOI
Day 8-2 hr EOI
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10

FIG..27

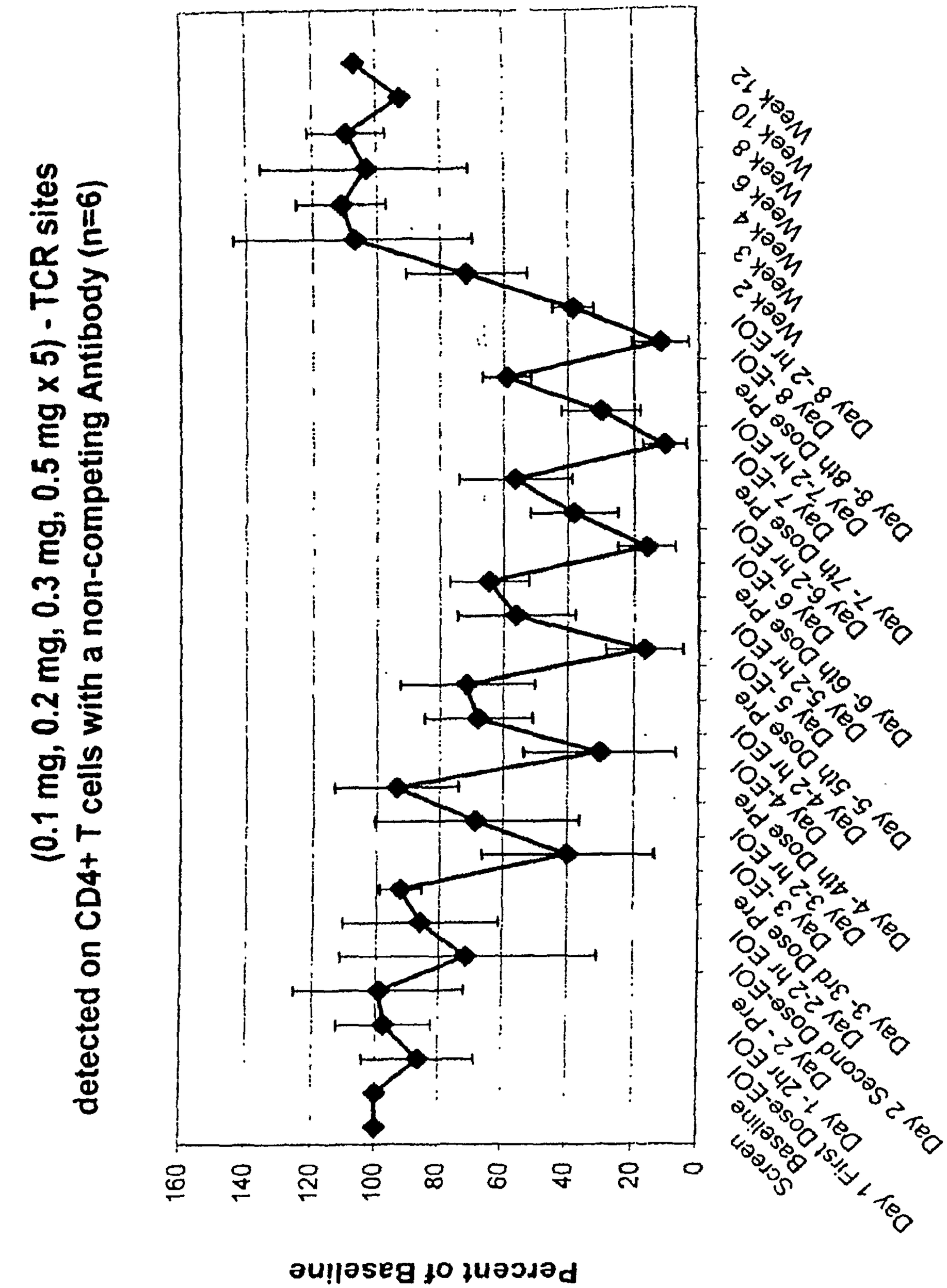
(0.1 mg, 0.2 mg, 0.3 mg, 0.5 mg x 5) - TCR sites detected on CD4+ T cells with a non-competing Antibody (n=6)
Percent of Baseline
160
140
120
100
80
60
40
20
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1- 2hr EOI
Day 2 - Pre EOI
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre EOI
Day 4-2 hr EOI
Day 5- 5th Dose Pre EOI
Day 5-2 hr EOI
Day 6- 6th Dose Pre EOI
Day 6-2 hr EOI
Day 7- 7th Dose Pre EOI
Day 7-2 hr EOI
Day 8- 8th Dose Pre EOI
Day 8-2 hr EOI
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12

FIG..28

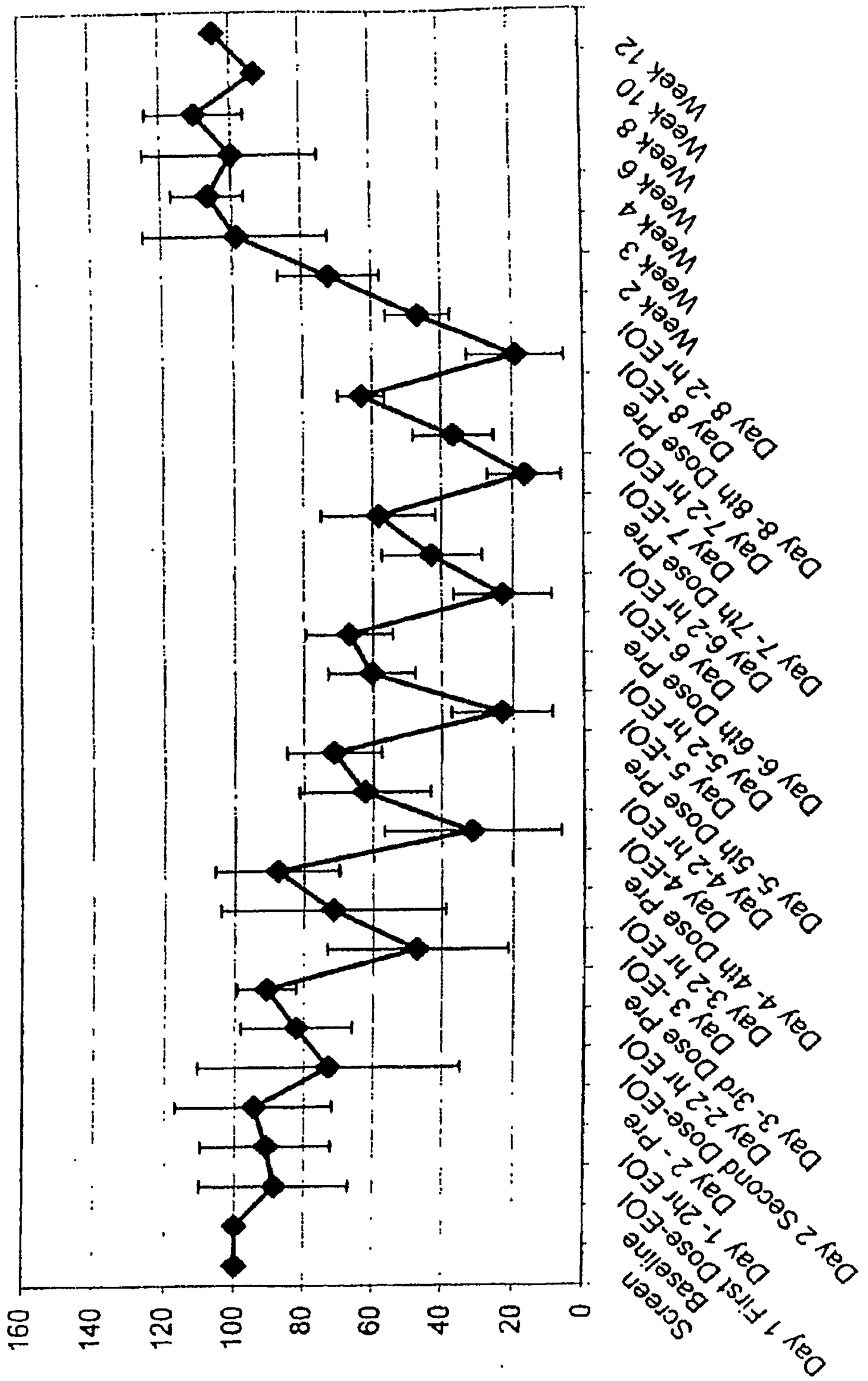
(0.1 mg, 0.2 mg, 0.3 mg, 0.5 mg x 5) - TCR sites
detected on CD8+ T cells with a non competing Antibody (n=6)
Percent of Baseline
160
140
120
100
80
60
40
20
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1- 2hr EOI
Day 2 - Pre EOI
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre EOI
Day 4-2 hr EOI
Day 5- 5th Dose Pre EOI
Day 5-2 hr EOI
Day 6- 6th Dose Pre EOI
Day 6-2 hr EOI
Day 7- 7th Dose Pre EOI
Day 7-2 hr EOI
Day 8- 8th Dose Pre EOI
Day 8-2 hr EOI
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12

FIG..29

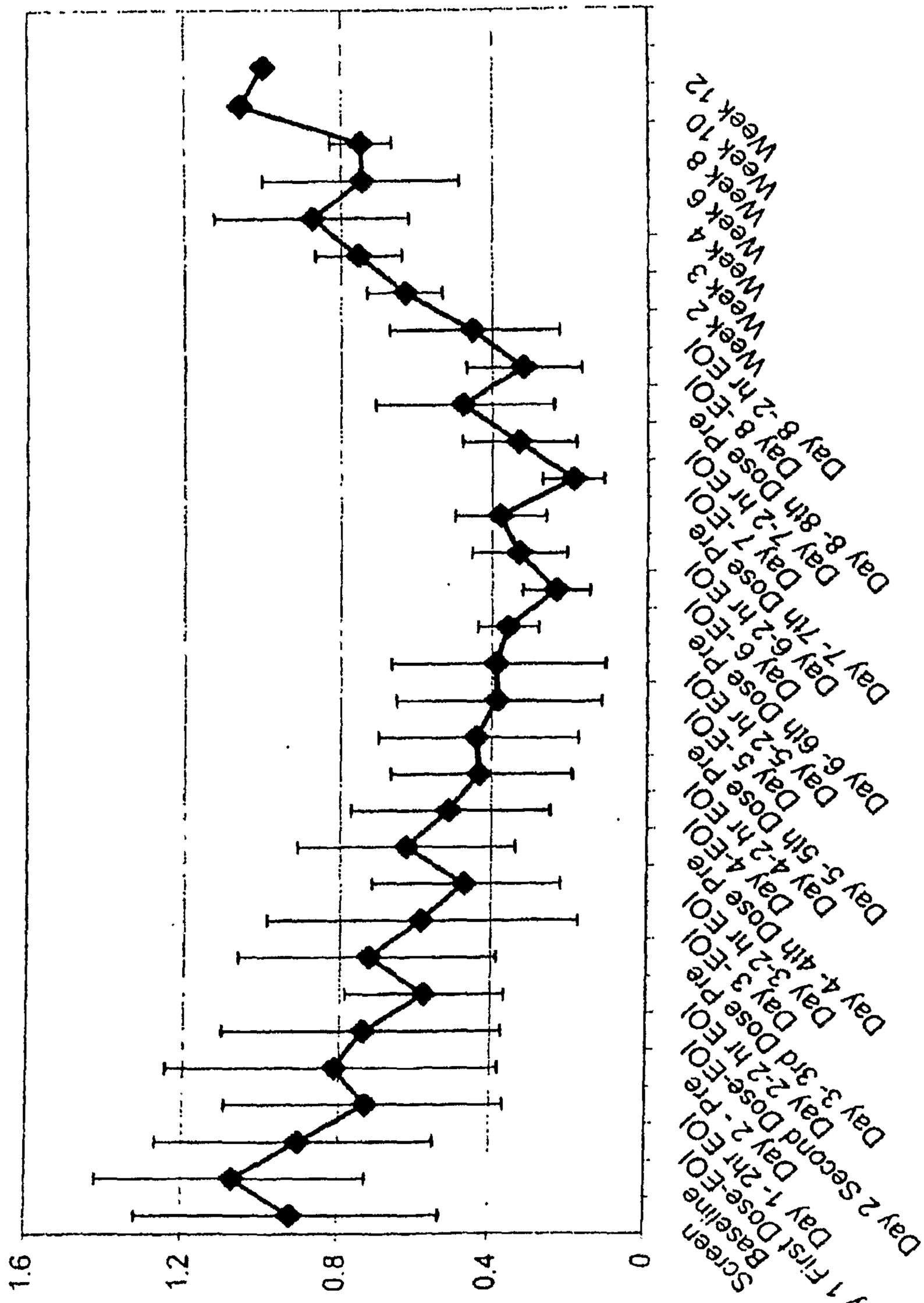
(0.1 mg, 0.2 mg, 0.3 mg, 0.5 mg x 5) - CD4+ T Cells
(Mean +/- Standard Deviation)(n=6)
Absolute cell count - (x10⁹/L)
1.6
1.2
0.8
0.4
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1- 2hr EOI
Day 2 - Pre EOI
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre EOI
Day 4-2 hr EOI
Day 5- 5th Dose Pre EOI
Day 5-2 hr EOI
Day 6- 6th Dose Pre EOI
Day 6-2 hr EOI
Day 7- 7th Dose Pre EOI
Day 7-2 hr EOI
Day 8- 8th Dose Pre EOI
Day 8-2 hr EOI
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12

FIG..30

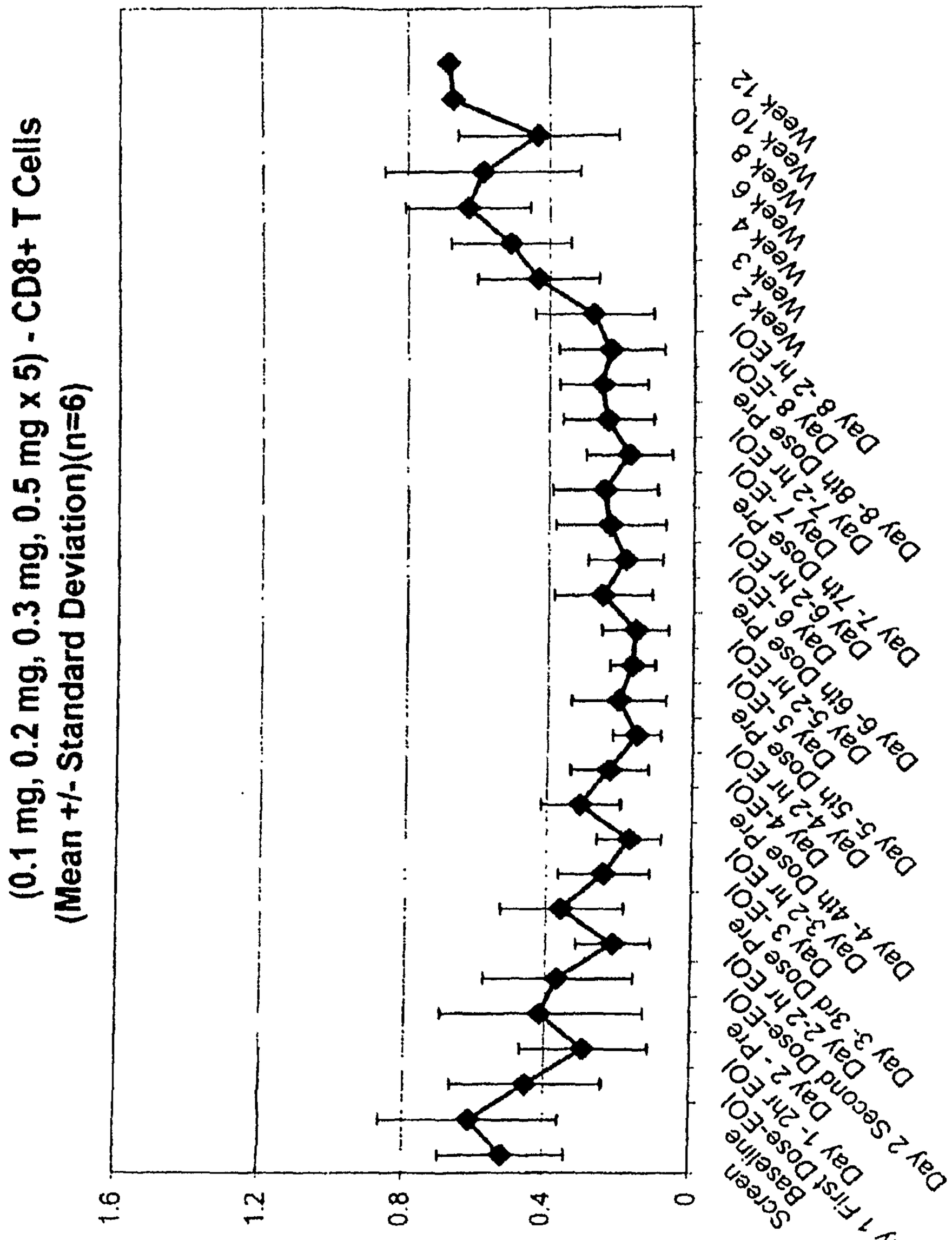
(0.1 mg, 0.2 mg, 0.3 mg, 0.5 mg x 5) - CD8+ T Cells
(Mean +/- Standard Deviation)(n=6)
Absolute cell count - (x10⁹/L)
0
0.4
0.8
1.2
1.6
Screen
Baseline
Day 1 First Dose-EOI
Day 1- 2hr EOI
Day 2 - Pre EOI
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre EOI
Day 4-2 hr EOI
Day 5- 5th Dose Pre
Day 5-2
Day 6-
Day 6- 6th Dose Pre-EOI
Day 6-2 hr EOI
Day 7 Pre-EOI
Day 7-2 hr EOI
Day 7- 7th Dose Pre EOI
Day 7-2 hr EOI
Day 8- 8th Dose Pre-EOI
Day 8 -2 hr EOI
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12

FIG..31

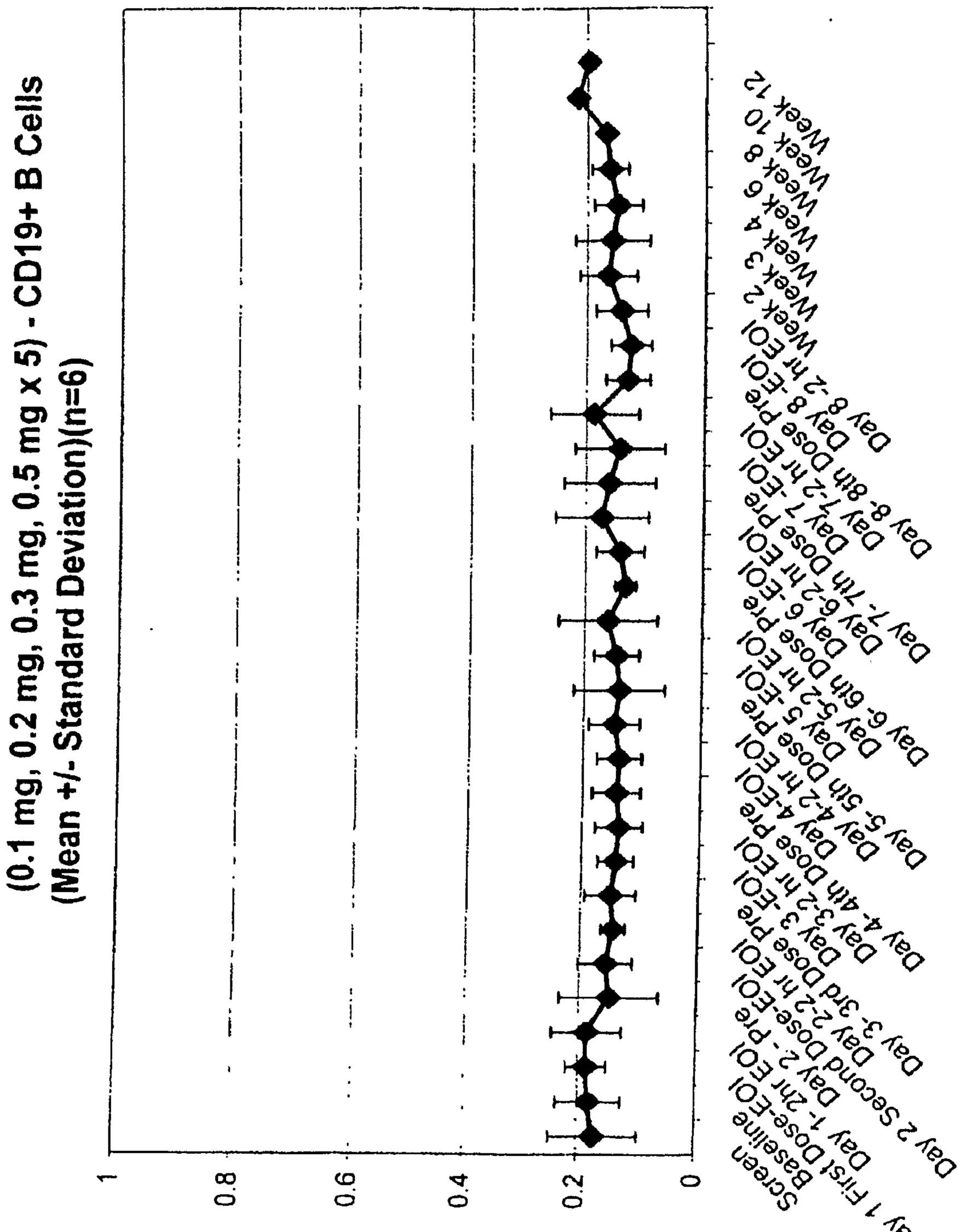
(0.1 mg, 0.2 mg, 0.3 mg, 0.5 mg x 5) - CD19+ B Cells
(Mean +/- Standard Deviation)(n=6)
Absolute cell count - (x10⁹/L)
1
0.8
0.6
0.4
0.2
0
Screen
Baseline
Day 1 First Dose-EOI
Day 1- 2hr EOI
Day 2 - Pre EOI
Day 2 Second Dose-EOI
Day 2-2 hr EOI
Day 3- 3rd Dose Pre EOI
Day 3-2 hr EOI
Day 4- 4th Dose Pre EOI
Day 4-2 hr EOI
Day 5- 5th Dose Pre EOI
Day 5-2 hr EOI
Day 6- 6th Dose Pre EOI
Day 6-2 hr EOI
Day 7- 7th Dose Pre EOI
Day 7-2 hr EOI
Day 8- 8th Dose Pre EOI
Day 8-2 hr EOI
Week 2
Week 3
Week 4
Week 6
Week 8
Week 10
Week 12

FIG..32

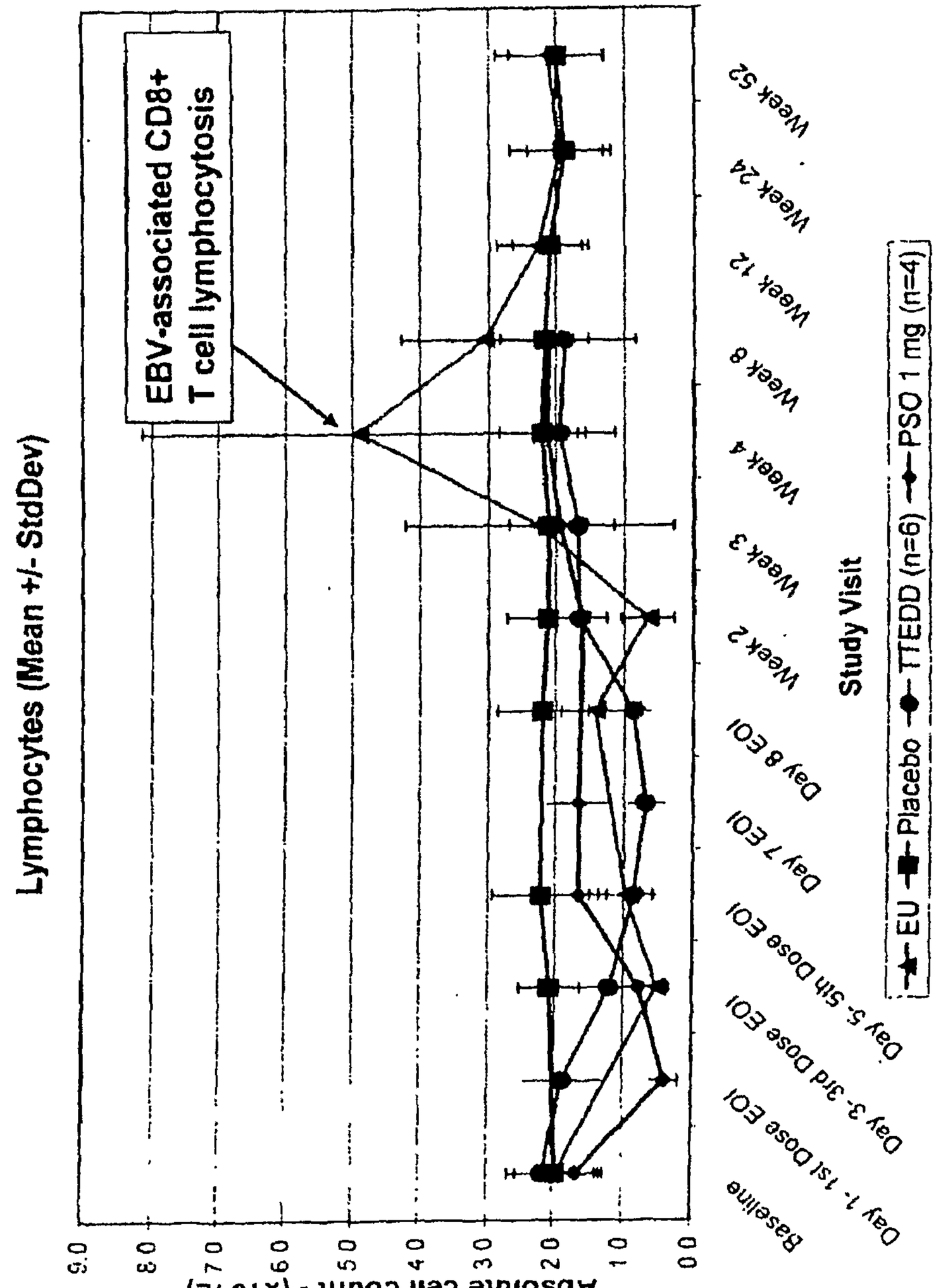
EBV-associated CD8+
T cell lymphocytosis
Lymphocytes (Mean +/- StdDev)
Absolute cell count - (x10⁹/L)
Study Visit
Baseline
Day 1- 1st Dose EOI
Day 3- 3rd Dose EOI
Day 5- 5th Dose EOI
Day 7 EOI
Day 8 EOI
Week 2
Week 3
Week 4
Week 8
Week 12
Week 24
Week 52
EU
Placebo
TTEDD (n=6)
PSO 1 mg (n=4)

FIG..33

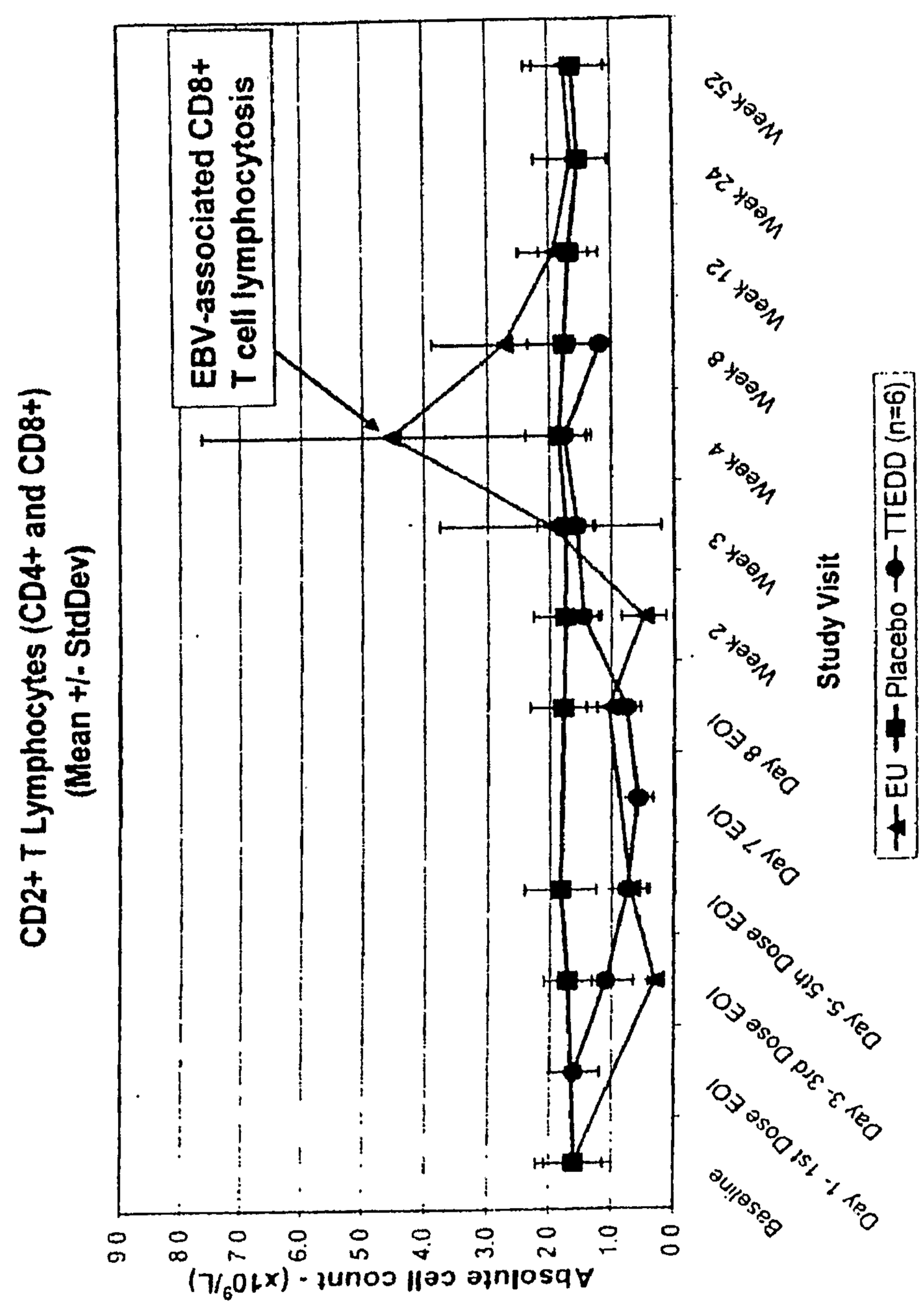
EBV-associated CD8+
T cell lymphocytosis
CD2+ T Lymphocytes (CD4+ and CD8+)
(Mean +/- StdDev)
Absolute cell count - (x10⁹/L)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Study Visit
Baseline
Day 1- 1st Dose EOI
Day 3- 3rd Dose EOI
Day 5- 5th Dose EOI
Day 7 EOI
Day 8 EOI
Week 2
Week 3
Week 4
Week 8
Week 12
Week 24
Week 52
EU
Placebo
TTEDD (n=6)

FIG..34

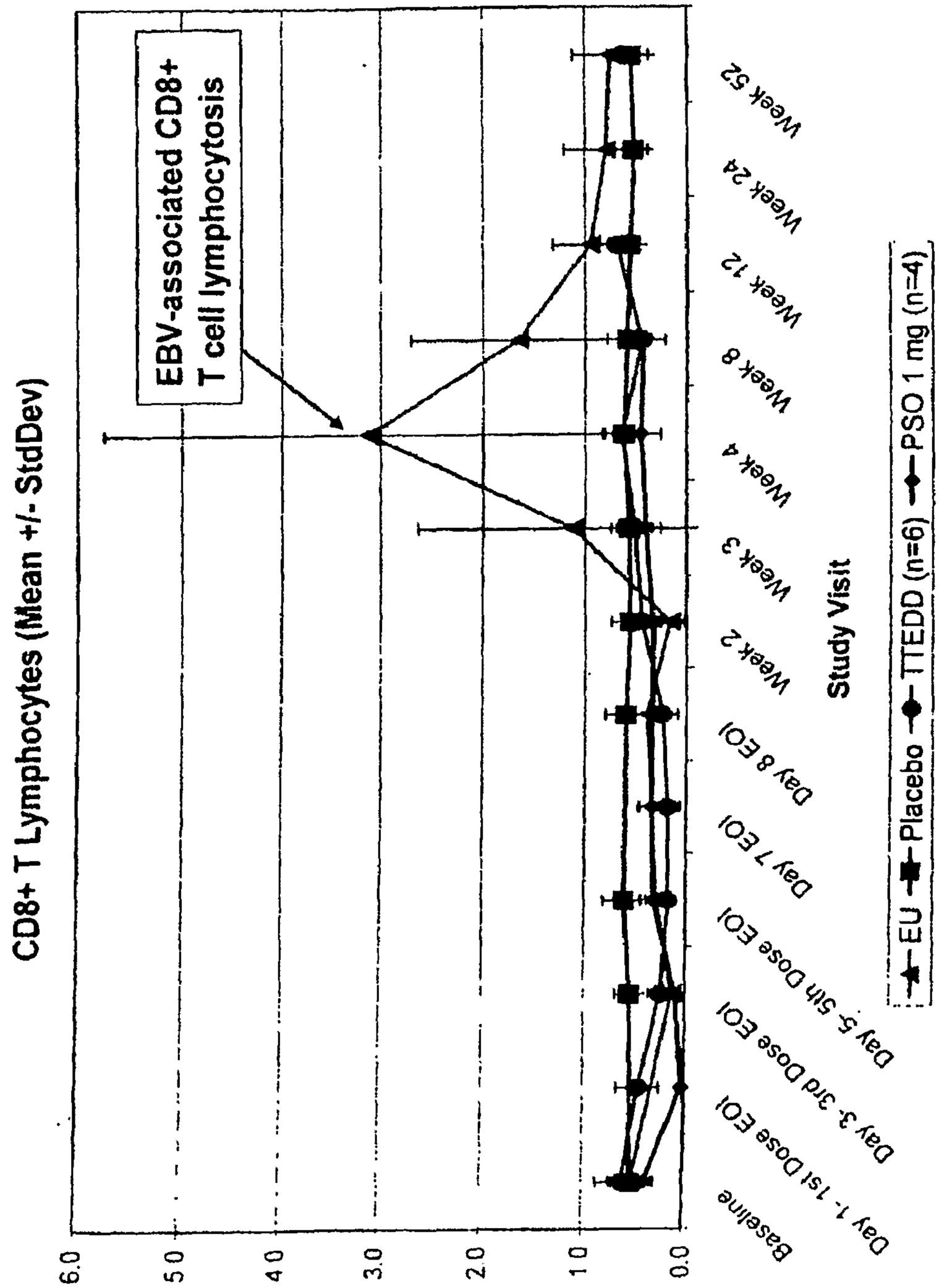
EBV-associated CD8+ T cell lymphocytosis
CD8+ T Lymphocytes (Mean +/- StdDev)
Absolute cell count - (x10⁹/L)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Study Visit
Baseline
Day 1- 1st Dose EOI
Day 3- 3rd Dose EOI
Day 5- 5th Dose EOI
Day 7 EOI
Day 8 EOI
Week 2
Week 3
Week 4
Week 8
Week 12
Week 24
Week 52
EU
Placebo
TTEDD (n=6)
PSO 1 mg (n=4)

FIG..35

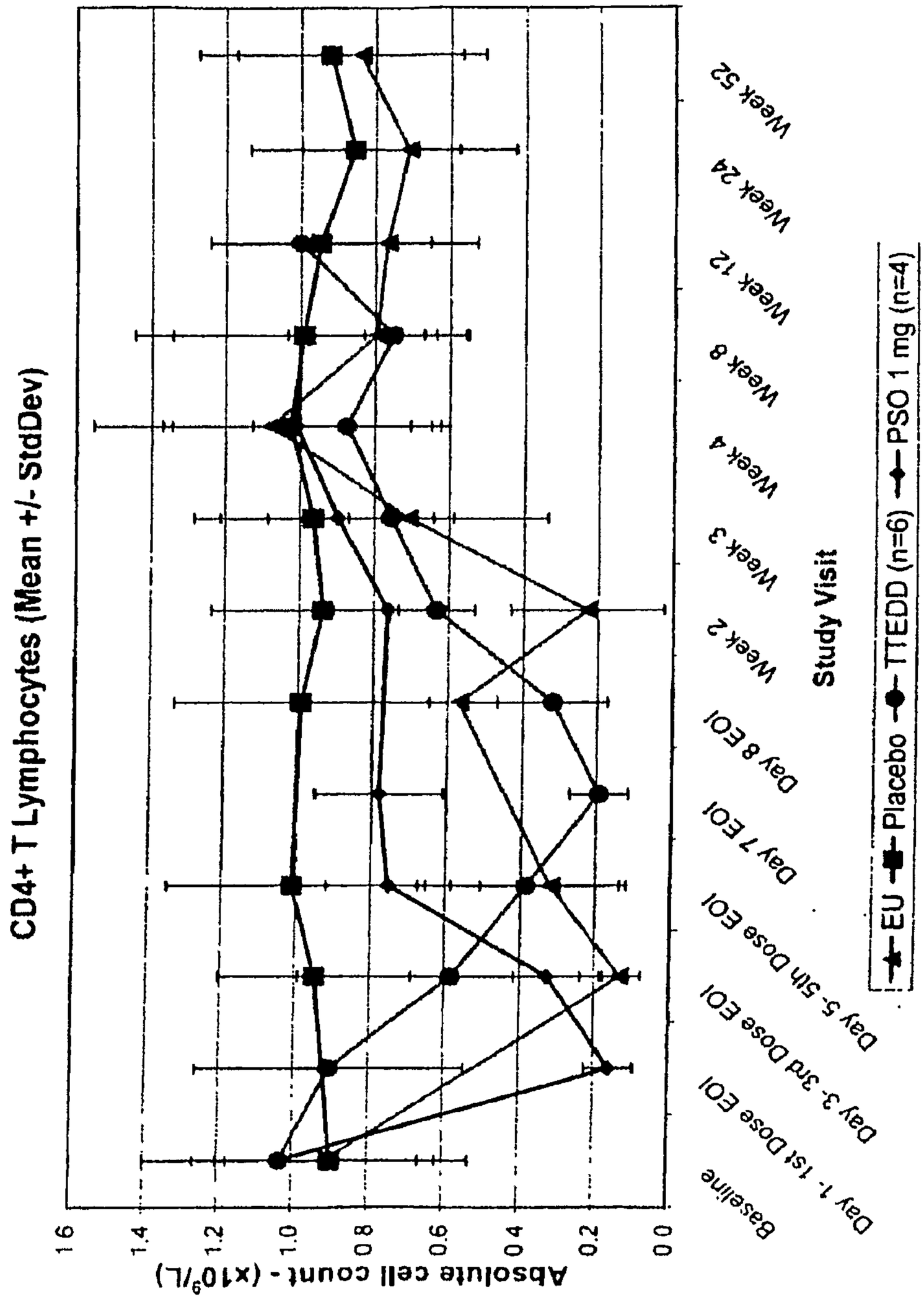
CD4+ T Lymphocytes (Mean +/- StdDev)
Absolute cell count - (x10⁹/L)
Study Visit
Baseline
Day 1- 1st Dose EOI
Day 3- 3nd Dose EOI
Day 5- 5th Dose EOI
Day 7 EOI
Day 8 EOI
Week 2
Week 3
Week 4
Week 8
Week 12
Week 24
Week 52
EU
Placebo
TTEDD (n=6)
PSO 1 mg (n=4)

FIG..36

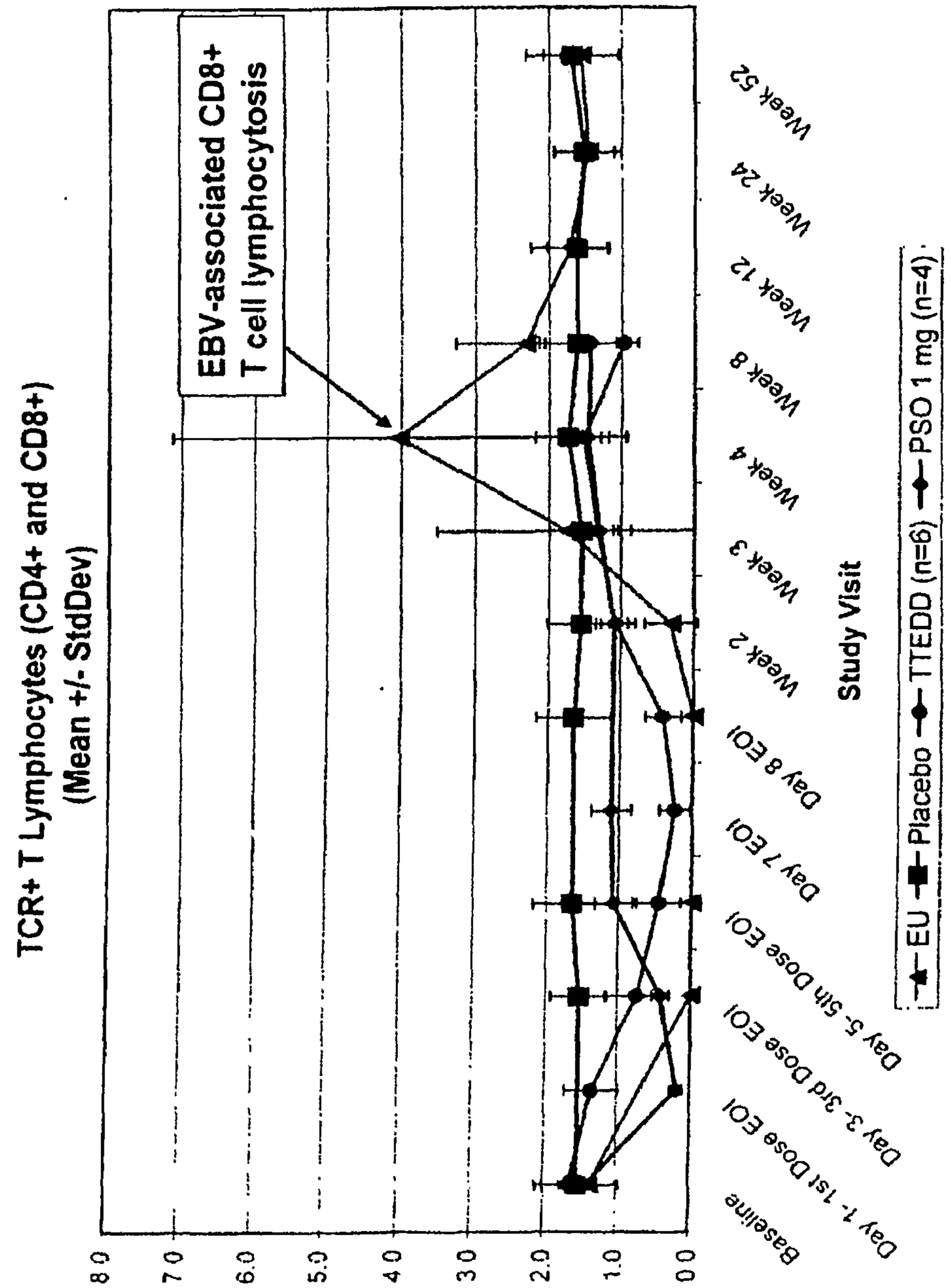
EBV-associated CD8+
T cell lymphocytosis
TCR+ T Lymphocytes (CD4+ and CD8+)
(Mean +/- StdDev)
Absolute cell count - (x10⁹/L)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Baseline
Day 1- 1st Dose EOI
Day 3- 3rd Dose EOI
Day 5- 5th Dose EOI
Day 7 EOI
Day 8 EOI
Week 2
Week 3
Week 4
Week 8
Week 12
Week 24
Week 52
Study Visit
EU
Placebo
TTEDD (n=6)
PSO 1 mg (n=4)

FIG..37

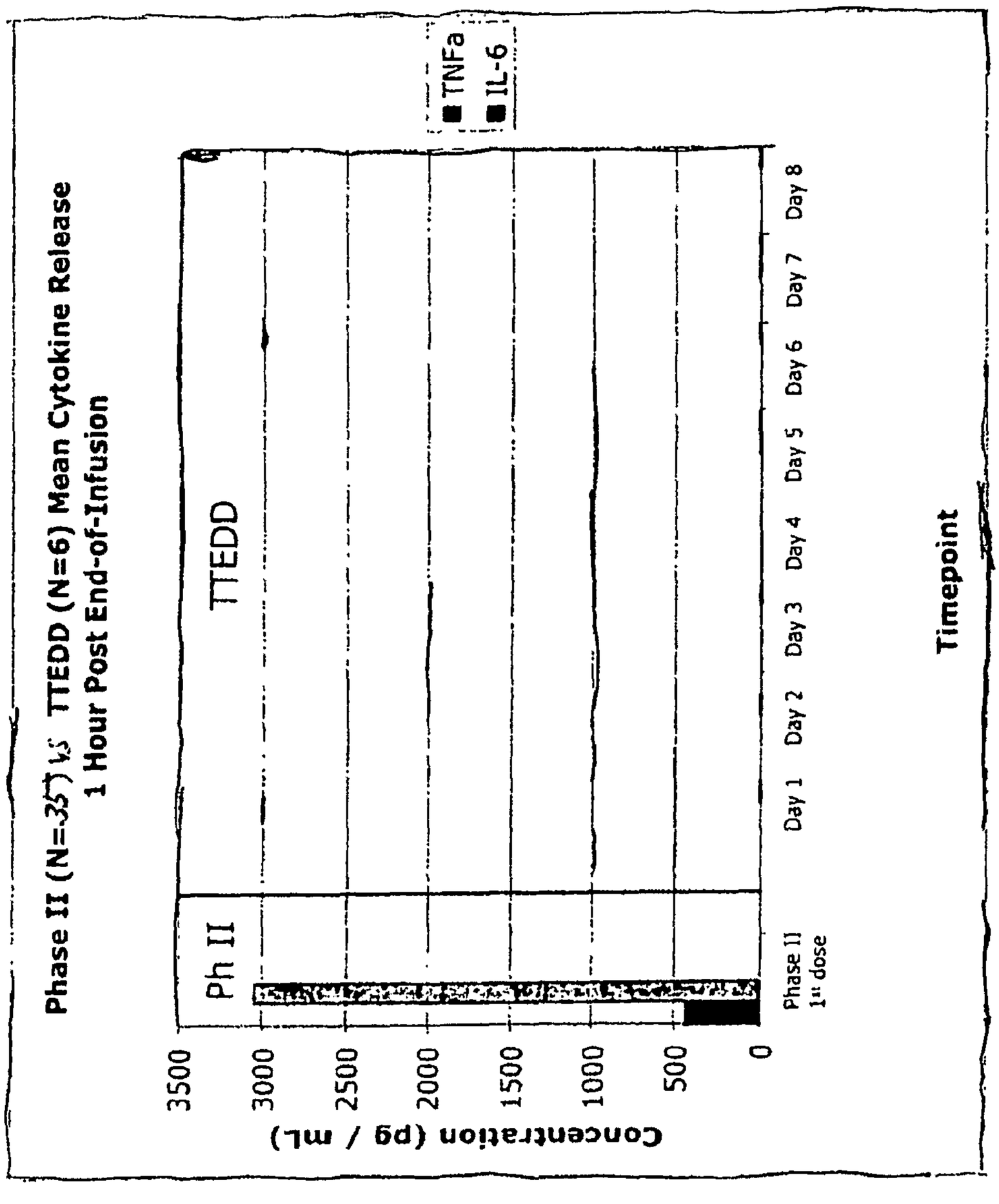
Phase II (N=35) vs TTEDD (N=6) Mean C
1 Hour Post End-of-Infusio
TNFa
IL-6
TTEDD
Ph II
3500
3000
2500
2000
1500
1000
500
0
Concentration (pg / mL)
Phase II
1st dose
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Timepoint

FIG..38