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(54) **Title:** PREVENTION OR MITIGATION OF T-CELL ENGAGING AGENT-RELATED ADVERSE EFFECTS

(57) **Abstract:** The present invention relates to the prevention or mitigation of adverse effects related to T cell engaging agents, such as cytokine release syndrome. Specifically, the invention relates to the prevention or mitigation of such side effects using an inhibitor of JAK and/or mTOR.

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Prevention or mitigation of T-cell engaging agent-related adverse effects

Field of the Invention

The present invention relates to the prevention or mitigation of adverse effects related to T cell engaging agents, such as cytokine release syndrome. Specifically, the invention relates to the
5 prevention or mitigation of such side effects using an inhibitor of JAK and/or mTOR.

Background

T cell engaging agents such as T cell bispecific antibodies (TCBs) or chimeric antigen receptor (CAR) expressing T cells (CAR-T cells) hold great promise as cancer immunotherapeutics.
10 However, treatment with T cell engaging agent is sometimes associated with safety liabilities due to on-target on-tumor, on-target off-tumor cytotoxic activity and cytokine release. One of the most common adverse effects reported for T cell engaging agents is Cytokine Release Syndrome (CRS). This complex clinical syndrome is characterized by fever, hypotension and respiratory deficiency and associated with the release of pro-inflammatory cytokines such as IL-6, TNF-α, IFN-γ, and
15 IL-10 (see e.g. Shimabukuro-Vornhagen et al., J Immunother Cancer (2018) 6, 56). Approaches to mitigate these life-threatening toxicities are greatly needed. The Src inhibitor dasatinib was identified as a potent candidate for prevention or mitigation of adverse effects of CAR-T cells (Weber et al., Blood Advances (2019) 3, 711-7; Mestermann et al., Sci Transl Med (2019) 11, eaau5907) as well as TCBs (Leclercq et al., J Immunother Cancer (2020) 8 (Suppl 3): A690
20 (abstract 653)). Dasatinib, however, switches off CAR-T cell functionality as well as TCB-induced T cell functionality entirely, without differentiation between desired and undesired activity of these agents. A way to prevent or mitigate adverse effects of T cell engaging agents while preserving their therapeutic efficacy would be highly desirable. Blockade of individual cytokines such as IL-6 or TNF-α was proposed as strategy for prevention of CRS without affecting TCB-induced T cell
25 activity (Li et al., Sci Transl Med 11, eaax8861 (2019)). Besides anti-IL-6 treatment (e.g. with

tocilizumab), glucocorticoids are also used in the management of CRS. Some patients are refractory to these approaches, however, reinforcing the need to develop novel approaches for CRS mitigation.

5 **Description of the Invention**

The present inventors have found that inhibitors of JAK and/or mTOR signaling may be used to mitigate CRS by T cell engaging therapies. mTOR inhibitors such as temsirolimus, sirolimus and everolimus, and JAK inhibitors such as ruxolitinib, were found to potently prevent TCB-induced cytokine release while retaining TCB-mediated target cell killing. The results provide evidence
10 that the mechanisms of TCB-dependent cytokine release and target cell killing can be uncoupled, and suggests mTOR and/or JAK inhibitors as attractive potential superior alternative or complementation to currently used strategies, such as steroids or IL-6/IL-6R blockade, for the mitigation of CRS associated with T cell engaging therapies.

Accordingly, in a first aspect, the present invention provides a T cell engaging agent for use in the
15 treatment of a disease in an individual, wherein said treatment comprises
(a) the administration of the T cell engaging agent to the individual, and
(b) the administration of an inhibitor of Janus kinase (JAK) and/or mammalian target of rapamycin (mTOR) signaling to the individual.

The invention further provides the use of a T cell engaging agent in the manufacture of a
20 medicament for the treatment of a disease in an individual, wherein said treatment comprises
(a) the administration of the T cell engaging agent to the individual, and
(b) the administration of an inhibitor of JAK and/or mTOR signaling to the individual.

The invention also provides a method for treatment of a disease in an individual, wherein said method comprises
25 (a) the administration of a T cell engaging agent to the individual, and
(b) the administration of an inhibitor of JAK and/or mTOR signaling to the individual.

According to any of the above aspects, the administration of the inhibitor of JAK and/or mTOR signaling may be for the prevention or mitigation of an adverse effect related to the administration of the T cell engaging agent.

In another aspect, the invention provides an inhibitor of JAK and/or mTOR signaling for use in the prevention or mitigation of an adverse effect related to the administration of a T cell engaging agent to an individual.

The invention further provides the use of an inhibitor of JAK and/or mTOR signaling in the manufacture of a medicament for the prevention or mitigation of an adverse effect related to the administration of a T cell engaging agent.

The invention also provides a method for preventing or mitigating an adverse effect related to the administration of a T cell engaging agent to an individual, comprising the administration of an inhibitor of JAK and/or mTOR signaling to the individual.

The T cell engaging agent for use, inhibitor of JAK and/or mTOR signaling for use, uses or methods described above and herein, may incorporate, singly or in combination, any of the features described in the following (unless the context dictates otherwise).

Terms are used herein as generally used in the art, unless otherwise defined herein.

In some aspects, the inhibitor of JAK and/or mTOR signaling is an mTOR inhibitor. In more specific aspects, the inhibitor of JAK and/or mTOR signaling is an mTOR kinase inhibitor, particularly a small molecule mTOR kinase inhibitor.

"mTOR" stands for mammalian target of rapamycin (also known as FK506-binding protein 12-rapamycin complex-associated protein 1 (FRAP1)), and is a serine/threonine-specific protein kinase that belongs to the family of phosphatidylinositol-3 kinase (PI3K) related kinases. It serves as core component of two distinct protein complexes, mTOR complex 1 (TORC1) and mTOR complex 2 (TORC2), which regulate different cellular processes. Human mTOR is described in UniProt entry P42345 (version 218). mTOR inhibitors are compounds that inhibit mTOR. The most established inhibitors of mTOR are the so-called rapalogs, which are derivatives of rapamycin. Rapalogs include sirolimus, temsirolimus, everolimus and ridaforolimus. A second generation of mTOR inhibitors are ATP-competitive mTOR kinase inhibitors, designed to compete with ATP in the catalytic site of mTOR.

Exemplary mTOR inhibitors that might be useful in the present invention are provided in **Table 1** below.

Table 1. mTOR inhibitors.

INN	Structure
Sirolimus	HO, O, N, O, O, O, OH, O, O, HO, O, O
Everolimus	HO, O, O, N, O, O, O, OH, O, O, HO, O, O
Temsirolimus	HO, OH, O, O, O, N, O, O, O, OH, O, O, HO, O, O
Ridaforolimus	P, O, O, O, N, O, O, O, OH, O, O, HO, O, O

In some aspects, the mTOR inhibitor is a derivative of rapamycin (also known as a rapalog).

In some aspects, the mTOR inhibitor is selected from the group consisting of sirolimus, temsirolimus, everolimus and ridaforolimus, particularly the group consisting of sirolimus, temsirolimus and everolimus.

In specific aspects, the mTOR inhibitor is sirolimus. In further specific aspects, the mTOR inhibitor is temsirolimus. In yet further specific aspects, the mTOR inhibitor is everolimus.

In some aspects, the inhibitor of JAK and/or mTOR signaling is a JAK inhibitor. In more specific aspects, the inhibitor of JAK and/or mTOR signaling is a JAK kinase inhibitor, particularly a small molecule JAK kinase inhibitor.

"JAK" stands for Janus kinase and refers to a family of intracellular, non-receptor tyrosine kinases that transduce cytokine-mediated signals via the JAK/STAT pathway. JAKs possess two near-identical phosphate-transferring domains, one exhibiting the kinase activity, and the other one negatively regulating the kinase activity of the first. The four JAK family members are JAK1, JAK2, JAK3 and TYK2 (tyrosine kinase 2). In particular aspects herein, JAK is JAK1 and/or JAK2 (JAK1/2). Human JAK1 and JAK2 are described in UniProt entries P23458 (version 221) and P60674 (version 224), respectively. JAK inhibitors (also sometimes referred to as jakinibs) are compounds that inhibit the activity of one or more of the JAK family of enzymes (JAK1, JAK2, JAK3, TYK2), thereby interfering with the the JAK/STAT signaling pathway.

Exemplary JAK inhibitors that might be useful in the present invention are provided in **Table 2** below.

Table 2. JAK inhibitors.

INN	Main specificity	Structure
Ruxolitinib	JAK1, JAK2	

Baricitinib	JAK1, JAK2	
Momelotinib	JAK1, JAK2	
Upadacitinib	JAK1	
Filgotinib	JAK1	
Abrocitinib	JAK1	

Itacitinib	JAK1	
Solcitinib	JAK1	
Oclacitinib	JAK1	
Fedratinib	JAK2	
Gandotinib	JAK2	

Lestaurtinib	JAK2	
Pacritinib	JAK2	
Peficitinib	pan-JAK (JAK3)	
Tofacitinib	pan-JAK (JAK3)	
Decernotinib	JAK3	

In some aspects, the JAK inhibitor is a JAK1 and/or JAK2 (JAK1/2) inhibitor. In some aspects, the JAK inhibitor is selected from the group consisting of ruxolitinib, baricitinib, momelotinib,

upadacitinib, filgotinib, abrocitinib, itacitinib, solcitinib, oclacitinib, fedratinib, gandotinib, lestaurtinib and pacritinib.

In particular aspects, the JAK inhibitor is a JAK1 and JAK2 inhibitor. In specific such aspects, the JAK inhibitor is selected from the group consisting of ruxolitinib, baricitinib and momelotinib.

5 In some aspects, the JAK inhibitor is a JAK1 inhibitor. In specific such aspects, the JAK inhibitor is selected from the group consisting of upadacitinib, filgotinib, abrocitinib, itacitinib, solcitinib and oclacitinib.

In some aspects, the JAK inhibitor is a JAK2 inhibitor. In specific such aspects, the JAK inhibitor is selected from the group consisting of fedratinib, gandotinib, lestaurtinib and pacritinib. In a 10 particular such aspect, the JAK inhibitor is fedratinib.

In some aspects, the JAK inhibitor is a pan-JAK inhibitor. In specfic such aspects, the JAK inhibitor is tofacitinib or peficitinib, particularly tofacitinib.

In particular aspects, the JAK inhibitor is ruxolitinib. In further particular aspects, the JAK inhibitor is baricitinib. In some aspects, the JAK inhibitor is tofacitinib. In some aspects, the JAK 15 inhibitor is fedratinib.

In particular aspects, the inhibitor of JAK and/or mTOR signaling is selected from the group consisting of sirolimus, temsirolimus, everolimus and ruxolitinib. In further particular aspects, the inhibitor of JAK and/or mTOR signaling is selected from the group consisting of sirolimus, temsirolimus, everolimus, ruxolitinib and baricitinib.

20 In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of an activity of the T cell engaging agent. In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling does not cause inhibition of another activity of the T cell engaging agent. In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of a first activity of the T cell engaging agent but does not cause inhibition of a second 25 activity of the T cell engaging agent. In some of these aspects, said inhibition is a complete inhibition.

In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of a first activity of the T cell engaging agent and inhibition of a second activity of the T cell engaging agent, wherein said inhibition of the first activity is stronger than said inhibition of the

second activity. In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of a first activity of the T cell engaging agent and inhibition of a second activity of the T cell engaging agent, wherein said inhibition of the first activity is a complete inhibition and said inhibition of the second activity is a partial inhibition.

5 "Activity" of a T cell engaging agent refers to responses in an individual's body caused by the T cell engaging agent. Such activity may include cellular response(s) of T cells, particularly CD4+ and/or CD8+ T cells, such as proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, and expression of activation markers, and/or effects on target cells, particularly target cells (e.g. tumor cells) expressing the target cell antigen of the T cell 10 engaging agent, such as lysis of target cells.

In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of cytokine secretion by immune cells, particularly T cells (induced by the T cell engaging agent). In some aspects, said cytokine is one or more cytokine selected from the group consisting of IL-6, IFN-γ, IL-10, TNF-α, GM-CSF, MCP-1 and IL-1β. Immune cells may include various immune 15 cell types, such as T cells, macrophages, monocytes, NK cells etc. In some aspects, said T cells are CD8+ T cells or CD4+ cells. In some aspects, said inhibition is a complete inhibition.

In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling does not cause inhibition of the activation of T cells (induced by the T cell engaging agent). In some aspects, said inhibition is a complete inhibition. In some aspects, (administration of) the inhibitor of JAK and/or 20 mTOR signaling causes inhibition of the activation of T cells (induced by the T cell engaging agent), wherein said inhibition is a partial inhibition.

"Activation of T cells" or "T cell activation" as used herein refers to one or more cellular response of a T lymphocyte, particularly a CD4+ or CD8+ T cell, selected from: proliferation, differentiation, cytotoxic effector molecule release, cytotoxic activity, and expression of activation 25 markers. Suitable assays to measure T cell activation are known in the art and described herein. In particular aspects, T cell activation is the expression of activation markers, particularly expression of CD25 and/or CD69 (optionally as measured by flow cytometry). In particular aspects, T cell activation is determined by measuring expression of CD25 and/or CD69 on the T cell, e.g. by flow cytometry.

In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling does not cause inhibition of the cytotoxic activity of T cells (induced by the T cell engaging agent). In some aspects, said inhibition is a complete inhibition. In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of the cytotoxic activity of T cells (induced by 5 the T cell engaging agent), wherein said inhibition is a partial inhibition.

"Cytotoxic activity" of a T cell refers to the induction of lysis (i.e. killing) of target cells by a T lymphocyte, particularly a CD4+ or CD8+ T cell. Cytotoxic activity typically involves degranulation of the T lymphocyte, associated with the release of cytotoxic effector molecules such as granzyme B and/or perforin from the T lymphocyte.

10 In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of cytokine secretion by T cells (induced by the T cell engaging agent) but does not cause inhibition of the activation and/or the cytotoxic activity of T cells (induced by the T cell engaging agent). In some of these aspects, said inhibition is a complete inhibition.

In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition 15 of cytokine secretion by T cells (induced by the T cell engaging agent) and inhibition of the activation and/or the cytotoxic activity of T cells (induced by the T cell engaging agent), wherein said inhibition of cytokine secretion is stronger than said inhibition of activation and/or cytotoxic activity. In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of cytokine secretion by T cells (induced by the T cell engaging agent) and inhibition of 20 the activation and/or the cytotoxic activity of T cells (induced by the T cell engaging agent), wherein said inhibition of cytokine secretion is a complete inhibition and said inhibition of activation and/or cytotoxic activity is a partial inhibition.

An inhibition herein may be a partial inhibition or a complete inhibition. A complete inhibition is a stronger inhibition than a partial inhibition. A partial inhibition in some aspects is an inhibition 25 by no more than 30%, no more than 40%, no more than 50%, no more than 60%, or no more than 70%. In some aspects, a partial inhibition is an inhibition by no more than 30%. In some aspects, a partial inhibition is an inhibition by no more than 40%. In some aspects, a partial inhibition is an inhibition by no more than 50%. In some aspects, a partial inhibition is an inhibition by no more than 60%. In some aspects, a partial inhibition is an inhibition by no more than 70%. A complete 30 inhibition in some aspects is an inhibition by at least 80%, at least 90%, or 100%. In some aspects, a complete inhibition is an inhibition by at least 80%. In some aspects, a complete inhibition is an

inhibition by at least 90%. In some aspects, a complete inhibition is an inhibition by 100%. In some aspects, a partial inhibition is an inhibition by no more than 50%, and a complete inhibition is an inhibition by at least 80%. In some aspects, a complete inhibition is clinically meaningful and/or statistically significant, and/or a partial inhibition is not clinically meaningful and/or 5 statistically significant.

In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes reduction of the serum level of one of more cytokine in the individual. In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes reduction of the secretion of one of more cytokine by immune cells, particularly T cells, in the individual. In some aspects, said one or more 10 cytokine is selected from the group consisting of IL-6, IFN-γ, IL-10, TNF-α, GM-CSF, MCP-1 and IL-1β. Immune cells may include various immune cell types, such as T cells, macrophages, monocytes, NK cells etc.

In some aspects, said reduction is sustained after the inhibitor of JAK and/or mTOR signaling has not been administered (to the individual) for a given amount of time. In some aspects, said amount 15 of time is about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 12 hours, 16 hours, 20 hours, 24 hours, 36 hours, 48 hours, 72 hours, or 96 hours. In some aspects, said reduction is sustained after a subsequent administration of the T cell engaging agent. Particularly, said reduction is sustained even after administration of the inhibitor of JAK and/or mTOR signaling is stopped / no further administration of the inhibitor of JAK and/or mTOR signaling is 20 made. Said reduction of the serum level/cytokine secretion is in particular as compared to the serum level/cytokine secretion in an individual (including the same individual) without administration of the inhibitor of JAK and/or mTOR signaling (i.e. in such case the serum level/cytokine secretion is reduced as compared to the serum level/cytokine secretion without/before administration of the inhibitor of JAK and/or mTOR signaling). Said reduction of 25 the serum level/cytokine secretion is in particular as compared to the serum level/cytokine secretion in an individual (including the same individual) with administration (in particular first administration) of the T cell engaging agent but without administration of the inhibitor of JAK and/or mTOR signaling (i.e. in such case the serum level/cytokine secretion is reduced as compared to the serum level/cytokine secretion with/after administration of the T cell engaging 30 agent but without/before administration of the inhibitor of JAK and/or mTOR signaling). Without said reduction, the serum level/cytokine secretion particularly may be elevated/increased in relation to the (administration of) the T cell engaging agent. In some aspects, said reduction is

clinically meaningful and/or statistically significant. In some aspects, said reduction is at least 30%, at least 40%, at least 50%, at least 60%, or at least 70%. In some aspects, said reduction is at least 30%. In some aspects, said reduction is at least 40%. In some aspects, said reduction is at least 50%. In some aspects, said reduction is at least 60%. In some aspects, said reduction is at least 5 70%.

In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of an adverse effect related to the administration of the T cell engaging agent. In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling does not cause inhibition of a desired effect related to the administration of the T cell engaging agent. In some aspects, 10 (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of an adverse effect related to the administration of the T cell engaging agent but does not cause inhibition of a desired effect related to the administration of the T cell engaging agent. In some of these aspects, said inhibition is a complete inhibition. In some of these aspects, said inhibition is clinically meaningful and/or statistically significant.

15 In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of an adverse effect related to the administration of the T cell engaging agent and inhibition of a desired effect related to the administration of the T cell engaging agent, wherein said inhibition of the adverse effect is stronger than said inhibition of the desired effect. In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of an adverse 20 effect related to the administration of the T cell engaging agent and inhibition of a desired effect related to the administration of the T cell engaging agent, wherein said inhibition of the adverse effect is a complete inhibition and said inhibition of the beneficial effect is a partial inhibition. In some aspects, (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of an adverse effect related to the administration of the T cell engaging agent and inhibition of a 25 desired effect related to the administration of the T cell engaging agent, wherein said inhibition of the adverse effect is a clinically meaningful and/or statistically significant inhibition and said inhibition of the beneficial effect is not a clinically meaningful and/or statistically significant inhibition.

A “desired effect” is a beneficial and desired effect resulting from medication in the treatment of 30 an individual, herein particularly with a T cell engaging agent, i.e. a therapeutic or prophylactic effect, such as e.g. killing of tumor cells, reduction or retardation of tumor growth, reduction of

tumor volume, reduction or prevention of tumor metastasis, increase of progression-free or overall survival, alleviation of disease symptoms, and the like.

An "adverse effect", which is sometimes also denoted as "side effect" or "adverse event" (especially in clinical studies) is a harmful and undesired effect resulting from medication in the treatment of an individual, herein particularly with a T cell engaging agent.

According to the invention, the adverse effect is related to the administration of the T cell engaging agent. In some aspects, the adverse effect is related to the first administration of the T cell engaging agent. In some aspects, the adverse effect occurs upon the first administration of the T cell engaging agent. In some aspects, the adverse effect occurs predominantly or only upon the first administration of the T cell engaging agent. In some aspects, the adverse effect occurs within 12 hours, 24 hours, 36 hours, 48 hours, 72 hours or 96 hours of the administration, particularly the first administration, of the T cell engaging agent. In some aspects, in particular wherein only a single administration of the T cell engaging is made (in the course of the treatment with the T cell engaging agent), the adverse effect occurs within 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 14 days or 21 days of the administration of the T cell engaging agent.

In some aspects, said adverse effect is cytokine release syndrome (CRS).

“Cytokine release syndrome” (abbreviated as “CRS”) refers to an increase in the levels of cytokines, such tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin-6 (IL-6), interleukin-10 (IL-10) and others, in the blood of a subject during or shortly after (e.g. within 1 day of) administration of a therapeutic agent (e.g. a T cell engaging agent), resulting in adverse symptoms. CRS is an adverse reaction to therapeutic agent and timely related to administration of the therapeutic agent. It typically occurs during or shortly after an administration of the therapeutic agent, i.e. typically within 24 hours after administration (typically infusion), predominantly at the first administration. In some instances, e.g. after the administration of CAR-T cells, CRS can also occur only later, e.g. several days after administration upon expansion of the CAR-T cells. The incidence and severity typically decrease with subsequent administrations. Symptoms may range from symptomatic discomfort to fatal events, and may include fever, chills, dizziness, hypertension, hypotension, hypoxia, dyspnea, restlessness, sweating, flushing, skin rash, tachycardia, tachypnoea, headache, tumour pain, nausea, vomiting and/or organ failure. CRS may be graded according to the Modified Cytokine Release Syndrome Grading System established by Lee et al., Blood (2014) 124: 188-195 or Lee et al., Biol Blood Marrow Transplant (2019) 25(4): 625-638

(each incorporated herein by reference in its entirety). For a review of CRS see e.g. Shimabukuro-Vornhagen et al., Journal for ImmunoTherapy of Cancer (2018) 6:56 (incorporated herein by reference in its entirety).

In some aspects, said adverse effect is fever, hypotension and/or hypoxia.

5 In some aspects, said adverse effect is an elevated serum level of one of more cytokine. Said elevated serum level is in particular as compared to the serum level in a healthy individual, and/or the serum level in an individual (including the same individual) without administration of the T cell engaging agent (i.e. in such case the serum level is elevated as compared to the serum level without administration of the T cell engaging agent). In some aspects, said one or more cytokine 10 is selected from the group consisting of IL-6, IFN-γ, IL-10, TNF-α, GM-CSF, MCP-1 and IL-1β.

In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is upon (clinical) manifestation of the adverse effect (in the individual). Said administration may be, for example, within about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 12 hours, 16 hours, 20 hours or 24 hours after manifestation of the adverse effect (i.e. the occurrence clinical 15 symptoms of the side effect, such as fever). In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is in response to the (clinical) manifestation of the adverse effect (in the individual).

In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is before the administration of the T cell engaging agent. In some aspects, administration of the inhibitor of 20 JAK and/or mTOR signaling is concurrent to the administration of the T cell engaging agent. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is after the administration of the T cell engaging agent. Where administration of the inhibitor of JAK and/or mTOR signaling is before or after the administration of the T cell engaging agent, such administration of the inhibitor of JAK and/or mTOR signaling may be, for example, within about 25 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 12 hours, 16 hours, 20 hours or 24 hours before or after, respectively, the administration of the T cell engaging agent. Administration of the inhibitor of JAK and/or mTOR signaling may be intermittently or continuously. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is oral. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is parenteral, 30 particularly intravenous.

In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at a dose sufficient to cause inhibition of an activity of the T cell engaging agent. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at a dose insufficient to cause inhibition of another activity of the T cell engaging agent. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at a dose sufficient to cause inhibition of a first activity of the T cell engaging agent but insufficient to cause inhibition of a second activity of the T cell engaging agent. In some of these aspects, said inhibition is a complete inhibition.

In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at a dose sufficient to cause inhibition of cytokine secretion by immune cells, particularly T cells (induced by the T cell engaging agent). In some aspects, said cytokine is one or more cytokine selected from the group consisting of IL-6, IFN-γ, IL-10, TNF-α, GM-CSF, MCP-1 and IL-1β. Immune cells may include various immune cell types, such as T cells, macrophages, monocytes, NK cells etc. In some aspects, said T cells are CD8+ T cells or CD4+ cells. In some aspects, said inhibition is a complete inhibition.

In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at a dose insufficient to cause inhibition of the activation of T cells (induced by the T cell engaging agent). In some aspects, said inhibition is a complete inhibition.

In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at a dose insufficient to cause inhibition of the cytotoxic activity of T cells (induced by the T cell engaging agent). In some aspects, said inhibition is a complete inhibition.

In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at a dose sufficient to causes inhibition of cytokine secretion by T cells (induced by the T cell engaging agent) but insufficient to cause inhibition of the activation and/or the cytotoxic activity of T cells (induced by the T cell engaging agent). In some of these aspects, said inhibition is a complete inhibition.

In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at a dose sufficient to cause reduction of the serum level of one of more cytokine in the individual. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at a dose sufficient to cause reduction of the secretion of one of more cytokine by immune cells, particularly T cells, in the individual. In some aspects, said one or more cytokine is selected from the group consisting of

IL-6, IFN-γ, IL-10, TNF-α, GM-CSF, MCP-1 and IL-1β. Immune cells may include various immune cell types, such as T cells, macrophages, monocytes, NK cells etc.

In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at a dose sufficient to cause inhibition of an adverse effect related to the administration of the T cell engaging agent. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at a dose insufficient to cause inhibition of a desired effect related to the administration of the T cell engaging agent. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at a dose sufficient to cause inhibition of an adverse effect related to the administration of the T cell engaging agent but insufficient to cause inhibition of a desired effect related to the administration of the T cell engaging agent. In some of these aspects, said inhibition is a complete inhibition. In some of these aspects, said inhibition is clinically meaningful and/or statistically significant.

In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is at an effective dose.

An "effective amount" or "effective dose" of an agent, e.g. a inhibitor of JAK and/or mTOR signaling or a T cell engaging agent, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

In some aspects, the administration of the inhibitor of JAK and/or mTOR signaling is at a dose equaling a dose strength available for the inhibitor of JAK and/or mTOR signaling. Typically, several dose strengths (i.e. dosage forms such as tablets or capsules with a specific amount of active ingredient) are available for a given inhibitor of JAK and/or mTOR signaling. Dosing the inhibitor of JAK and/or mTOR signaling at such (commercially) available dose strengths will be most convenient. For example, if the inhibitor of JAK and/or mTOR signaling is everolimus, it may preferably be administred at a dose of 2.5 mg, 5 mg, 7.5 mg or 10 mg (administration preferably being oral administration). For example, if the inhibitor of JAK and/or mTOR signaling is sirolimus, it may preferably be administered at a dose of 0.5 mg, 1 mg or 2 mg (administration preferably being oral administration). For example, if the inhibitor of JAK and/or mTOR signaling is ruxolitinib, it may preferably be administred at a dose of 5 mg, 10 mg, 15 mg, 20 mg or 25 mg (administration preferably being oral administration). If the inhibitor of JAK and/or mTOR signaling is temsirolimus, it may be administred for example at a dose of 12.5 mg or 25 mg

(administration preferably being intravenous administration, particularly using a solution of 25mg/ml active ingredient).

In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is daily. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is once daily. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is once daily at a dose as mentioned hereinabove. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is for the period of time during which the adverse effect persists (i.e. administration of the inhibitor of JAK and/or mTOR signaling is from manifestation of the adverse effect until reduction or disappearance of the adverse effect). In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is stopped after the adverse effect is prevented or mitigated. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is stopped after reduction or disappearance of the adverse effect. Said reduction particularly is clinically meaningful and/or statistically significant. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is once, twice, three times, four times, five times, six times, seven times, eight times, nine times or ten times, particularly once, twice, three times, four times, five times, six times, seven times, eight times, nine times or ten times in the course of the treatment of the individual with the T cell engaging agent. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days or 10 days. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is once daily for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days or 10 days. The administration of the inhibitor of JAK and/or mTOR signaling is generally associated with the administration of the T cell engaging agent. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is associated with the first administration of the T cell engaging agent. Said first administration is particularly the first administration of the T cell engaging agent in the course of the treatment of the individual with the T cell engaging agent. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is concurrent with the first administration of the T cell engaging agent. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is prior to the first administration of the T cell engaging agent. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is subsequent to the first administration of the T cell engaging agent. In some aspects, administration of the inhibitor of JAK and/or mTOR signaling is subsequent to the first administration of the T cell engaging agent and prior to a second administration of the T cell engaging agent. Where administration of the inhibitor of JAK and/or mTOR signaling is prior or subsequent to the (first) administration of

the T cell engaging agent, such administration of the inhibitor of JAK and/or mTOR signaling may be, for example, within about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 12 hours, 16 hours, 20 hours, 24 hours, 48 hours or 72 hours before or after, respectively, the administration of the T cell engaging agent.

5 In some aspects, the administration of the T cell engaging agent is for a longer period of time than the administration of the inhibitor of JAK and/or mTOR signaling. In some aspects, the administration of the T cell engaging agent continues after the administration of the inhibitor of JAK and/or mTOR signaling is stopped. In some aspects, the administration of the T cell engaging agent is a single administration or a repeated administration. In the course of the treatment of the 10 individual with the T cell engaging agent, the T cell engaging agent may be administered once or several times. For example, treatment of the individual with the T cell engaging agent may comprise multiple treatment cycles which each comprise one or more administrations of the T cell engaging agent. In some aspects, the administration of the T cell engaging agent comprises a first and a second administration.

15 For use in the present invention, the T cell engaging agent would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to 20 medical practitioners.

In some aspects, the administration of the T cell engaging agent is at an effective dose. For systemic administration, an effective dose can be estimated initially from *in vitro* assays, such as cell culture assays. A dose can then be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture. Such information can be 25 used to more accurately determine useful doses in humans. Initial dosages can also be estimated from *in vivo* data, *e.g.*, animal models, using techniques that are well known in the art. Dosage amount and interval may be adjusted individually to provide plasma levels of the T cell engaging agent which are sufficient to maintain therapeutic effect. Usual patient dosages for administration by injection range from about 0.1 to 50 mg/kg/day, typically from about 0.5 to 1 mg/kg/day. 30 Therapeutically effective plasma levels may be achieved by administering multiple doses each day. Levels in plasma may be measured, for example, by HPLC.

An effective amount of the T cell engaging agent may be administered for prevention or treatment of disease. The appropriate route of administration and dosage of the T cell engaging agent may be determined based on the type of disease to be treated, the type of the T cell engaging agent, the severity and course of the disease, the clinical condition of the individual, the individual's clinical history and response to the treatment, and the discretion of the attending physician. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

The T cell engaging agent and the inhibitor of JAK and/or mTOR signaling can be administered by any suitable route, and may be administered by the same route of administration or by different routes of administration. In some aspects, the administration of the T cell engaging agent is parenteral, particularly intravenous.

In some aspects, the administration of the T cell engaging agent is the first administration of the T cell engaging agent to the individual, particularly the first administration of the T cell engaging agent in the course of the treatment of the individual with the T cell engaging agent.

In some aspects, (administration of) the T cell engaging agent induces (i.e. causes or increases) the activation of T cells. In some aspects, (administration of) the T cell engaging agent induces cytotoxic activity of T cells. In some aspects, (administration of) the T cell engaging agent induces cytokine secretion by T cells. In some aspects, cytokine is one or more cytokine selected from the group consisting of IL-2, IL-6, IFN-γ, IL-10, TNF-α and GM-CSF. In some aspects, said T cells are CD8+ T cells or CD4+ cells.

In some aspects, administration of the T cell engaging agent results in activation of T cells, particularly cytotoxic T cells, particularly at the site of the cancer (e.g. within a solid tumor cancer). Said activation may comprise proliferation of T cells, differentiation of T cells, cytokine secretion by T cells, cytotoxic effector molecule release from T cells, cytotoxic activity of T cells, and expression of activation markers by T cells. In some aspects, the administration of the T cell engaging agent results in an increase of T cell, particularly cytotoxic T cell, numbers at the site of the cancer (e.g. within a solid tumor cancer).

By "T cell engaging agent" is meant an immunotherapeutic agent that exerts its effect through the activity of T cells, particularly cytotoxic T cells. Such activity of T cells may include cellular response(s) of T cells, particularly CD4+ and/or CD8+ T cells, such as proliferation, differentiation, expression of activation markers, cytokine secretion, cytotoxic effector molecule release and/or cytotoxic activity. T cell engaging agents as contemplated herein typically comprise an antigen binding moiety that enables their binding to a target cell antigen on a target cell such as a tumor cell. Such T cell engaging agents exert effects on their target cell, such as lysis of the target cell, through the activity of T cells. Exemplary T cell engaging agents include T cell bispecific antibodies, chimeric antigen receptor (CAR) expressing T cells (CAR-T cells), and T cell receptor (TCR)-based approaches such as ImmTACs ("Immune mobilising monoclonal T-cell receptors Against Cancer"; bispecific fusion proteins of an engineered TCR and an antibody fragment, capable of binding to a T cell and a target cell) or TCR-modified T cells featuring engineered T cell receptors capable of binding to a specific antigenic determinant on a target cell (TCR-T cells).

In particular aspects of the present invention, the T cell engaging agent is a T cell bispecific antibody.

In other aspects, the T cell engaging agent is a CAR-T cell. In some aspects, the CAR-T cell is a universal CAR-T cell. By "universal" CAR-T cell is meant a CAR-T cell that binds to a target cell antigen through an adaptor molecule, such as an antibody, that binds to the target cell antigen. A universal CAR-T cell expresses a CAR comprising an antigen binding moiety that binds to the adaptor molecule, and the adaptor molecule binds to the target cell antigen. Through different adaptor molecules (binding to different target cell antigens), a universal CAR-T cell can bind to different target cell antigens, without the need for expression of a different CAR for each target cell antigen. The adaptor molecule is a molecule that (i) can be bound by the CAR, and (ii) can bind to a target cell antigen, such as, for example, an antibody that binds to the target cell antigen and comprises an Fc region that can be bound by the CAR. In some aspects, the CAR-T cell expresses a CAR comprising an antigen binding moiety that binds to an antibody Fc region, particularly an IgG Fc region, more particularly an IgG_1 Fc region, and particularly a human Fc region. In some aspects, the CAR-T expresses a CAR comprising an antigen binding moiety that binds to an IgG Fc region, particularly a human IgG_1 Fc region, comprising the amino acid substitution P329G (Kabat EU index numbering). In particular such aspects, the antigen binding moiety is a scFv. In other aspects, the CAR-T expresses a CAR comprising an antigen binding moiety that binds to a wild-type Fc region, particularly a wild-type human IgG_1 Fc region. In

particular such aspects, the antigen binding moiety is CD16 or an Fc-binding fragment thereof (for example, the extracellular domain of CD16).

In some aspects, the T cell engaging agent is an ImmTAC. In some aspects, the T cell engaging agent is a TCR-T cell.

5 In the following, the T cell bispecific antibody that may be used in the present invention is described.

By "T cell bispecific antibody" is meant an antibody that is able to bind, including simultaneously bind, to a T cell (typically via an antigenic determinant expressed on the T cell, such as CD3) and to a target cell (typically via an antigenic determinant expressed on the target cell, such as CEA, 10 CD19, CD20 or HLA-A2/MAGE-A4).

In preferred aspects according to the invention, the T cell bispecific antibody is capable of simultaneous binding to the antigenic determinant on the T cell (i.e. a first antigen such as CD3) and the antigenic determinant on the target cell (i.e. a second antigen such as CEA, CD19, CD20 or HLA-A2/MAGE-A4). In some aspects, the T cell bispecific antibody is capable of crosslinking 15 the T cell and the target cell by simultaneous binding to CD3 and a target cell antigen. In even more preferred aspects, such simultaneous binding results in lysis of the target cell, particularly a target cell antigen (e.g. CEA, CD19, CD20 or HLA-A2/MAGE-A4)-expressing tumor cell. In some aspects, such simultaneous binding results in activation of the T cell. In some aspects, such simultaneous binding results in a cellular response of the T cell, selected from the group of: 20 proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, and expression of activation markers. In some aspects, binding of the T cell bispecific antibody to CD3 without simultaneous binding to the target cell antigen does not result in T cell activation. In some aspects, the T cell bispecific antibody is capable of re-directing cytotoxic activity of a T cell to a target cell. In preferred aspects, said re-direction is independent of MHC-25 mediated peptide antigen presentation by the target cell and and/or specificity of the T cell.

The term "bispecific" means that the antibody is able to bind to at least two distinct antigenic determinants. Typically, a bispecific antibody comprises two antigen binding sites, each of which is specific for a different antigenic determinant. In certain aspects, the bispecific antibody is capable of simultaneously binding two antigenic determinants, particularly two antigenic 30 determinants expressed on two distinct cells.

As used herein, the term "antigenic determinant" is synonymous with "antigen" and "epitope", and refers to a site (e.g. a contiguous stretch of amino acids or a conformational configuration made up of different regions of non-contiguous amino acids) on a polypeptide macromolecule to which an antigen binding moiety binds, forming an antigen binding moiety-antigen complex. Useful
5 antigenic determinants can be found, for example, on the surfaces of tumor cells, on the surfaces of virus-infected cells, on the surfaces of other diseased cells, on the surface of immune cells, free in blood serum, and/or in the extracellular matrix (ECM).

As used herein, the term "antigen binding moiety" refers to a polypeptide molecule that binds, including specifically binds, to an antigenic determinant. In some aspects, an antigen binding
10 moiety is able to direct the entity to which it is attached (e.g. a second antigen binding moiety) to a target site, for example to a specific type of tumor cell bearing the antigenic determinant. In further aspects, an antigen binding moiety is able to activate signaling through its target antigen, for example a T cell receptor complex antigen. Antigen binding moieties include antibodies and fragments thereof as further defined herein. Particular antigen binding moieties include an antigen
15 binding domain of an antibody, comprising an antibody heavy chain variable region and an antibody light chain variable region. In certain aspects, the antigen binding moieties may comprise antibody constant regions as further defined herein and known in the art. Useful heavy chain constant regions include any of the five isotypes: α, δ, ε, γ, or μ. Useful light chain constant regions include any of the two isotypes: κ and λ.

20 By "specific binding" is meant that the binding is selective for the antigen and can be discriminated from unwanted or non-specific interactions. The term “bind” or “binding” herein generally refers to “specific binding”. The ability of an antigen binding moiety to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance (SPR) technique
25 (analyzed e.g. on a BIAcore instrument) (Liljeblad et al., Glyco J 17, 323-329 (2000)), and traditional binding assays (Heeley, Endocr Res 28, 217-229 (2002)). In some aspects, the extent of binding of an antigen binding moiety to an unrelated protein is less than about 10% of the binding of the antigen binding moiety to the antigen as measured, e.g., by SPR. In certain aspects, an antigen binding moiety that binds to the antigen, or an antibody comprising that antigen binding
30 moiety, has a dissociation constant (K_D) of $\leq$ 1 μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

"Affinity" refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g., a receptor) and its binding partner (e.g., a ligand). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., an antigen binding moiety and an antigen, or
5 a receptor and its ligand). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D), which is the ratio of dissociation and association rate constants (k_{off} and k_{on}, respectively). Thus, equivalent affinities may comprise different rate constants, as long as the ratio of the rate constants remains the same. Affinity can be measured by well established methods known in the art, including those described herein. A particular method
10 for measuring affinity is Surface Plasmon Resonance (SPR).

"CD3" refers to any native CD3 from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed CD3 as well as any form of CD3 that results from processing in the cell. The term also encompasses naturally
15 occurring variants of CD3, e.g., splice variants or allelic variants. In some aspects, CD3 is human CD3, particularly the epsilon subunit of human CD3 (CD3ε). The amino acid sequence of human CD3ε is shown in UniProt (www.uniprot.org) accession no. P07766 (version 144), or NCBI (www.ncbi.nlm.nih.gov/) RefSeq NP_000724.1. See also SEQ ID NO: 1. The amino acid sequence of cynomolgus [Macaca fascicularis] CD3ε is shown in NCBI GenBank no.
20 BAB71849.1. See also SEQ ID NO: 2.

A "target cell antigen" as used herein refers to an antigenic determinant presented on the surface of a target cell, for example a cell in a tumor such as a cancer cell or a cell of the tumor stroma (in that case a "tumor cell antigen"). Preferably, the target cell antigen is not CD3, and/or is expressed on a different cell than CD3. In some aspects, the target cell antigen is CEA, particularly human
25 CEA. In some aspects, the target cell antigen is CD20, particularly human CD20. In other aspects, the target cell antigen is HLA-A2/MAGE-A4, particularly human HLA-A2/MAGE-A4. In some aspects, the target cell antigen is CD19, particularly human CD19.

As used herein, the terms "first", "second" or "third" with respect to antigen binding moieties etc., are used for convenience of distinguishing when there is more than one of each type of moiety.
30 Use of these terms is not intended to confer a specific order or orientation of the bispecific antibody unless explicitly so stated.

The term “valent” as used herein denotes the presence of a specified number of antigen binding sites in an antibody. As such, the term “monovalent binding to an antigen” denotes the presence of one (and not more than one) antigen binding site specific for the antigen in the antibody.

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

The terms “full length antibody,” “intact antibody,” and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab’-SH, $F(ab')_2$, diabodies, linear antibodies, single-chain antibody molecules (e.g. scFv), and single-domain antibodies. For a review of certain antibody fragments, see Hudson et al., Nat Med 9, 129-134 (2003). For a review of scFv fragments, see e.g. Plückthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased *in vivo* half-life, see U.S. Patent No. 5,869,046. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat Med 9, 129-134 (2003); and Hollinger et al., Proc Natl Acad Sci USA 90, 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat Med 9, 129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain aspects, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see e.g. U.S. Patent No. 6,248,516 B1). Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. E. coli or phage), as described herein.

The term “variable region” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain

and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, e.g., Kindt et al., Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity. As used herein in connection with variable region sequences, "Kabat numbering" refers to the numbering system set forth by Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).

As used herein, the amino acid positions of all constant regions and domains of the heavy and light chain are numbered according to the Kabat numbering system described in Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991), referred to as “numbering according to Kabat” or “Kabat numbering” herein. Specifically the Kabat numbering system (see pages 647-660 of Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)) is used for the light chain constant domain CL of kappa and lambda isotype and the Kabat EU index numbering system (see pages 661-723) is used for the heavy chain constant domains (CH1, Hinge, CH2 and CH3), which is herein further clarified by referring to “numbering according to Kabat EU index” in this case.

The term “hypervariable region” or “HVR”, as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and which determine antigen binding specificity, for example “complementarity determining regions” (“CDRs”). Generally, antibodies comprise six CDRs; three in the VH (HCDR1, HCDR2, HCDR3), and three in the VL (LCDR1, LCDR2, LCDR3). Exemplary CDRs herein include:

(a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));

(b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991)); and

(c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)).

Unless otherwise indicated, the CDRs are determined according to Kabat et al., *supra*. One of skill in the art will understand that the CDR designations can also be determined according to Chothia, *supra*, McCallum, *supra*, or any other scientifically accepted nomenclature system.

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR)
5 residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following order in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The “class” of an antibody or immunoglobulin refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG,
10 and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

A “Fab molecule” refers to a protein consisting of the VH and CH1 domain of the heavy chain (the “Fab heavy chain”) and the VL and CL domain of the light chain (the “Fab light chain”) of
15 an immunoglobulin.

By a “crossover” Fab molecule (also termed “Crossfab”) is meant a Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged (i.e. replaced by each other), i.e. the crossover Fab molecule comprises a peptide chain composed of the light chain variable domain VL and the heavy chain constant domain 1 CH1 (VL-CH1, in N-
20 to C-terminal direction), and a peptide chain composed of the heavy chain variable domain VH and the light chain constant domain CL (VH-CL, in N- to C-terminal direction). For clarity, in a crossover Fab molecule wherein the variable domains of the Fab light chain and the Fab heavy chain are exchanged, the peptide chain comprising the heavy chain constant domain 1 CH1 is referred to herein as the “heavy chain” of the (crossover) Fab molecule. Conversely, in a crossover
25 Fab molecule wherein the constant domains of the Fab light chain and the Fab heavy chain are exchanged, the peptide chain comprising the heavy chain variable domain VH is referred to herein as the “heavy chain” of the (crossover) Fab molecule.

In contrast thereto, by a “conventional” Fab molecule is meant a Fab molecule in its natural format, i.e. comprising a heavy chain composed of the heavy chain variable and constant domains (VH-

CH1, in N- to C-terminal direction), and a light chain composed of the light chain variable and constant domains (VL-CL, in N- to C-terminal direction).

The term “immunoglobulin molecule” refers to a protein having the structure of a naturally occurring antibody. For example, immunoglobulins of the IgG class are heterotetrameric 5 glycoproteins of about 150,000 daltons, composed of two light chains and two heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable domain (VH), also called a variable heavy domain or a heavy chain variable region, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable domain (VL), also called a variable light domain or a 10 light chain variable region, followed by a constant light (CL) domain, also called a light chain constant region. The heavy chain of an immunoglobulin may be assigned to one of five types, called α (IgA), δ (IgD), ε (IgE), γ (IgG), or μ (IgM), some of which may be further divided into subtypes, e.g. γ_1 (IgG_1), γ_2 (IgG_2), γ_3 (IgG_3), γ_4 (IgG_4), α_1 (IgA_1) and α_2 (IgA_2). The light chain of an immunoglobulin may be assigned to one of two types, called kappa (κ) and lambda (λ), based 15 on the amino acid sequence of its constant domain. An immunoglobulin essentially consists of two Fab molecules and an Fc domain, linked via the immunoglobulin hinge region.

The term “Fc domain” or “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc 20 region of an IgG heavy chain might vary slightly, the human IgG heavy chain Fc region is usually defined to extend from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, antibodies produced by host cells may undergo post-translational cleavage of one or more, particularly one or two, amino acids from the C-terminus of the heavy chain. Therefore an antibody produced by a host cell by expression of a specific nucleic acid molecule encoding a full-25 length heavy chain may include the full-length heavy chain, or it may include a cleaved variant of the full-length heavy chain. This may be the case where the final two C-terminal amino acids of the heavy chain are glycine (G446) and lysine (K447, numbering according to Kabat EU index). Therefore, the C-terminal lysine (Lys447), or the C-terminal glycine (Gly446) and lysine (K447), of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino 30 acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991 (see also above).

A “subunit” of an Fc domain as used herein refers to one of the two polypeptides forming the dimeric Fc domain, i.e. a polypeptide comprising C-terminal constant regions of an immunoglobulin heavy chain, capable of stable self-association. For example, a subunit of an IgG Fc domain comprises an IgG CH2 and an IgG CH3 constant domain.

5 A “modification promoting the association of the first and the second subunit of the Fc domain” is a manipulation of the peptide backbone or the post-translational modifications of an Fc domain subunit that reduces or prevents the association of a polypeptide comprising the Fc domain subunit with an identical polypeptide to form a homodimer. A modification promoting association as used herein particularly includes separate modifications made to each of the two Fc domain subunits 10 desired to associate (i.e. the first and the second subunit of the Fc domain), wherein the modifications are complementary to each other so as to promote association of the two Fc domain subunits. For example, a modification promoting association may alter the structure or charge of one or both of the Fc domain subunits so as to make their association sterically or electrostatically favorable, respectively. Thus, (hetero)dimerization occurs between a polypeptide comprising the 15 first Fc domain subunit and a polypeptide comprising the second Fc domain subunit, which might be non-identical in the sense that further components fused to each of the subunits (e.g. antigen binding moieties) are not the same. In some aspects the modification promoting association comprises an amino acid mutation in the Fc domain, specifically an amino acid substitution. In particular aspects, the modification promoting association comprises a separate amino acid 20 mutation, specifically an amino acid substitution, in each of the two subunits of the Fc domain.

The term “effector functions” refers to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular 25 phagocytosis (ADCP), cytokine secretion, immune complex-mediated antigen uptake by antigen presenting cells, down regulation of cell surface receptors (e.g. B cell receptor), and B cell activation.

“Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with 30 the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes

of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, Clustal W, Megalign (DNASTAR) software or the FASTA program package. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the ggsearch program of the FASTA package version 36.3.8c or later with a BLOSUM50 comparison matrix. The FASTA program package was authored by W. R. Pearson and D. J. Lipman (1988), "Improved Tools for Biological Sequence Analysis", PNAS 85:2444-2448; W. R. Pearson (1996) "Effective protein sequence comparison" Meth. Enzymol. 266:227- 258; and Pearson et. al. (1997) Genomics 46:24-36, and is publicly available from http://fasta.bioch.virginia.edu/fasta_www2/fasta_down.shtml. Alternatively, a public server accessible at http://fasta.bioch.virginia.edu/fasta_www2/index.cgi can be used to compare the sequences, using the ggsearch (global protein:protein) program and default options (BLOSUM50; open: -10; ext: -2; Ktup = 2) to ensure a global, rather than local, alignment is performed. Percent amino acid identity is given in the output alignment header.

An "activating Fc receptor" is an Fc receptor that following engagement by an Fc domain of an antibody elicits signaling events that stimulate the receptor-bearing cell to perform effector functions. Human activating Fc receptors include FcγRIIIa (CD16a), FcγRI (CD64), FcγRIIa (CD32), and FcαRI (CD89).

"Reduced binding", for example reduced binding to an Fc receptor, refers to a decrease in affinity for the respective interaction, as measured for example by SPR. For clarity, the term includes also reduction of the affinity to zero (or below the detection limit of the analytic method), i.e. complete abolishment of the interaction. Conversely, "increased binding" refers to an increase in binding affinity for the respective interaction.

By "fused" is meant that the components (e.g. a Fab molecule and an Fc domain subunit) are linked by peptide bonds, either directly or via one or more peptide linkers.

In particular aspects, the T cell bispecific antibody binds to CD3 and a target cell antigen. Accordingly, in some aspects, the T cell bispecific antibody comprises an antigen binding moiety that binds to CD3 and an antigen binding moiety that binds to a target cell antigen.

In some aspects, the first and/or the second antigen binding moiety is a Fab molecule. In some aspects, the first antigen binding moiety is a crossover Fab molecule wherein either the variable or the constant regions of the Fab light chain and the Fab heavy chain are exchanged. In such aspects, the second antigen binding moiety preferably is a conventional Fab molecule.

5 In some aspects wherein the first and the second antigen binding moiety of the T cell bispecific antibody are both Fab molecules, and in one of the antigen binding moieties (particularly the first antigen binding moiety) the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other,

i) in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is 10 substituted by a positively charged amino acid (numbering according to Kabat), and wherein in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 or the amino acid at position 213 is substituted by a negatively charged amino acid (numbering according to Kabat EU index); or

ii) in the constant domain CL of the second antigen binding moiety the amino acid at position 124 15 is substituted by a positively charged amino acid (numbering according to Kabat), and wherein in the constant domain CH1 of the second antigen binding moiety the amino acid at position 147 or the amino acid at position 213 is substituted by a negatively charged amino acid (numbering according to Kabat EU index).

The T cell bispecific antibody does not comprise both modifications mentioned under i) and ii). 20 The constant domains CL and CH1 of the antigen binding moiety having the VH/VL exchange are not replaced by each other (i.e. remain unexchanged).

In more specific aspects,

i) in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to 25 Kabat), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index); or

ii) in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to

Kabat), and in the constant domain CH1 of the second antigen binding moiety the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In some aspects, in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the second antigen binding moiety the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In further aspects, in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the second antigen binding moiety the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In preferred aspects, in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the second antigen binding moiety the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In some aspects, in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) (numbering according to Kabat), and in the constant domain CH1 of the second antigen binding moiety the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In some aspects, in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and in the constant

domain CH1 of the second antigen binding moiety the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In particular aspects, if amino acid substitutions according to the above aspects are made in the
5 constant domain CL and the constant domain CH1 of the second antigen binding moiety, the constant domain CL of the second antigen binding moiety is of kappa isotype.

In some aspects, the first and the second antigen binding moiety are fused to each other, optionally via a peptide linker.

In some aspects, the first and the second antigen binding moiety are each a Fab molecule and either
10 (i) the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety, or (ii) the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety.

In some aspects, the T cell bispecific antibody provides monovalent binding to CD3.

15 In particular aspects, the T cell bispecific antibody comprises a single antigen binding moiety that binds to CD3, and two antigen binding moieties that bind to the target cell antigen. Thus, in some aspects, the T cell bispecific antibody comprises a third antigen binding moiety, particularly a Fab molecule, more particularly a conventional Fab molecule, that binds to the target antigen. The third antigen binding moiety may incorporate, singly or in combination, all of the features described
20 herein in relation to the second antigen binding moiety (e.g. the CDR sequences, variable region sequences, and/or amino acid substitutions in the constant regions). In some aspects, the third antigen moiety is identical to the first antigen binding moiety (e.g. is also a conventional Fab molecule and comprises the same amino acid sequences).

In particular aspects, the T cell bispecific antibody further comprises an Fc domain composed of
25 a first and a second subunit. In some aspects, the Fc domain is an IgG Fc domain. In particular aspects, the Fc domain is an IgG_1 Fc domain. In other aspects, the Fc domain is an IgG_4 Fc domain. In more specific aspects, the Fc domain is an IgG_4 Fc domain comprising an amino acid substitution at position S228 (Kabat EU index numbering), particularly the amino acid substitution S228P. This amino acid substitution reduces *in vivo* Fab arm exchange of IgG_4 antibodies (see
30 Stubenrauch et al., Drug Metabolism and Disposition 38, 84-91 (2010)). In further particular

aspects, the Fc domain is a human Fc domain. In particularly preferred aspects, the Fc domain is a human IgG_1 Fc domain. An exemplary sequence of a human IgG_1 Fc region is given in SEQ ID NO: 3.

In some aspects wherein the first, the second and, where present, the third antigen binding moiety
5 are each a Fab molecule, (a) either (i) the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, or (ii) the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second
10 antigen binding moiety and the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain; and (b) the third antigen binding moiety, where present, is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

In some aspects, the T cell bispecific antibody essentially consists of the first, the second and the
15 third antigen binding moiety (particularly Fab molecule), the Fc domain composed of a first and a second subunit, and optionally one or more peptide linkers.

The components of the T cell bispecific antibody may be fused to each other directly or, preferably, via one or more suitable peptide linkers. Where fusion of a Fab molecule is to the N-terminus of a subunit of the Fc domain, it is typically via an immunoglobulin hinge region.

20 The antigen binding moieties may be fused to the Fc domain or to each other directly or through a peptide linker, comprising one or more amino acids, typically about 2-20 amino acids. Peptide linkers are known in the art and are described herein. Suitable, non-immunogenic peptide linkers include, for example, $(G_4S)_n$, $(SG_4)_n$, $(G_4S)_n$, $G_4(SG_4)_n$ or $(G_4S)_nG_5$ peptide linkers. “n” is generally an integer from 1 to 10, typically from 2 to 4. In some aspects, said peptide linker has a length of
25 at least 5 amino acids, in some aspects a length of 5 to 100, in further aspects of 10 to 50 amino acids. In some aspects said peptide linker is $(GxS)_n$ or $(GxS)_nG_m$ with G=glycine, S=serine, and (x=3, n= 3, 4, 5 or 6, and m=0, 1, 2 or 3) or (x=4, n=1, 2, 3, 4 or 5 and m= 0, 1, 2, 3, 4 or 5), in some aspects x=4 and n=2 or 3, in further aspects x=4 and n=2, in yet further aspects x=4, n=1 and m=5. In some aspects, said peptide linker is $(G_4S)_2$. In other aspects, said peptide linker is G_4SG_5.
30 Additionally, linkers may comprise (a portion of) an immunoglobulin hinge region. Particularly

where a Fab molecule is fused to the N-terminus of an Fc domain subunit, it may be fused via an immunoglobulin hinge region or a portion thereof, with or without an additional peptide linker.

In particular aspects, the Fc domain comprises a modification promoting the association of the first and the second subunit of the Fc domain. The site of most extensive protein-protein interaction between the two subunits of a human IgG Fc domain is in the CH3 domain. Thus, in some aspects, said modification is in the CH3 domain of the Fc domain.

In specific aspects, said modification promoting the association of the first and the second subunit of the Fc domain is a so-called “knob-into-hole” modification, comprising a “knob” modification in one of the two subunits of the Fc domain and a “hole” modification in the other one of the two subunits of the Fc domain. The knob-into-hole technology is described e.g. in US 5,731,168; US 7,695,936; Ridgway et al., Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001). Generally, the method involves introducing a protuberance (“knob”) at the interface of a first polypeptide and a corresponding cavity (“hole”) in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine).

Accordingly, in some aspects, an amino acid residue in the CH3 domain of the first subunit of the Fc domain is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and an amino acid residue in the CH3 domain of the second subunit of the Fc domain is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable. Preferably said amino acid residue having a larger side chain volume is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y), and tryptophan (W). Preferably said amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine (S), threonine (T), and valine (V). The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, e.g. by site-specific mutagenesis, or by peptide synthesis.

In specific such aspects, in the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V) and optionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numbering according to Kabat EU index). In further aspects, in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C) (particularly the serine residue at position 354 is replaced with a cysteine residue), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numbering according to Kabat EU index). In preferred aspects, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W, and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S, L368A and Y407V (numbering according to Kabat EU index).

In some aspects, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

In particular aspects, the Fc receptor is an Fcγ receptor. In some aspects, the Fc receptor is a human Fc receptor. In some aspects, the Fc receptor is an activating Fc receptor. In specific aspects, the Fc receptor is an activating human Fcγ receptor, more specifically human FcγRIIIa, FcγRI or FcγRIIa, most specifically human FcγRIIIa. In some aspects, the effector function is one or more selected from the group of complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and cytokine secretion. In particular aspects, the effector function is ADCC.

Typically, the same one or more amino acid substitution is present in each of the two subunits of the Fc domain. In some aspects, the one or more amino acid substitution reduces the binding affinity of the Fc domain to an Fc receptor. In some aspects, the one or more amino acid substitution reduces the binding affinity of the Fc domain to an Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold.

In some aspects, the Fc domain comprises an amino acid substitution at a position selected from the group of E233, L234, L235, N297, P331 and P329 (numberings according to Kabat EU index). In more specific aspects, the Fc domain comprises an amino acid substitution at a position selected

from the group of L234, L235 and P329 (numberings according to Kabat EU index). In some aspects, the Fc domain comprises the amino acid substitutions L234A and L235A (numberings according to Kabat EU index). In some such aspects, the Fc domain is an IgG_1 Fc domain, particularly a human IgG_1 Fc domain. In some aspects, the Fc domain comprises an amino acid
5 substitution at position P329. In more specific aspects, the amino acid substitution is P329A or P329G, particularly P329G (numberings according to Kabat EU index). In some aspects, the Fc domain comprises an amino acid substitution at position P329 and a further amino acid substitution at a position selected from E233, L234, L235, N297 and P331 (numberings according to Kabat EU index). In more specific aspects, the further amino acid substitution is E233P, L234A, L235A,
10 L235E, N297A, N297D or P331S. In particular aspects, the Fc domain comprises amino acid substitutions at positions P329, L234 and L235 (numberings according to Kabat EU index). In more particular aspects, the Fc domain comprises the amino acid mutations L234A, L235A and P329G (“P329G LALA”, “PGLALA” or “LALAPG”). Specifically, in preferred aspects, each subunit of the Fc domain comprises the amino acid substitutions L234A, L235A and P329G
15 (Kabat EU index numbering), i.e. in each of the first and the second subunit of the Fc domain the leucine residue at position 234 is replaced with an alanine residue (L234A), the leucine residue at position 235 is replaced with an alanine residue (L235A) and the proline residue at position 329 is replaced by a glycine residue (P329G) (numbering according to Kabat EU index). In some such aspects, the Fc domain is an IgG_1 Fc domain, particularly a human IgG_1 Fc domain.

20 In some aspects, the target cell antigen of the T cell bispecific antibody is carcinoembryonic antigen (CEA).

“Carcinoembryonic antigen” or “CEA” (also known as Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5)) refers to any native CEA from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and
25 rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed CEA as well as any form of CEA that results from processing in the cell. The term also encompasses naturally occurring variants of CEA, e.g., splice variants or allelic variants. In some aspects, CEA is human CEA. The amino acid sequence of human CEA is shown in UniProt (www.uniprot.org) accession no. P06731, or NCBI (www.ncbi.nlm.nih.gov/) RefSeq
30 NP_004354.2. In some aspects, CEA is cell membrane-bound CEA. In some aspects, CEA is CEA expressed on the surface of a cell, e.g. a cancer cell.

Useful T cell bispecific antibodies for the present invention that bind to CEA are described e.g. in PCT publication no. WO 2014/131712 (incorporated herein by reference in its entirety).

Is some aspects, the T cell bispecific antibody comprises a first antigen binding moiety that binds to CD3, and a second antigen binding moiety that binds to CEA.

5 In some aspects, the first antigen binding moiety comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 4, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 6; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9.

In some aspects, the second antigen binding moiety comprises a heavy chain variable region 10 comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 12, the HCDR2 of SEQ ID NO: 13, and the HCDR3 of SEQ ID NO: 14; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 15, the LCDR2 of SEQ ID NO: 16 and the LCDR3 of SEQ ID NO: 17.

In some aspects, the T cell bispecific antibody comprises

15 (i) a first antigen binding moiety that binds to CD3 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 4, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 6; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9; and

(ii) a second antigen binding moiety that binds to CEA and comprises a heavy chain variable region 20 comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 12, the HCDR2 of SEQ ID NO: 13, and the HCDR3 of SEQ ID NO: 14; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 15, the LCDR2 of SEQ ID NO: 16 and the LCDR3 of SEQ ID NO: 17.

In some aspects, the first antigen binding moiety comprises a heavy chain variable region sequence 25 that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 10 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 11. In some aspects, the first antigen binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 10 and the light chain variable region sequence of SEQ ID NO: 11.

In some aspects, the second antigen binding moiety comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 18 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 19. In some 5 aspects, the second antigen binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 18 and the light chain variable region sequence of SEQ ID NO: 19.

In some aspects, the T cell bispecific antibody comprises a third antigen binding moiety that binds to CEA and/or an Fc domain composed of a first and a second subunit, as described herein.

In preferred aspects, the T cell bispecific antibody comprises
10 (i) a first antigen binding moiety that binds to CD3, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 4, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 6; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9, wherein the first antigen binding moiety is a crossover Fab molecule wherein either the variable 15 or the constant regions, particularly the constant regions, of the Fab light chain and the Fab heavy chain are exchanged;
(ii) a second and a third antigen binding moiety that bind to CEA, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 12, the HCDR2 of SEQ ID NO: 13, and the HCDR3 of SEQ ID NO: 14; and a light chain variable region comprising 20 the light chain CDR (LCDR) 1 of SEQ ID NO: 15, the LCDR2 of SEQ ID NO: 16 and the LCDR3 of SEQ ID NO: 17, wherein the second and third antigen binding moiety are each a Fab molecule, particularly a conventional Fab molecule;
(iii) an Fc domain composed of a first and a second subunit,
wherein the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to 25 the N-terminus of the Fab heavy chain of the first antigen binding moiety, and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

In some aspects, the first antigen binding moiety of the T cell bispecific antibody (that binds to 30 CEA and CD3) comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 10 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the

amino acid sequence of SEQ ID NO: 11. In some aspects, the first antigen binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 10 and the light chain variable region sequence of SEQ ID NO: 11.

In some aspects, the second and (where present) third antigen binding moiety of the T cell bispecific antibody (that binds to CEA and CD3) comprise a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 18 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 19. In some aspects, the second and (where present) third antigen binding moiety comprise the heavy chain variable region of SEQ ID NO: 18 and the light chain variable region of SEQ ID NO: 19.

The Fc domain according to the above aspects may incorporate, singly or in combination, all of the features described hereinabove in relation to Fc domains.

In some aspects, the Fc domain of the T cell bispecific antibody (that binds to CEA and CD3) comprises a modification promoting the association of the first and the second subunit of the Fc domain, and/or the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

In some aspects, the antigen binding moieties and the Fc region are fused to each other by peptide linkers, particularly by peptide linkers as in SEQ ID NO: 21 and SEQ ID NO: 23.

In some aspects, the T cell bispecific antibody (that binds to CEA and CD3) comprises a polypeptide (particularly two polypeptides) comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 20, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 21, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 22, and a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 23. In some aspects, the T cell bispecific antibody (that binds to CEA and CD3) comprises a polypeptide (particularly two polypeptides) comprising the sequence of SEQ ID NO: 20, a polypeptide comprising the sequence of SEQ ID NO: 21, a polypeptide comprising the sequence of SEQ ID NO: 22, and a polypeptide comprising the sequence of SEQ ID NO: 23.

In preferred aspects, the T cell bispecific antibody is cibisatamab (WHO Drug Information (International Nonproprietary Names for Pharmaceutical Substances), Recommended INN: List 80, 2018, vol. 32, no. 3, p. 438).

In some aspects, the target cell antigen of the T cell bispecific antibody is CD20.

5 "CD20", also known as "B-lymphocyte antigen B1", refers to any native CD20 from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed CD20 as well as any form of CD20 that results from processing in the cell. The term also encompasses naturally occurring variants of CD20, e.g., splice
10 variants or allelic variants. In some aspects, CD20 is human CD20. Human CD20 is described in UniProt (www.uniprot.org) accession no. P11836 (entry version 200), and an amino acid sequence of human CD20 is also shown in SEQ ID NO: 36.

Useful T cell bispecific antibodies for the present invention that bind to CD20 are described e.g. in PCT publication no. WO 2016/020309 (incorporated herein by reference in its entirety).

15 In some aspects, the T cell bispecific antibody comprises a first antigen binding moiety that binds to CD3, and a second antigen binding moiety that binds to CD20.

In some aspects, the first antigen binding moiety comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 4, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 6; and a light chain variable region comprising the light chain CDR
20 (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9.

In some aspects, the second antigen binding moiety comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 24, the HCDR2 of SEQ ID NO: 25, and the HCDR3 of SEQ ID NO: 26; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 27, the LCDR2 of SEQ ID NO: 28 and the LCDR3 of SEQ ID
25 NO: 29.

In some aspects, the T cell bispecific antibody comprises
(i) a first antigen binding moiety that binds to CD3 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 4, the HCDR2 of SEQ ID NO: 5, and

the HCDR3 of SEQ ID NO: 6; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9; and (ii) a second antigen binding moiety that binds to CD20 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 24, the HCDR2 of SEQ ID
5 NO: 25, and the HCDR3 of SEQ ID NO: 26; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 27, the LCDR2 of SEQ ID NO: 28 and the LCDR3 of SEQ ID NO: 29.

In some aspects, the first antigen binding moiety comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of
10 SEQ ID NO: 10 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 11. In some aspects, the first antigen binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 10 and the light chain variable region sequence of SEQ ID NO: 11.

In some aspects, the second antigen binding moiety comprises a heavy chain variable region
15 sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 30 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 31. In some aspects, the second antigen binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 30 and the light chain variable region sequence of SEQ ID NO: 31.

20 In some aspects, the T cell bispecific antibody comprises a third antigen binding moiety that binds to CD20 and/or an Fc domain composed of a first and a second subunit, as described herein.

In preferred aspects, the T cell bispecific antibody comprises
(i) a first antigen binding moiety that binds to CD3, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 4, the HCDR2 of SEQ ID NO: 5, and
25 the HCDR3 of SEQ ID NO: 6; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9, wherein the first antigen binding moiety is a crossover Fab molecule wherein either the variable or the constant regions, particularly the variable regions, of the Fab light chain and the Fab heavy chain are exchanged;
30 (ii) a second and a third antigen binding moiety that bind to CD20, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 24, the HCDR2 of

SEQ ID NO: 25, and the HCDR3 of SEQ ID NO: 26; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 27, the LCDR2 of SEQ ID NO: 28 and the LCDR3 of SEQ ID NO: 29, wherein the second and third antigen binding moiety are each a Fab molecule, particularly a conventional Fab molecule;

5 (iii) an Fc domain composed of a first and a second subunit,

wherein the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety, and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third antigen binding moiety is fused at the C-terminus 10 of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

In some aspects, the first antigen binding moiety of the T cell bispecific antibody (that binds to CD20 and CD3) is a crossover Fab molecule wherein the variable regions of the Fab light chain and the Fab heavy chain are exchanged, and wherein the second and (where present) third antigen binding moiety of the T cell bispecific antibody is a conventional Fab molecule wherein in the 15 constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and in the constant domain CH1 the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino 20 acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

Particularly, in the above aspects, in the constant domain CL of the second and the third Fab molecule under (ii) the amino acid at position 124 may be substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 may be substituted by lysine (K) or arginine 25 (R), particularly by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the second and the third Fab molecule under (ii) the amino acid at position 147 may be substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 may be substituted by glutamic acid (E) (numbering according to Kabat EU index).

In some aspects, the first antigen binding moiety of the T cell bispecific antibody (that binds to 30 CD20 and CD3) comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 10 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the

amino acid sequence of SEQ ID NO: 11. In some aspects, the first antigen binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 10 and the light chain variable region sequence of SEQ ID NO: 11.

In some aspects, the second and (where present) third antigen binding moiety of the T cell bispecific antibody (that binds to CD20 and CD3) comprise a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 30 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 31. In some aspects, the second and (where present) third antigen binding moiety comprise the heavy chain variable region of SEQ ID NO: 30 and the light chain variable region of SEQ ID NO: 31.

The Fc domain according to the above aspects may incorporate, singly or in combination, all of the features described hereinabove in relation to Fc domains.

In some aspects, the Fc domain of the T cell bispecific antibody (that binds to CD20 and CD3) comprises a modification promoting the association of the first and the second subunit of the Fc domain, and/or the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

In some aspects, the antigen binding moieties and the Fc region are fused to each other by peptide linkers, particularly by peptide linkers as in SEQ ID NO: 33 and SEQ ID NO: 35.

In some aspects, the T cell bispecific antibody (that binds to CD20 and CD3) comprises a polypeptide (particularly two polypeptides) comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 32, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 33, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 34, and a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 35. In some aspects, the T cell bispecific antibody (that binds to CD20 and CD3) comprises a polypeptide (particularly two polypeptides) comprising the sequence of SEQ ID NO: 32, a polypeptide comprising the sequence of SEQ ID NO: 33, a polypeptide comprising the sequence of SEQ ID NO: 34, and a polypeptide comprising the sequence of SEQ ID NO: 35.

In preferred aspects, the T cell bispecific antibody is glofitamab (WHO Drug Information (International Nonproprietary Names for Pharmaceutical Substances), Recommended INN: List 83, 2020, vol. 34, no. 1, p. 39).

In some aspects, the target cell antigen of the T cell bispecific antibody is HLA-A2/MAGE-A4.

5 "MAGE-A4" stands for "Melanoma-associated antigen 4", which is a member of the MAGE family of Cancer Testis Antigens (CTAs). The MAGE-A family of proteins encompasses 12 highly homologous genes clustered at Xq26-28 and characterized by the presence of a conserved domain (MAGE Homology Domain, MHD). Human MAGE-A4 is described in UniProt (www.uniprot.org) accession no. P43358 (entry version 163), and an amino acid sequence of 10 human MAGE-A4 is also shown in SEQ ID NO: 57 herein. "MAGE-A4" as used herein, refers to any native MAGE-A4 from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed MAGE-A4 as well as any form of MAGE-A4 that results from processing in the cell. The term also encompasses naturally 15 occurring variants of MAGE-A4, e.g., splice variants or allelic variants. In one aspect, MAGE-A4 is human MAGE-A4, particularly the protein of SEQ ID NO: 57.

By "MAGE-A4 $_{p230-239}$" or "p230-239 peptide" is meant the MAGE-A4 derived peptide having the amino acid sequence GVYDGREHTV (SEQ ID NO: 58; position 230-239 of the MAGE-A4 protein of SEQ ID NO: 57).

20 "HLA-A2", "HLA-A*02", "HLA-A02", or "HLA-A*2" (used interchangeably) refers to a human leukocyte antigen serotype in the HLA-A serotype group. The HLA-A2 protein (encoded by the respective HLA gene) constitutes the α chain of the respective class I MHC (major histocompatibility complex) protein, which further comprises a β2 microglobulin subunit. A specific HLA-A2 protein is HLA-A201 (also referred to as HLA-A0201, HLA-A02.01, or HLA-25 A*02:01). In specific aspects, the HLA-A2 protein described herein is HLA-A201. An exemplary sequence of human HLA-A2 is given in SEQ ID NO: 59.

"HLA-A2/MAGE-A4" refers to a complex of a HLA-A2 molecule and a MAGE-A4 derived peptide (also referred to herein as a "MAGE-A4 peptide"), specifically the p230-239 peptide ("HLA-A2/MAGE-A4 $_{p230-239}$").

Useful T cell bispecific antibodies for the present invention that bind to HLA-A2/MAGE-A4 are described e.g. in PCT application no. PCT/EP2020/086614 (incorporated herein by reference in its entirety).

In some aspects, the T cell bispecific antibody comprises a first antigen binding moiety that binds to CD3, and a second antigen binding moiety that binds to HLA-A2/MAGE-A4, particularly HLA-A2/MAGE-A4 $_{p230-239}$.

In some aspects, the first antigen binding moiety comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 37, the HCDR2 of SEQ ID NO: 38, and the HCDR3 of SEQ ID NO: 39; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 40, the LCDR2 of SEQ ID NO: 41 and the LCDR3 of SEQ ID NO: 42.

In some aspects, the second antigen binding moiety comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 45, the HCDR2 of SEQ ID NO: 46, and the HCDR3 of SEQ ID NO: 47; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 48, the LCDR2 of SEQ ID NO: 49 and the LCDR3 of SEQ ID NO: 50.

In some aspects, the T cell bispecific antibody comprises

(i) a first antigen binding moiety that binds to CD3 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 37, the HCDR2 of SEQ ID NO: 38, and the HCDR3 of SEQ ID NO: 39; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 40, the LCDR2 of SEQ ID NO: 41 and the LCDR3 of SEQ ID NO: 42; and

(ii) a second antigen binding moiety that binds to HLA-A2/MAGE-A4 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 45, the HCDR2 of SEQ ID NO: 46, and the HCDR3 of SEQ ID NO: 47; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 48, the LCDR2 of SEQ ID NO: 49 and the LCDR3 of SEQ ID NO: 50.

In some aspects, the first antigen binding moiety comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 43 and a light chain variable region sequence that is at least about 95%, 96%, 97%,

98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 44. In some aspects, the first antigen binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 43 and the light chain variable region sequence of SEQ ID NO: 44.

In some aspects, the second antigen binding moiety comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 51 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 52. In some aspects, the second antigen binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 51 and the light chain variable region sequence of SEQ ID NO: 52.

In some aspects, the T cell bispecific antibody comprises a third antigen binding moiety that binds to HLA-A2/MAGE-A4 and/or an Fc domain composed of a first and a second subunit, as described herein.

In preferred aspects, the T cell bispecific antibody comprises
(i) a first antigen binding moiety that binds to CD3, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 37, the HCDR2 of SEQ ID NO: 38, and the HCDR3 of SEQ ID NO: 39; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 40, the LCDR2 of SEQ ID NO: 41 and the LCDR3 of SEQ ID NO: 42, wherein the first antigen binding moiety is a crossover Fab molecule wherein either the variable or the constant regions, particularly the variable regions, of the Fab light chain and the Fab heavy chain are exchanged;
(ii) a second and a third antigen binding moiety that bind to HLA-A2/MAGE-A4, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 45, the HCDR2 of SEQ ID NO: 46, and the HCDR3 of SEQ ID NO: 47; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 48, the LCDR2 of SEQ ID NO: 49 and the LCDR3 of SEQ ID NO: 50, wherein the second and third antigen binding moiety are each a Fab molecule, particularly a conventional Fab molecule;
(iii) an Fc domain composed of a first and a second subunit,
wherein the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety, and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

In some aspects, the first antigen binding moiety of the T cell bispecific antibody (that binds to HLA-A2/MAGE-A4 and CD3) is a crossover Fab molecule wherein the variable regions of the Fab light chain and the Fab heavy chain are exchanged, and wherein the second and (where present) third antigen binding moiety of the T cell bispecific antibody is a conventional Fab molecule wherein in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and in the constant domain CH1 the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

Particularly, in the above aspects, in the constant domain CL of the second and the third Fab molecule under (ii) the amino acid at position 124 may be substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 may be substituted by lysine (K) or arginine (R), particularly by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the second and the third Fab molecule under (ii) the amino acid at position 147 may be substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 may be substituted by glutamic acid (E) (numbering according to Kabat EU index).

In some aspects, the first antigen binding moiety of the T cell bispecific antibody (that binds to HLA-A2/MAGE-A4 and CD3) comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 43 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 44. In some aspects, the first antigen binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 43 and the light chain variable region sequence of SEQ ID NO: 44.

In some aspects, the second and (where present) third antigen binding moiety of the T cell bispecific antibody (that binds to HLA-A2/MAGE-A4 and CD3) comprise a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 51 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 52. In some aspects, the second and (where present) third antigen binding moiety comprise the heavy chain variable region of SEQ ID NO: 51 and the light chain variable region of SEQ ID NO: 52.

The Fc domain according to the above aspects may incorporate, singly or in combination, all of the features described hereinabove in relation to Fc domains.

In some aspects, the Fc domain of the T cell bispecific antibody (that binds to HLA-A2/MAGE-A4 and CD3) comprises a modification promoting the association of the first and the second subunit of the Fc domain, and/or the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

In some aspects, the antigen binding moieties and the Fc region are fused to each other by peptide linkers, particularly by peptide linkers as in SEQ ID NO: 54 and SEQ ID NO: 56.

In some aspects, the T cell bispecific antibody (that binds to HLA-A2/MAGE-A4 and CD3) comprises a polypeptide (particularly two polypeptides) comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 53, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 54, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 55, and a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 56. In some aspects, the T cell bispecific antibody (that binds to HLA-A2/MAGE-A4 and CD3) comprises a polypeptide (particularly two polypeptides) comprising the sequence of SEQ ID NO: 53, a polypeptide comprising the sequence of SEQ ID NO: 54, a polypeptide comprising the sequence of SEQ ID NO: 55, and a polypeptide comprising the sequence of SEQ ID NO: 56.

In some aspects, the target cell antigen of the T cell bispecific antibody is CD19.

"CD19" stands for cluster of differentiation 19 (also known as B-lymphocyte antigen CD19 or B-lymphocyte surface antigen B4) and refers to any native CD19 from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed CD19 as well as any form of CD19 that results from processing in the cell. The term also encompasses naturally occurring variants of CD19, e.g., splice variants or allelic variants. In some aspects, CD19 is human CD19. See for the human protein UniProt (www.uniprot.org) accession no. P15391 (version 211), or NCBI (www.ncbi.nlm.nih.gov/) RefSeq NP_001761.3. An exemplary sequence of human CD19 is given in SEQ ID NO: 60.

Useful T cell bispecific antibodies for the present invention that bind to CD19 are described e.g. in EP application nos. 20181056.1 and 20180968.8 (incorporated herein by reference in their entirety).

In some aspects, the T cell bispecific antibody comprises a first antigen binding moiety that binds to CD3, and a second antigen binding moiety that binds to CD19.

In some aspects, the first antigen binding moiety comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 61, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 62; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9.

In other aspects, the first antigen binding moiety comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 64, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 65; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9.

In some aspects, the second antigen binding moiety comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 67, the HCDR2 of SEQ ID NO: 68, and the HCDR3 of SEQ ID NO: 69; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 70, the LCDR2 of SEQ ID NO: 71 and the LCDR3 of SEQ ID NO: 72.

In some aspects, the T cell bispecific antibody comprises
(i) a first antigen binding moiety that binds to CD3 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 61, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 62, or a heavy chain variable region comprising the HCDR1 of SEQ ID NO: 64, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 65; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9; and
(ii) a second antigen binding moiety that binds to CD19 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 67, the HCDR2 of SEQ ID NO: 68, and the HCDR3 of SEQ ID NO: 69; and a light chain variable region comprising the light

chain CDR (LCDR) 1 of SEQ ID NO: 70, the LCDR2 of SEQ ID NO: 71 and the LCDR3 of SEQ ID NO: 72.

In some aspects, the first antigen binding moiety comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 63 or a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 66, and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 35. In some aspects, the first antigen binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 63 or the heavy chain variable region sequence of SEQ ID NO: 66, and the light chain variable region sequence of SEQ ID NO: 11.

In some aspects, the second antigen binding moiety comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 73 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 74. In some aspects, the second antigen binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 73 and the light chain variable region sequence of SEQ ID NO: 74.

In some aspects, the T cell bispecific antibody comprises a third antigen binding moiety that binds to CD19 and/or an Fc domain composed of a first and a second subunit, as described herein.

In preferred aspects, the T cell bispecific antibody comprises

(i) a first antigen binding moiety that binds to CD3, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 61, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 62, or a heavy chain variable region comprising the HCDR1 of SEQ ID NO: 64, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 65; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9, wherein the first antigen binding moiety is a crossover Fab molecule wherein either the variable or the constant regions, particularly the variable regions, of the Fab light chain and the Fab heavy chain are exchanged;

(ii) a second and a third antigen binding moiety that bind to CD19, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 67, the HCDR2 of SEQ ID NO: 68, and the HCDR3 of SEQ ID NO: 69; and a light chain variable region comprising

the light chain CDR (LCDR) 1 of SEQ ID NO: 70, the LCDR2 of SEQ ID NO: 71 and the LCDR3 of SEQ ID NO: 72, wherein the second and third antigen binding moiety are each a Fab molecule, particularly a conventional Fab molecule;
(iii) an Fc domain composed of a first and a second subunit,
wherein the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety, and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

In some aspects, the first antigen binding moiety of the T cell bispecific antibody (that binds to CD19 and CD3) is a crossover Fab molecule wherein the variable regions of the Fab light chain and the Fab heavy chain are exchanged, and wherein the second and (where present) third antigen binding moiety of the T cell bispecific antibody is a conventional Fab molecule wherein in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and in the constant domain CH1 the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

Particularly, in the above aspects, in the constant domain CL of the second and the third Fab molecule under (ii) the amino acid at position 124 may be substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 may be substituted by lysine (K) or arginine (R), particularly by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the second and the third Fab molecule under (ii) the amino acid at position 147 may be substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 may be substituted by glutamic acid (E) (numbering according to Kabat EU index).

In some aspects, the first antigen binding moiety of the T cell bispecific antibody (that binds to CD19 and CD3) comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 63 or a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 66, and a light chain variable region sequence that is at least

about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 11. In some aspects, the first antigen binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 63 or the heavy chain variable region sequence of SEQ ID NO: 66, and the light chain variable region sequence of SEQ ID NO: 11.

5 In some aspects, the second and (where present) third antigen binding moiety of the T cell bispecific antibody (that binds to CD19 and CD3) comprise a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 73 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 74. In some aspects, the 10 second and (where present) third antigen binding moiety comprise the heavy chain variable region of SEQ ID NO: 73 and the light chain variable region of SEQ ID NO: 74.

The Fc domain according to the above aspects may incorporate, singly or in combination, all of the features described hereinabove in relation to Fc domains.

In some aspects, the Fc domain of the T cell bispecific antibody (that binds to CD19 and CD3) 15 comprises a modification promoting the association of the first and the second subunit of the Fc domain, and/or the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

In some aspects, the antigen binding moieties and the Fc region are fused to each other by peptide linkers, particularly by peptide linkers as in SEQ ID NO: 75, SEQ ID NO: 76 and SEQ ID NO: 77.

20 In some aspects, the T cell bispecific antibody (that binds to CD19 and CD3) comprises a polypeptide (particularly two polypeptides) comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 78, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 75, a polypeptide comprising a sequence that is at least 80%, 85%, 25 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 77, and a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 79. In some aspects, the T cell bispecific antibody (that binds to CD19 and CD3) comprises a polypeptide (particularly two polypeptides) comprising the sequence of SEQ ID NO: 78, a polypeptide comprising the sequence of SEQ ID NO: 75, a polypeptide

comprising the sequence of SEQ ID NO: 77, and a polypeptide comprising the sequence of SEQ ID NO: 79.

In other aspects, the T cell bispecific antibody (that binds to CD19 and CD3) comprises a polypeptide (particularly two polypeptides) comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 78, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 76, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 77, and a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 80. In some aspects, the T cell bispecific antibody (that binds to CD19 and CD3) comprises a polypeptide (particularly two polypeptides) comprising the sequence of SEQ ID NO: 78, a polypeptide comprising the sequence of SEQ ID NO: 76, a polypeptide comprising the sequence of SEQ ID NO: 77, and a polypeptide comprising the sequence of SEQ ID NO: 80.

In some aspects, the disease (to be treated by the T cell engaging agent) is cancer.

As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention in an attempt to alter the natural course of a disease in the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis.

The term “cancer” refers to the physiological condition in mammals that is typically characterized by unregulated cell proliferation. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma and leukemia. More non-limiting examples of cancers include haematological cancer such as leukemia, bladder cancer, brain cancer, head and neck cancer, pancreatic cancer, biliary cancer, thyroid cancer, lung cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer, endometrial cancer, esophageal cancer, colon cancer, colorectal cancer, rectal cancer, gastric cancer, prostate cancer, skin cancer, squamous cell carcinoma, sarcoma, bone cancer, and kidney cancer. Other cell proliferation disorders include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum,

endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic region, and urogenital system. Also included are pre-cancerous conditions or lesions and cancer metastases.

5 In some aspects, the cancer is a cancer expressing the target cell antigen of the T cell engaging agent (e.g. the T cell bispecific antibody).

In some aspects, the cancer is a carcinoembryonic antigen (CEA)-expressing cancer (in particular in aspects, wherein the target cell antigen of the T cell engaging agent, e.g. T cell bispecific antibody, is CEA). By "CEA-positive cancer" or "CEA-expressing cancer" is meant a cancer 10 characterized by expression or overexpression of CEA on cancer cells. The expression of CEA may be determined for example by an immunohistochemistry (IHC) or flow cytometric assay. In some aspects, the cancer expresses CEA. In some aspects, the cancer expresses CEA in at least 20%, preferably at least 50% or at least 80% of tumor cells as determined by immunohistochemistry (IHC) using an antibody specific for CEA.

15 In some aspects, the cancer is colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, prostate cancer, or other cancers described herein.

In particular aspects, the cancer is a cancer selected from the group consisting of colorectal cancer, lung cancer, pancreatic cancer, breast cancer, and gastric cancer. In preferred aspects, the cancer 20 is colorectal cancer (CRC). In some aspects, the colorectal cancer is metastatic colorectal cancer (mCRC). In some aspects, the colorectal cancer is microsatellite-stable (MSS) colorectal cancer. In some aspects, the colorectal cancer is microsatellite-stable metastatic colorectal cancer (MSS mCRC).

In some aspects, the cancer is a CD20-expressing cancer (in particular in aspects, wherein the 25 target cell antigen of the T cell engaging agent, e.g. T cell bispecific antibody, is CD20). By "CD20-positive cancer" or "CD20-expressing cancer" is meant a cancer characterized by expression or overexpression of CD20 in cancer cells. The expression of CD20 may be determined for example by quantitative real-time PCR (measuring CD20 mRNA levels), flow cytometry, immunohistochemistry (IHC) or western blot assays. In some aspects, the cancer expresses CD20. 30 In some aspects, the cancer expresses CD20 in at least 20%, preferably at least 50% or at least

80% of tumor cells as determined by immunohistochemistry (IHC) using an antibody specific for CD20.

In some aspects, the cancer is a B-cell cancer, particularly a CD20-positive B-cell cancer. In some aspects, the cancer is selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), marginal zone lymphoma (MZL), Multiple myeloma (MM) or Hodgkin lymphoma (HL). In particular aspects, the cancer is selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL) and marginal zone lymphoma (MZL). In more particular aspects, the cancer is NHL, particularly relapsed/refractory (r/r) NHL. In some aspects, the cancer is DLBCL. In some aspects, the cancer is FL. In some aspects, the cancer is MCL. In some aspects, the cancer is MZL.

In some aspects, the cancer is a MAGE-A4-expressing cancer (in particular in aspects, wherein the target cell antigen of the T cell engaging agent, e.g. T cell bispecific antibody, is HLA-A2/MAGE-A4). By “MAGE-A4-positive cancer” or “MAGE-A4-expressing cancer” is meant a cancer characterized by expression or overexpression of MAGE-A4 in cancer cells.

In some aspects, the cancer is a cancer selected from the group consisting of lung cancer, head and neck cancer, bladder cancer, esophageal cancer, skin cancer, gastric cancer and ovarian cancer.

In some aspects, the cancer is a CD19-expressing cancer (in particular in aspects, wherein the target cell antigen of the T cell engaging agent, e.g. T cell bispecific antibody, is CD19). By “CD19-positive cancer” or “CD19-expressing cancer” is meant a cancer characterized by expression or overexpression of CD19 in cancer cells. The expression of CD19 may be determined for example by quantitative real-time PCR (measuring CD19 mRNA levels), flow cytometry, immunohistochemistry (IHC) or western blot assays. In some aspects, the cancer expresses CD19. In some aspects, the cancer expresses CD19 in at least 20%, preferably at least 50% or at least 80% of tumor cells as determined by immunohistochemistry (IHC) using an antibody specific for CD19.

In some aspects, the cancer is a B-cell cancer, particularly a CD19-positive B-cell cancer. In some aspects, the cancer is a B-cell lymphoma or a B-cell leukemia. In some aspects, the cancer is non-

Hodgkin lymphoma (NHL), acute lymphoblastic leukemia (ALL) or chronic lymphocytic leukemia (CLL).

In some aspects, the cancer is treatable by the T cell engaging agent. In some aspects, the T cell engaging agent is indicated for the treatment of the cancer.

In some aspects, the cancer is a solid tumor cancer. By a “solid tumor cancer” is meant a malignancy that forms a discrete tumor mass (including also tumor metastasis) located at specific location in the patient’s body, such as sarcomas or carcinomas (as opposed to e.g. blood cancers such as leukemia, which generally do not form solid tumors). Non-limiting examples of solid tumor cancers include bladder cancer, brain cancer, head and neck cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer, endometrial cancer, esophageal cancer, colon cancer, colorectal cancer, rectal cancer, gastric cancer, prostate cancer, skin cancer, squamous cell carcinoma, bone cancer, liver cancer and kidney cancer. Other solid tumor cancers that are contemplated in the context of the present invention include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic, skin, soft tissue, muscles, spleen, thoracic region, and urogenital system. Also included are pre-cancerous conditions or lesions and cancer metastases.

In some aspects wherein the target cell antigen of the T cell engaging agent, e.g. T cell bispecific antibody, is CD19, the disease (to be treated by the T cell bispecific antibody) is an autoimmune disease. In specific aspects, the autoimmune disease is lupus, in particular systemic lupus erythematosus (SLE) or lupus nephritis (LN).

An “individual” or “subject” herein is a mammal. Mammals include, but are not limited to, domesticated animals (e.g. cows, sheep, cats, dogs, and horses), primates (e.g. humans and non-human primates such as monkeys), rabbits, and rodents (e.g. mice and rats). In certain aspects, the individual or subject is a human. In some aspects, the individual has a disease, particularly a disease treatable or to be treated by the T cell engaging agent. In some aspects, the individual has cancer, particularly a cancer treatable or to be treated by the T cell engaging agent. In particular, an individual herein is any single human subject eligible for treatment who is experiencing or has experienced one or more signs, symptoms, or other indicators of cancer. In some aspects, the individual has cancer or has been diagnosed with cancer, in particular any of the cancers described

hereinabove. In some aspects, the individual has locally advanced or metastatic cancer or has been diagnosed with locally advanced or metastatic cancer. The individual may have been previously treated with a T cell engaging agent (e.g. a T cell bispecific antibody) or another drug, or not so treated. In particular aspects, the patient has not been previously treated with a T cell engaging agent (e.g. a T cell bispecific antibody). The patient may have been treated with a therapy comprising one or more drugs other than T cell engaging agent (e.g. other than a T cell bispecific antibody) before the T cell engaging agent therapy is commenced.

In some aspects, the individual has an elevated serum level of one of more cytokine. In some aspects, said elevated serum level is related to the administration of the T cell engaging agent to the individual. Said elevated serum level is in particular as compared to the serum level in a healthy individual, and/or the serum level in an individual (including the same individual) without administration of the T cell engaging agent (i.e. in such case the serum level is elevated as compared to the serum level without administration of the T cell engaging agent). In some aspects, said one or more cytokine is selected from the group consisting of IL-6, IFN-γ, IL-10, TNF-α, GM-CSF, MCP-1 and IL-1β.

A cytokine according to any of the aspects of the invention may be one or more cytokine selected from the group consisting of interleukin (IL)-6, interferon (IFN)-γ, IL-10, tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), monocyte chemoattractant protein (MCP)-1, IL-1β, IL-8, IL-4 and IL-2. In some aspects, the cytokine is one or more cytokine selected from the group consisting of IL-6, IFN-γ, IL-10, TNF-α, GM-CSF, MCP-1 and IL-1β. In some aspects, the cytokine is one or more cytokine selected from the group consisting of IL-6, IFN-γ, IL-10, TNF-α and GM-CSF. In some aspects, the cytokine is one or more cytokine selected from the group consisting of IL-6, IFN-γ, IL-10 and TNF-α. In some aspects, the cytokine is one or more cytokine selected from the group consisting of IL-6, IFN-γ and IL-10. In some aspects, the cytokine is IL-6. In some aspects, the cytokine is IFN-γ. In some aspects, the cytokine is IL-10. In some aspects, the cytokine is TNF-α. In some aspects, the cytokine is GM-CSF. In some aspects, the cytokine is MCP-1. In some aspects, the cytokine is IL-1β. In some aspects, the cytokine is IL-8. In some apsects, the cytokine is IL-4. In some aspects, the cytokine is IL-2.

Preferably, a T cell according to any of the aspects of the invention is a cytotoxic T cell. In some aspects, the T cell is a $CD4^+$ or a $CD8^+$ T cell. In some aspects, the T cell is a $CD8^+$ T cell. In some aspects, the T cell is a $CD4^+$ T cell.

In some aspects, the treatment with or administration of the T cell engaging agent may result in a response in the individual. In some aspects, the response may be a complete response. In some aspects, the response may be a sustained response after cessation of the treatment. In some aspects, the response may be a complete response that is sustained after cessation of the treatment. In other aspects, the response may be a partial response. In some aspects, the response may be a partial response that is sustained after cessation of the treatment. In some aspects, the treatment with or administration of the T cell engaging agent and the inhibitor of JAK and/or mTOR signaling may improve the response as compared to treatment with or administration of the T cell engaging agent alone (i.e. without the inhibitor of JAK and/or mTOR signaling). In some aspects, the treatment or administration of the T cell engaging agent and the inhibitor of JAK and/or mTOR signaling may increase response rates in a patient population, as compared to a corresponding patient population treated with the T cell engaging agent alone (i.e. without the inhibitor of JAK and/or mTOR signaling).

The T cell engaging agent may be used alone or together with other agents in a therapy. For instance, a T cell engaging agent may be co-administered with at least one additional therapeutic agent. In certain aspects, an additional therapeutic agent is an anti-cancer agent, e.g. a chemotherapeutic agent, an inhibitor of tumor cell proliferation, or an activator of tumor cell apoptosis.

The inhibitor of JAK and/or mTOR signaling may be used alone or together with one or more other agents for the prevention of mitigation of an adverse effect, particularly CRS, related to the administration of the T cell engaging agent. The inhibitor of JAK and/or mTOR signaling may for example be used together with an IL-6R antagonist (e.g. tocilizumab), a steroid (e.g. a corticosteroid such as methylprednisolone or dexamethasone) or a TNF-α antagonist (e.g. etanercept).

Amino Acid Sequences

	Sequence	**SEQ ID NO**
Human CD3	MQSGTHWRVLGLCLLSVGVWGQDGNEEMGGITQTPYKVS ISGTTVILTCPQYPGSEILWQHNDKNIGGDEDDKNIGSDEDH LSLKEFSELEQSGYYVCYPRGSKPEDANFYLYLRARVCENC MEMDVMSVATIVIVDICITGGLLLLVYYWSKNRKAKAKPV	1

	TRGAGAGGRQRGQNKERPPPVPNPDYEPIRKGQRDLYSGL NQRRI	
Cynomolgus CD3	MQSGTRWRVLGLCLLSIGVWGQDGNEEMGSITQTPYQVSI SGTTVILTCSQHLGSEAQWQHNGKNKEDSGDRLFLPEFSE MEQSGYYVCYPRGSNPEDASHHLYLKARVCENCMEMDV MAVATIVIVDICITLGLLLLVYYWSKNRKAKAKPVTRGAG AGGRQRGQNKERPPPVPNPDYEPIRKGQQDLYSGLNQRRI	2
hIgG1 Fc region	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSP	3
CD3 HCDR1	TYAMN	4
CD3 HCDR2	RIRSKYNNYATYYADSVKG	5
CD3 HCDR3	HGNFGNSYVSWFAY	6
CD3 LCDR1	GSSTGAVTTSNYAN	7
CD3 LCDR2	GTNKRAP	8
CD3 LCDR3	ALWYSNLWV	9
CD3 VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQA PGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNT LYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQG TLVTVSS	10
CD3 VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQE KPGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQ PEDEAEYYCALWYSNLWVFGGGTKLTVL	11
CEA HCDR1	EFGMN	12
CEA HCDR2	WINTKTGEATYVEEFKG	13
CEA HCDR3	WDFAYYVEAMDY	14
CEA LCDR1	KASAAVGTYVA	15
CEA LCDR2	SASYRKR	16
CEA LCDR3	HQYYTYPLFT	17
CEA VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQ APGQGLEWMGWINTKTGEATYVEEFKGRVTFTTDTSTSTA	18

	YMELRSLRSDDTAVYYCARWDFAYYVEAMDYWGQGTTV TVSS	
CEA VL	DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVAWYQQKP GKAPKLLIYSASYRKRGVPSRFSGSGSGTDFTLTISSLQPED FATYYCHQYYTYPLFTFGQGTKLEIK	19
CEA VL-CL	DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVAWYQQKP GKAPKLLIYSASYRKRGVPSRFSGSGSGTDFTLTISSLQPED FATYYCHQYYTYPLFTFGQGTKLEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC	20
CEA VH-CH1-Fc(hole, PGLALA)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQ APGQGLEWMGWINTKTGEATYVEEFKGRVTFTTDTSTSTA YMELRSLRSDDTAVYYCARWDFAYYVEAMDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPPSRDE LTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK	21
CD3 VL-CH1	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQE KPGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQ PEDEAEYYCALWYSNLWVFGGGTKLTVLSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSC	22
CEA VH-CH1-CD3 VH-CL-Fc(knob, PGLALA)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQ APGQGLEWMGWINTKTGEATYVEEFKGRVTFTTDTSTSTA YMELRSLRSDDTAVYYCARWDFAYYVEAMDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSEVQLL ESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGL EWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQM NSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS SASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGECDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLP PCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK	23

	TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK	
CD20 HCDR1	YSWIN	24
CD20 HCDR2	RIFPGDGDTDYNGKFKG	25
CD20 HCDR3	NVFDGYWLVY	26
CD20 LCDR1	RSSKSLLHSNGITYLY	27
CD20 LCDR2	QMSNLVS	28
CD20 LCDR3	AQNLELPYT	29
CD20 VH	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWINWVRQ APGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTSTA YMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVS S	30
CD20 VL	DIVMTQTPLSLPVTPGEPASISCRSSKSLLHSNGITYLYWYL QKPGQSPQLLIYQMSNLVSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCAQNLELPYTFGGGTKVEIK	31
CD20 VL-CL(RK)	DIVMTQTPLSLPVTPGEPASISCRSSKSLLHSNGITYLYWYL QKPGQSPQLLIYQMSNLVSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCAQNLELPYTFGGGTKVEIKRTVAAPSVFIFPP SDRKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC	32
CD20 VH-CH1(EE)-Fc(hole, PGLALA)	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWINWVRQ APGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTSTA YMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPPSRDELT KNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSP	33
CD3 VH-CL	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQA PGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNT LYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQG TLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	34

CD20 VH-CH1(EE)-CD3 VL-CH1-Fc(knob, PGLALA)	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWINWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP	35
Human CD20	MTTPRNSVNGTFPAEPMKGPIAMQSGPKPLFRRMSSLVGPTQSFFMRESKTLGAVQIMNGLFHIALGGLLMIPAGIYAPICVTVWYPLWGGIMYIISGSLLAATEKNSRKCLVKGKMIMNSLSLFAAISGMILSIMDILNIKISHFLKMESLNFIRAHTPYINIYNCEPANPSEKNSPSTQYCYSIQSLFLGILSVMLIFAFFQELVIAGIVENEWKRTCSRPKSNIVLLSAEEKKEQTIEIKEEVVGLTETSSQPKNEEDIEIIPIQEEEEEETETNFPEPPQDQESSPIENDSSP	36
CD3 HCDR1	GYTMN	37
CD3 HCDR2	LINPYKGVSTYNQKFKD	38
CD3 HCDR3	SGYYGDSDWYFDV	39
CD3 LCDR1	RASQDIRNYLN	40
CD3 LCDR2	YTSRLES	41
CD3 LCDR3	QQGNTLPWT	42
CD3 VH	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSS	43
CD3 VL	DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIK	44
MAGE-A4 HCDR1	KAMS	45

MAGE-A4 HCDR2	SISPSGGSTYYNDNVLG	46
MAGE-A4 HCDR3	DVGFFDE	47
MAGE-A4 LCDR1	RASQSISSYLA	48
MAGE-A4 LCDR2	DASIRDI	49
MAGE-A4 LCDR3	QQYSSYPYT	50
MAGE-A4 VH	AQLVESGGGLVQPGGSLRLSCAASAYFSFKAMSWVRQAP GKGLEWVGSISPSGGSTYYNDNVLGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKDVGFFDEWGQGTLVTVSS	51
MAGE-A4 VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQKPG KAPKLLIYDASIRDIGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQYSSYPYTFGQGTKLEIK	52
MAGE-A4 VL-CL(RK)	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQKPG KAPKLLIYDASIRDIGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQYSSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDRKLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC	53
MAGE-A4 VH-CH1(EE)-Fc(hole, PGLALA)	AQLVESGGGLVQPGGSLRLSCAASAYFSFKAMSWVRQAP GKGLEWVGSISPSGGSTYYNDNVLGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKDVGFFDEWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALGAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSL SCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL VSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP	54
CD3 VH-CL	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQ APGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTA YLQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTL VTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC	55
MAGE-A4 VH-CH1(EE)-	AQLVESGGGLVQPGGSLRLSCAASAYFSFKAMSWVRQAP GKGLEWVGSISPSGGSTYYNDNVLGRFTISRDNSKNTLYLQ	56

CD3 VL-CH1-Fc(knob, PGLALA)	MNSLRAEDTAVYYCAKDVGFFDEWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP	
Human MAGE-A4	MSSEQKSQHCKPEEGVEAQEEALGLVGAQAPTTEEQEAAVSSSSPLVPGTLEEVPAAESAGPPQSPQGASALPTTISFTCWRQPNEGSSSQEEEGPSTSPDAESLFREALSNKVDELAHFLLRKYRAKELVTKAEMLERVIKNYKRCFPVIFGKASESLKMIFGIDVKEVDPASNTYTLVTCLGLSYDGLLGNNQIFPKTGLLIIVLGTIAMEGDSASEEEIWEELGVMGVYDGREHTVYGEPRKLLTQDWVQENYLEYRQVPGSNPARYEFLWGPRALAETSYVKVLEHVVRVNARVRIAYPSLREAALLEEEEGV	57
p230-239 peptide	GVYDGREHTV	58
HLA-A2	GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWE	59
Human CD19	MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPFLKLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGELFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSLNQSLSQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARDMWVMETGLLLPRATAQDAGKYYCHRGNLTMSFHLEITARPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRRKRKRMTDPTRRFFKVTPPPGSGPQNQYGNVLSLPTPTSGLGRAQRWAAGLGGTAPSYGNPSSDVQADGALGSRSPPGVGPEEEEGEGYEEPDSEEDSEFYENDSNLGQDQLSQDGSGYENPEDEPLGPEDEDSFSNAESYENEDEELTQPVARTMDFLSPHGSA	60

	WDPSREATSLGSQSYEDMRGILYAAPQLRSIRGQPGPNHEE DADSYENMDNPDGPDPAWGGGGRMGTWSTR	
CD3 HCDR1	SYAMN	61
CD3 HCDR3	HTTFPSSYVSYYGY	62
CD3 VH	EVQLLESGGGLVQPGGSLRLSCAASGFQFSSYAMNWVRQA PGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNT LYLQMNSLRAEDTAVYYCVRHTTFPSSYVSYYGYWGQGT LVTVSS	63
CD3 HCDR1	SYAMN	64
CD3 HCDR3	ASNFPASYVSYFAY	65
CD3 VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQA PGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNT LYLQMNSLRAEDTAVYYCVRASNFPASYVSYFAYWGQGT LVTVSS	66
CD19 HCDR1	DYIMH	67
CD19 HCDR2	YINPYNDGSKYTEKFQG	68
CD19 HCDR3	GTYYYGPQLFDY	69
CD19 LCDR1	KSSQSLETSTGTTYLN	70
CD19 LCDR2	RVSKRFS	71
CD19 LCDR3	LQLLEDPYT	72
CD19 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIMHWVRQ APGQGLEWMGYINPYNDGSKYTEKFQGRVTMTSDTSISTA YMELSRLRSDDTAVYYCARGTYYYGPQLFDYWGQGTTVT VSS	73
CD19 VL	DIVMTQTPLSLSVTPGQPASISCKSSQSLETSTGTTYLNWYL QKPGQSPQLLIYRVSKRFSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCLQLLEDPYTFGQGTKLEIK	74
CD19 VH-CH1(EE) – CD3 VL-CH1 – Fc (knob, PGLALA)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIMHWVRQ APGQGLEWMGYINPYNDGSKYTEKFQGRVTMTSDTSISTA YMELSRLRSDDTAVYYCARGTYYYGPQLFDYWGQGTTVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDEKVEPKSCDGGGGSGGGGSQAVVT QEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQA FRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEA EYYCALWYSNLWVFGGGTKLTVLSSASTKGPSVFPLAPSS	75

	KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIE KTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSP	
CD19 VH-CH1(EE) – CD3 VL-CH1 – Fc (knob, PGLALA)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIMHWVRQ APGQGLEWMGYINPYNDGSKYTEKFQGRVTMTSDTSISTA YMELSRLRSDDTAVYYCARGTYYYGPQLFDYWGQGTTVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDEKVEPKSCDGGGGSGGGGGQAVVT QEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQA FRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEA EYYCALWYSNLWVFGGGTKLTVLSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIE KTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSP	76
CD19 VH-CH1(EE) –Fc (hole, PGLALA)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIMHWVRQ APGQGLEWMGYINPYNDGSKYTEKFQGRVTMTSDTSISTA YMELSRLRSDDTAVYYCARGTYYYGPQLFDYWGQGTTVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPPSRDELT KNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSP	77
CD19 VL-CL(RK)	DIVMTQTPLSLSVTPGQPASISCKSSQSLETSTGTTYLNWYL QKPGQSPQLLIYRVSKRFSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCLQLLEDPYTFGQGTKLEIKRTVAAPSVFIFPPS DRKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC	78
CD3 VH-CL	EVQLLESGGGLVQPGGSLRLSCAASGFQFSSYAMNWVRQA PGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNT LYLQMNSLRAEDTAVYYCVRHTTFPSSYVSYYGYWGQGT	79

	LVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	
CD3 VH-CL	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQA PGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNT LYLQMNSLRAEDTAVYYCVRASNFPASYVSYFAYWGQGT LVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	80

Brief description of the Drawings

Figure 1. Assay set-up. MKN45 NucLightRed (NLR) target cells were co-cultured with 10 nM CEA-TCB, mTOR or JAK inhibitor and peripheral blood mononuclear cells (PBMCs), E:T = 50 000 PBMCs : 5 000 target cells. Kinetics of target cell killing was followed using an Incucyte® system (1 scan every 3 hours, zoom 10 x, phase and red 400 ms acquisition time).

Figure 2. Real-time killing of MKN45 NLR cells by 10 nM CEA-TCB in the presence of sirolimus (A), everolimus (B) and temisirolimus (C) concentrations ranging from 0 nM to 1000 nM in the assay described in Figure 1. %Killing was measured by normalizing total red area with values at t = 0 hour and target cells + PBMCs + mTOR inhibitor control wells for each time point. Means of technical replicates + SEM for 1 representative donor.

Figure 3. Effect of escalating concentrations of sirolimus (A), everolimus (B) and temsirolimus (C) on TCB-mediated target cell killing measured at 72h in the assay described in Figure 1. %Killing at 72h was measured by normalizing total red area with values at t = 0 hour and target cells + PBMCs + mTOR inhibitor control wells for each time point. Means of technical replicates +/- SD for 1 representative donor.

Figure 4. Effect of escalating concentrations of everolimus (A), sirolimus (B) and temsirolimus (C) on PBMC viability at 72h in the assay described in Figure 1. Technical replicates were pooled and viability of PBMCs was measured by flow cytometry using a Live/Dead™ Fixable Aqua Dead Cell Stain. 1 representative donor.

Figure 5. Effect of escalating concentrations of everolimus on CD69 expression on CD4+ (A) and CD8+ (C) T cells and on CD25 expression on CD4+ (B) and CD8+ (D) T cells at 72h after

treatment with 10 nM CEA-TCB in the assay of Figure 1. Technical replicates were pooled and expression of CD69 and CD25 on CD4+ and CD8+ T cells was measured by flow cytometry at 72h. 1 representative donor.

Figure 6. Effect of escalating concentrations of sirolimus on CD69 expression on CD4+ (A) and CD8+ (C) T cells and on CD25 expression on CD4+ (B) and CD8+ (D) T cells at 72h after treatment with 10 nM CEA-TCB in the assay of Figure 1. Technical replicates were pooled and expression of CD69 and CD25 on CD4+ and CD8+ T cells was measured by flow cytometry at 72h. 1 representative donor.

Figure 7. Effect of escalating concentrations of temsirolimus on CD69 expression on CD4+ (A) and CD8+ (C) T cells and on CD25 expression on CD4+ (B) and CD8+ (D) T cells at 72h after treatment with 10 nM CEA-TCB in the assay of Figure 1. Technical replicates were pooled and expression of CD69 and CD25 on CD4+ and CD8+ T cells was measured by flow cytometry at 72h. 1 representative donor.

Figure 8. Effect of escalating concentrations of sirolimus, everolimus, and temsirolimus on cytokine release (IFN-γ (A), IL-2 (B), TNF-α (C), IL-6 (D), GM-CSF (E), IL-8 (F), IL-4 (G), IL-10 (H), MCP-1 (I)) measured at 72h after treatment with 10 nM CEA-TCB in the assay of Figure 1. Supernatants from technical replicates were pooled and cytokines were analyzed by Luminex. 1 representative donor.

Figure 9. (A) Real-time killing of MKN45 NLR cells by 10 nM CEA-TCB in the presence of ruxolitinib concentrations ranging from 0 nM to 100 nM in the assay of Figure 1. (B) Effect of escalating concentrations of ruxolitinib on target cell killing measured at 69h after treatment with 10 nM CEA-TCB in the assay of Figure 1. %Killing was measured by normalizing total red area with values at t = 0 hour and target cells + PBMCs + ruxolitinib control wells for each time point. Means of technical replicates + SEM for one representative donor (A). Mean of n=3 donors +/- SD.

Figure 10. Effect of escalating concentrations of ruxolitinib on PBMC viability at 69h in the assay of Figure 1. Technical replicates were pooled and viability of PBMCs was measured by flow cytometry using a Live/Dead™ Fixable Aqua Dead Cell Stain. 1 representative donor.

Figure 11. Effect of escalating concentrations of ruxolitinib on CD25 expression on CD8+ (A) and CD4+ (B) T cells and on CD69 expression on CD8+ (C) and CD4+ (D) T cells at 69h, after

treatment with 10 nM CEA-TCB in the assay of Figure 1. Technical replicates were pooled and the expression of CD25 and CD69 on CD4+ and CD8+ T cells was measured by flow cytometry at 69h. Mean of n=3 donors +/- SD.

Figure 12. Effect of escalating concentrations of ruxolitinib on cytokine release (IFN-γ (A), IL-2 (B), TNF-α (C), IL-6 (D), GM-CSF (E), IL-8 (F), IL-4 (G), IL-10 (H), MCP-1 (I)) induced by 10 nM CEA-TCB at 69h in the assay of Figure 1. Supernatants from technical replicates were pooled at 69h and cytokines were analyzed by Luminex. Mean of n=3 donors +/- SD.

Figure 13. *In vitro* killing assay set-up. Cell Trace™ Violet (CTV) labelled WSU DLCL2 tumor cells were co-cultured together with PBMCs [E:T=200'000:20'000], CD20-TCB and in the presence of escalating doses of ruxolitinib, temsirolimus, sirolimus and everolimus ranging from 0 nM to 1000 nM.

Figure 14. Effect of escalating concentrations of ruxolitinib (A), temsirolimus (B), sirolimus (C) and everolimus (D) on CTV WSU DLCL2 tumor cell killing in the assay of Figure 13 for 1 nM CD20-TCB. At 24 hours, the tumor cells and PBMCs from technical replicates were pooled and stained with a LIVE/DEAD™ Near-IR dead cell dye to allow exclusion of dead CTV labelled WSU DLCL2 tumor cells by flow cytometry. Mean of n=3 donors +/- SEM.

Figure 15. Effect of escalating concentrations of ruxolitinib on CD25 expression on CD4+ (B) and CD8+ (D) T cells as well as CD69 expression on CD4+ (A) and CD8+ (C) T cells in the assay of Figure 13 for 1 nM CD20-TCB. At 24 hours, the tumor cells and PBMCs from technical replicates were pooled and expression of CD69 and CD25 on CD4+ and CD8+ T cells was measured by flow cytometry. Mean of n=3 donors +/- SEM.

Figure 16. Effect of escalating concentrations of temsirolimus on CD25 expression on CD4+ (B) and CD8+ (D) T cells as well as CD69 expression on CD4+ (A) and CD8+ (C) T cells in the assay of Figure 13 for 1 nM CD20-TCB. At 24 hours, the tumor cells and PBMCs from technical replicates were pooled and expression of CD69 and CD25 on CD4+ and CD8+ T cells was measured by flow cytometry. Mean of n=3 donors +/- SEM.

Figure 17. Effect of escalating concentrations of sirolimus on CD25 expression on CD4+ (B) and CD8+ (D) T cells as well as CD69 expression on CD4+ (A) and CD8+ (C) T cells in the assay of Figure 13 for 1 nM CD20-TCB. At 24 hours, the tumor cells and PBMCs from technical replicates

were pooled and expression of CD69 and CD25 on CD4+ and CD8+ T cells was measured by flow cytometry. Mean of n=3 donors +/- SEM.

Figure 18. Effect of escalating concentrations of everolimus on CD25 expression on CD4+ (B) and CD8+ (D) T cells as well as CD69 expression on CD4+ (A) and CD8+ (C) T cells in the assay of Figure 13 for 1 nM CD20-TCB. At 24 hours, the tumor cells and PBMCs from technical replicates were pooled and expression of CD69 and CD25 on CD4+ and CD8+ T cells was measured by flow cytometry. Mean of n=3 donors +/- SEM.

Figure 19. Effect of escalating concentrations of ruxolitinib on IFN-γ (A), IL-2 (B), TNF-α (C), GM-CSF (D) and IL-6 (E) release in the assay of Figure 13 for 1 nM CD20-TCB. At 24 hours, the supernatants from technical replicates were pooled and cytokines were analyzed by Luminex. Mean of n=3 donors +/- SEM.

Figure 20. Effect of escalating concentrations of temsirolimus on IFN-γ (A), IL-2 (B), TNF-α (C), GM-CSF (D) and IL-6 (E) release in the assay of Figure 13 for 1 nM CD20-TCB. At 24 hours, the supernatants from technical replicates were pooled and cytokines were analyzed by Luminex. Mean of n=3 donors +/- SEM.

Figure 21. Effect of escalating concentrations of sirolimus on IFN-γ (A), IL-2 (B), TNF-α (C), GM-CSF (D) and IL-6 (E) release in the assay of Figure 13 for 1 nM CD20-TCB. At 24 hours, the supernatants from technical replicates were pooled and cytokines were analyzed by Luminex. Mean of n=3 donors +/- SEM.

Figure 22. Effect of escalating concentrations of everolimus on IFN-γ (A), IL-2 (B), TNF-α (C), GM-CSF (D) and IL-6 (E) release in the assay of Figure 13 for 1 nM CD20-TCB. At 24 hours, the supernatants from technical replicates were pooled and cytokines were analyzed by Luminex. Mean of n=3 donors +/- SEM.

Figure 23. Real-time killing of MKN45 NLR cells by 10 nM CEA-TCB in the presence of 5 μg/mL anti-TNF-α antibody (aTNF-α; Biolegend #502922 (antibody Mab11)), 5 μg/mL anti-IL-6R antibody (aIL-6R; Roche in-house), 1 μM dexamethasone (dexa), 0.1 μM dexamethasone, 50 nM dasatinib (dasa), 50 nM ruxolitinib (ruxo), 50 nM temsirolimus (temsi), 40 nM sirolimus (siro), 50 nM everolimus (evero) in the assay of Figure 1. %Killing was measured by normalizing total red area with values at t = 0 hour and target cells + PBMCs + corresponding coumpound control wells for each time point. Means of technical replicates + SD for 1 representative donor.

Figure 24. Effect of anti-TNF-α antibody (aTNF-α), anti-IL-6R antibody (aIL-6R), dexamethasone (dexa), dasatinib (dasa), ruxolitinib (ruxo), temsirolimus (temsi), sirolimus (siro), everolimus (evero) on CD69 (A) and CD25 (B) expression on CD4+ T cells induced by 10 nM CEA-TCB in the assay of Figure 1. Technical replicates were pooled and expression of CD69 and CD25 on CD4+ T cells was measured by flow cytometry at 66h. Mean of n=3 donors +/-SD

Figure 25. Effect of anti-TNF-α antibody (aTNF-α), anti-IL-6R antibody (aIL-6R), dexamethasone (dexa), dasatinib (dasa), ruxolitinib (ruxo), temsirolimus (temsi), sirolimus (siro), everolimus (evero) on CD69 (A) and CD25 (B) expression on CD8+ T cells induced by 10 nM CEA-TCB in the assay of Figure 1. Technical replicates were pooled and expression of CD69 and CD25 on CD8+ T cells was measured by flow cytometry at 66h. Mean of n=3 donors +/- SD.

Figure 26. Effect of anti-TNF-α antibody (aTNF-α), anti-IL-6R antibody (aIL-6R), dexamethasone (dexa), dasatinib (dasa), ruxolitinib (ruxo), temsirolimus (temsi), sirolimus (siro), everolimus (evero) on cytokine release (IFN-γ (A), IL-2 (B), TNF-α (C), IL-4 (D), IL-8 (E), IL-10 (F), GM-CSF (G), MCP-1 (H)) induced by 10 nM CEA-TCB in the assay of Figure 1. Supernatants from technical replicates were pooled at 66h and cytokines were analyzed by Luminex. Mean of n=3 donors +/- SD.

Figure 27. Effect of anti-TNF-α antibody (aTNF-α), anti-IL-6R antibody (aIL-6R), dexamethasone (dexa), dasatinib (dasa), ruxolitinib (ruxo), temsirolimus (temsi), sirolimus (siro), everolimus (evero) on CD20-TCB-induced B cell killing. WSU target cells were co-cultured with PBMCs (E:T = 200 000:20 000), escalating CD20-TCB concentrations and corresponding compound. Technical replicates were pooled at 24h and CD19+ B cells were measured by flow cytometry. Dead B cells were excluded from CD19+ B cells using Live/Dead™ Fixable Aqua Dead Cell Stain. 1 representative donor.

Figure 28. Effect of anti-TNF-α antibody (aTNF-α), anti-IL-6R antibody (aIL-6R), dexamethasone (dexa), dasatinib (dasa), ruxolitinib (ruxo), temsirolimus (temsi), sirolimus (siro), everolimus (evero) on CD20-TCB-induced B cell killing. WSU target cells were co-cultured with PBMCs (E:T = 200 000:20 000), 1 nM CD20-TCB and corresponding compound. Technical replicates were pooled at 24h and CD19+ B cells were measured by flow cytometry. Dead B cells were excluded from CD19+ B cells using Live/Dead™ Fixable Aqua Dead Cell Stain. Mean of n=3 donors +/- SD.

Figure 29. Effect of anti-TNF-α antibody (aTNF-α), anti-IL-6R antibody (aIL-6R), dexamethasone (dexa), dasatinib (dasa), ruxolitinib (ruxo), temsirolimus (temsi), sirolimus (siro), everolimus (evero) on CD20-TCB-induced T cell activation. WSU target cells were co-cultured with PBMCs (E:T = 200 000:20 000), escalating CD20-TCB concentrations and corresponding compound. Technical replicates were pooled at 24h and expression of CD69 on CD4+ (A) and CD8+ (C) T cells and CD25 on CD4+ (B) and CD8+ (D) T cells was measured by flow cytometry. 1 representative donor.

Figure 30. Effect of anti-TNF-α antibody (aTNF-α), anti-IL-6R antibody (aIL-6R), dexamethasone (dexa), dasatinib (dasa), ruxolitinib (ruxo), temsirolimus (temsi), sirolimus (siro), everolimus (evero) on CD69 (A) and CD25 (B) expression on CD4+ T cells . WSU target cells were co-cultured with PBMCs (E:T = 200 000:20 000), 1 nM CD20-TCB and corresponding compound. Technical replicates were pooled at 24h and expression of CD69 and CD25 on CD4+ T cells was measured by flow cytometry. Mean of n=3 donors +/- SD.

Figure 31. Effect of anti-TNF-α antibody (aTNF-α), anti-IL-6R antibody (aIL-6R), dexamethasone (dexa), dasatinib (dasa), ruxolitinib (ruxo), temsirolimus (temsi), sirolimus (siro), everolimus (evero) on CD69 (A) and CD25 (B) expression on CD8+ T cells . WSU target cells were co-cultured with PBMCs (E:T = 200 000:20 000), 1 nM CD20-TCB and corresponding compound. Technical replicates were pooled at 24h and expression of CD69 and CD25 on CD8+ T cells was measured by flow cytometry. Mean of n=3 donors +/- SD.

Figure 32. Effect of anti-TNF-α antibody (aTNF-α), anti-IL-6R antibody (aIL-6R), dexamethasone (dexa), dasatinib (dasa), ruxolitinib (ruxo), temsirolimus (temsi), sirolimus (siro), everolimus (evero) on CD20-TCB- induced cytokine release (TNF-α (A), IFN-γ (B), IL-2 (C), IL-1β (D), IL-6 (E), IL-4 (F), IL-10 (G), GM-CSF (H)). WSU target cells were co-cultured with PBMCs (E:T = 200 000:20 000), escalating CD20-TCB concentrations and corresponding compound. Supernatant from technical replicates were pooled at 24h and cytokines were analyzed by Luminex. 1 representative donor.

Figure 33. Effect of anti-TNF-α antibody (aTNF-α), anti-IL-6R antibody (aIL-6R), dexamethasone (dexa), dasatinib (dasa), ruxolitinib (ruxo), temsirolimus (temsi), sirolimus (siro), everolimus (evero) on CD20-TCB- induced cytokine release (TNF-α (A), IFN-γ (B), IL-2 (C), IL-1β (D), IL-6 (E), IL-4 (F), IL-10 (G), GM-CSF (H)). WSU target cells were co-cultured with PBMCs (E:T = 200 000:20 000), 1 nM CD20-TCB and corresponding compound. Supernatant

from technical replicates were pooled at 24h and cytokines were analyzed by Luminex. Mean of n=3 donors +/- SD.

Figure 34. *In vitro* killing assay set-up. Cell Trace™ Violet (CTV) labelled WSU DLCL2 tumor cells were co-cultured together with PBMCs [E:T=200'000:20'000] and stimulated with CD20-TCB for 18h. At 18h, 100 nM ruxolitinib, 100 nM temsirolimus, 100 nM sirolimus or 100 nM everolimus was added in the system.

Figure 35. CTV labelled WSU DLCL2 target cell killing at 18h in the assay of Figure 34 before the addition of ruxolitinib, temsirolimus, sirolimus and everolimus for 3 representative donors (D1-D3). At 18 hours, the tumor cells and PBMCs from technical replicates were pooled and stained with a LIVE/DEAD™ Near-IR dead cell dye to allow exclusion of dead CTV labelled WSU DLCL2 tumor cells by flow cytometry.

Figure 36. CD25 expression on CD8+ (A) and CD4+ (B) T cells at 18h in the assay of Figure 34 before the addition of ruxolitinib, temsirolimus, sirolimus and everolimus for 3 representative donors (D1-D3). At 18hours, the technical replicates were pooled and expression of CD25 was measured on CD4+ and CD8+ T cells by flow cytometry.

Figure 37. CTV labelled WSU DLCL2 target cell killing at 44h in the assay of Figure 34 after the addition of ruxolitinib, temsirolimus, sirolimus and everolimus. At 44 hours, the tumor cells and PBMCs from technical replicates were pooled and stained with a LIVE/DEAD™ Near-IR dead cell dye to allow exclusion of dead CTV labelled WSU DLCL2 tumor cells by flow cytometry. 1 representative donor.

Figure 38. Expression of CD25 expression on CD4+ (B) and CD8+ (D) T cells and CD69 expression on CD4+ (A) and CD8+ (C) T cells after the addition of ruxolitinib, temsirolimus, sirolimus and everolimus in the assay of Figure 34. At 44 hours, the tumor cells and PBMCs from technical replicates were pooled and expression of CD25 and CD69 on CD4+ and CD8+ T cells was measured by flow cytometry. 1 representative donor.

Figure 39. IL-2 (A), IFN-γ (B), TNF-α (C), IL-6 (D), IL-1β (E), GM-CSF (F) levels before (18h) and after (44h) addition of ruxolitinib, temsirolimus, sirolimus and everolimus in the assay of Figure 34. At 18 hours and 44 hours, the supernatants from technical replicates were pooled and cytokines were analyzed by Luminex. 1 representative donor.

Figure 40. CTV labelled WSU DLCL2 target cell killing before (18h) and after (44h) addition of ruxolitinib, temsirolimus, sirolimus and everolimus for 1 nM CD20-TCB in the assay of Figure 34. At 18 hours and 44 hours, the tumor cells and PBMCs from technical replicates were pooled and stained with a LIVE/DEAD™ Near-IR dead cell dye to allow exclusion of dead CTV labelled
5 WSU DLCL2 tumor cells by flow cytometry. Mean of n=3 donors +/- SEM.

Figure 41. Expression of CD69 (A) and CD25 (B) on CD4+ T cells before (18h) and after (44h) the addition of ruxolitinib, temsirolimus, sirolimus and everolimus for 1 nM CD20-TCB in the assay of Figure 34. At 18 hours and 44 hours, the tumor cells and PBMCs from technical replicates were pooled and expression of CD25 and CD69 on CD4+ T cells was measured by flow cytometry.
10 Mean of n=3 donors +/- SEM.

Figure 42. Expression of CD69 (A) and CD25 (B) on CD8+ T cells before (18h) and after (44h) the addition of ruxolitinib, temsirolimus, sirolimus and everolimus for 1 nM CD20-TCB in the assay of Figure 34. At 18 hours and 44 hours, the tumor cells and PBMCs from technical replicates were pooled and expression of CD25 and CD69 on CD8+ T cells was measured by flow cytometry.
15 Mean of n=3 donors +/- SEM.

Figure 43. IFN-γ (A), IL-2 (B), TNF-α (C), IL-6 (D) and IL-1β (E) levels before (18h) and after (44h) addition of ruxolitinib, temsirolimus, sirolimus and everolimus for 1 nM CD20-TCB in the assay of Figure 34. At 18 hours and 44 hours, the supernatants from technical replicates were pooled and cytokines were analyzed by Luminex. Mean of n=3 donors +/- SEM.

20 **Figure 44.** Real time killing of A375 NucLightRed (NLR) cells by 8 nM MAGEA4-TCB in the presence of escalating concentrations of ruxolitinib ranging from 0 nM to 1000 nM. A375 NLR target cells were co-cultured with MAGEA4-TCB (8 nM), ruxolitinib and PBMCs, E:T = 50 000 PBMCs : 5000 target cells. The killing was followed using an Incucyte® (1 scan every 3 hours, zoom 10 x, phase and red 400 ms acquisition time). %Killing was measured by normalizing total
25 red area with values at t = 0 hour and target cells + PBMCs + ruxolitinib control wells for each time point. Means of technical replicates + SD for 1 representative donor.

Figure 45. Effect of escalating concentrations (c) of ruxolitinib on IFN-γ (A), IL-2 (B), TNF-α (C), GM-CSF (D), IL-6 (E), IL-1β (F), IL-8 (G), MCP-1 (H) and IL-10 (I) levels induced by 8 nM MAGEA4-TCB. At 72 hours, the supernatants were collected and cytokines were analyzed by
30 cytometric bead array (CBA). Mean of technical replicates +/-SD for 1 representative donor.

Figure 46. Real time killing of A375 NucLightRed (NLR) cells by 8 nM MAGEA4-TCB in the presence of escalating concentrations of sirolimus (A), temsirolimus (B) and everolimus (C) ranging from 0 nM to 1000 nM. A375 NLR target cells were co-cultured with 8 nM MAGEA4-TCB, mTOR inhibitors and PBMCs, E:T = 50 000 PBMCs : 5000 target cells. The killing was followed using an Incucyte® (1 scan every 3 hours, zoom 10 x, phase and red 400 ms acquisition time). %Killing was measured by normalizing total red area with values at t = 0 hour and target cells + PBMCs + mTOR inhibitors control wells for each time point. Means of technical replicates + SEM for 1 representative donor.

Figure 47. Effect of escalating concentrations (c) of sirolimus, temsirolimus and everolimus on IFN-γ (A), IL-2 (B), TNF-α (C), GM-CSF (D), IL-6 (E), IL-1β (F), IL-8 (G), MCP-1 (H) and IL-10 (I) levels induced by 8 nM MAGEA4-TCB. At 72 hours, the supernatants were collected and cytokines were analyzed by CBA. Mean of technical replicates +/-SD for 1 representative donor.

Figure 48. Effect of escalating concentrations of baricitinib on CTV labelled WSU DLCL2 target cell killing induced by CD20-TCB. At 24 hours, the tumor cells and PBMCs from technical replicates were pooled and stained with a LIVE/DEAD™ Near-IR dye to allow exclusion of dead CTV labelled WSU DLCL2 tumor cells by flow cytometry. 1 representative donor.

Figure 49. Effect of escalating concentrations of baricitinib on CTV labelled WSU DLCL2 target cell killing induced by 1 nM CD20-TCB. At 24 hours, the tumor cells and PBMCs from technical replicates were pooled and stained with a LIVE/DEAD™ Near-IR dye to allow exclusion of dead CTV labelled WSU DLCL2 tumor cells by flow cytometry. Mean of n=2 donors.

Figure 50. Effect of escalating concentrations of baricitinib on CD69 on CD4+ (A) and CD8+ (B) T cells and CD25 expression on CD4+ (C) and CD8+ (D) T cells induced by CD20-TCB. At 24 hours, the technical replicates were pooled and the expression of CD69 and CD25 was measured on CD4+ and CD8+ T cells by flow cytometry. 1 representative donor.

Figure 51. Effect of escalating concentrations of baricitinib on CD69 (A) and CD25 (B) expression on CD4+ T cells for 1 nM CD20-TCB. At 24 hours, the technical replicates were pooled and the expression of CD69 and CD25 was measured on CD4+ T cells by flow cytometry. Mean of n=2 donors.

Figure 52. Effect of escalating concentrations of baricitinib on CD69 (A) and CD25 (B) expression on CD8+ T cells for 1 nM CD20-TCB. At 24 hours, the technical replicates were pooled and

expression of CD69 and CD25 was measured on CD8+ T cells by flow cytometry. Mean of n=2 donors.

Figure 53. Effect of escalating concentrations of baricitinib on IFN-γ (A), IL-2 (B), TNF-α (C), GM-CSF (D), IL-6 (E), IL-8 (F) levels in a killing assay for a dose-response of CD20-TCB. At 24 hours, the supernatants from technical replicates were pooled and cytokines were analyzed by Luminex. 1 representative donor.

Figure 54. Effect of escalating concentrations of baricitinib on IFN-γ (A), IL-2 (B), TNF-α (C), GM-CSF (D), IL-6 (E), IL-8 (F) levels for 1 nM CD20-TCB. At 24 hours, the supernatants from technical replicates were pooled and cytokines were analyzed by Luminex. Mean of n=2 donors.

Figure 55. Real-time killing of MKN45 NLR cells by 1 nM CEA-TCB in the presence of baricitinib (A) and ruxolitinib (B) concentrations ranging from 0 nM to 1000 nM. MKN45 NLR target cells were co-cultured with PBMCs (E:T = 50 000 PBMCs:5000 target cells) in medium supplemented with 1 nM CEA-TCB and the JAK inhibitors. The killing was followed using an Incucyte® (1 scan every 3 hours, zoom 10 x, phase and red 400 ms acquisition time). %Killing was measured by normalizing total red area with values at t = 0 hour and target cells + PBMCs + ruxolitinib or baricitinib control wells for each time point. Means of technical replicates + SEM for 1 representative donor.

Figure 56. Effect of escalating concentrations of baricitinib vs. ruxolitinib on CD25 expression on CD4+ (A) and CD8+ (B) T cells at 72 hours, after treatment with 10 nM CEA-TCB. Technical replicates were pooled and the expression of CD25 on CD4+ and CD8+ T cells was measured by flow cytometry at 69 hours. Mean of n=3 donors +/- SD.

Figure 57. Effect of escalating concentrations of baricitinib vs. ruxolitinib on IFN-γ (A), IL-2 (B), TNF-α (C), GM-CSF (D), IL-6 (E), IL-8 (F) release after treatment with 10 nM CEA-TCB. At 24 hours, the supernatants from technical replicates were pooled and cytokines were analyzed by Luminex. % inhibition was calculated by normalizing the cytokines levels for each kinase inhibitor concentration to the condition where no kinase inhibitor was added. Mean of n=3 donors +/- SEM.

Figure 58. Real time killing of A375 NucLightRed (NLR) cells by 25 nM MAGEA4-TCB in the presence of escalating concentrations of baricitinib (A) and ruxolitinib (B) ranging from 0 nM to 100 nM. A375 NLR target cells were co-cultured with PBMCs (E:T = 50 000 PBMCs : 5000 target cells) in medium supplemented with 25 nM MAGEA4-TCB and the JAK inhibitors. The killing

was followed using an Incucyte® (1 scan every 3 hours, zoom 10 x, phase and red 400 ms acquisition time). Killing [%] was measured by normalizing total red area with values at t = 0 hour and target cells + PBMCs + JAK inhibitors control wells for each time point. Means of technical replicates +/- SD for 1 representative donor.

5 **Figure 59.** Effect of escalating concentrations (0-100 nM) of baricitinib on GM-CSF (A), IL-2 (B), IFN-γ (C), TNF-α (D), IL-1β (E) and IL-6 (F) levels induced by 25 nM MAGEA4-TCB. At 69 hours, the supernatants were collected and cytokines were analyzed by CBA. Mean of technical replicates +/-SD for 1 representative donor.

Figure 60. Effect of escalating concentrations (0-100 nM) of ruxolitinib on GM-CSF (A), IL-2 10 (B), IFN-γ (C), TNF-α (D), IL-1β (E) and IL-6 (F) levels induced by 25 nM MAGEA4-TCB. At 69 hours, the supernatants were collected and cytokines were analyzed by CBA. Mean of technical replicates +/-SD for 1 representative donor.

Figure 61. CTV labelled WSU tumor cell killing by PGLALA CAR-T cells (A) and CD16 CAR-T cells (B) in the presence and absence of 100 nM ruxolitinib or 100 nM sirolimus. PGLALA 15 CAR-T cells and CD16 CAR-T cells were co-cultured together with CTV labelled WSU tumor cells (E:T=10:1) and escalating concentrations of anti-CD20 IgG with either PGLALA-Fc (for PGLALA CAR-T cells) or wild-type Fc (for CD16 CAR-T cells) in the presence and absence of 100 nM ruxolitinib (ruxo) or 100 nM sirolimus (siro). At 24 hours, the technical replicates were pooled and stained with a LIVE/DEAD™ Near-IR dye to allow exclusion of dead CTV labelled 20 WSU DLCL2 tumor cells by flow cytometry. 1 representative donor.

Figure 62. Effect of 100 nM ruxolitinib and 100 nM sirolimus on GM-CSF (A), IFN-γ (B), IL-2 (C) and TNF-α (D) induced by PGLALA CAR-T cells. PGLALA CAR-T cells were co-cultured together with CTV labelled WSU tumor cells (E:T=10:1) and escalating concentrations of PGLALA-Fc anti-CD20 IgG in the presence and absence of 100 nM ruxolitinib (ruxo) or 100 nM 25 sirolimus (siro) respectively. At 24 hours, the supernatants from technical replicates were pooled and cytokines were analyzed by Luminex. Mean of n=2 donors.

Figure 63. Effect of 100 nM ruxolitinib and 100 nM sirolimus on GM-CSF (A), IFN-γ (B), IL-2 (C) and TNF-α (D) induced by CD16 CAR-T cells. CD16 CAR-T cells were co-cultured together with CTV labelled WSU tumor cells (E:T=10:1) and escalating concentrations of wild-type Fc 30 anti-CD20 IgG in the presence and absence of 100 nM ruxolitinib (ruxo) or 100 nM sirolimus

(siro). At 24 hours, the supernatants from technical replicates were pooled and cytokines were analyzed by Luminex. Mean of n=2 donors.

Figure 64. *In vitro* killing assay set-up. PBMCs were co-cultured with CellTrace™ Violet (CTV) labelled SUDLH-8 tumor cells (E:T=10:1) in the presence of escalating concentrations of CD19-
5 TCB in media supplemented with the different kinase inhibitors (100 nM) for 24 hrs.

Figure 65. Effect of 100 nM dasatinib (Src inhibitor), sirolimus (mTOR inhibitor) and ruxolitinib (JAK1/2 inhibitor) on CD19-TCB-induced SUDLH-8 killing (A) and T cell activation (B, C, D, E) in the assay of Figure 64 (24 hrs). Representative flow cytometry plots of dead CTV labelled SUDLH-8 cells (A) excluded from live cells using a Live/Dead stain, and of CD69 expression on
10 CD4+ (B) and CD8+ (C) T cells, and CD25 expression on CD4+ (D) and CD8+ (E) T cells. 1 representative donor out of 3, 10 nM CD19-TCB.

Figure 66. Effect of 100 nM dasatinib (dasa), 100 nM sirolimus (siro), 100 nM temsirolimus (temsi), 100 nM everolimus (evero) and 100 nM ruxolitinib (ruxo) on CD19-TCB-dependent killing of CTV labelled SUDLH-8 cells in the assay of Figure 64. The killing of CTV labelled
15 SUDLH-8 cells was measured by flow cytometry at 24 hrs using a Live/Dead stain allowing for exclusion of dead cells. Mean of n=3 donors + standard deviation (SD).

Figure 67. Effect of 100 nM dasatinib (dasa), 100 nM sirolimus (siro), 100 nM temsirolimus (temsi), 100 nM everolimus (evero) and 100 nM ruxolitinib (ruxo) on CD19-TCB-dependent T cell activation in the assay of Figure 64. The expression of CD69 and CD25 on CD4+ (A, B) and
20 CD8+ (C, D) T cells was measured by flow cytometry at 24 hrs. Mean of n=3 donors + SD.

Figure 68. Effect of 100 nM dasatinib (dasa), 100 nM sirolimus (siro), 100 nM temsirolimus (temsi), 100 nM everolimus (evero) and 100 nM ruxolitinib (ruxo) on CD19-TCB-dependent cytokine release in the assay of Figure 64. The levels of IL-2 (A), IFN-γ (B), TNF-α (C), IL-6 (D) and GM-CSF (E) were measured in the supernatants by Luminex (24 hrs). 1 representative donor
25 out of 3.

Figure 69. *In vitro* killing assay set-up. PBMCs were co-cultured with CTV labelled SUDLH-8 cells (E:T=10:1) in the presence of escalating concentrations of CD19-TCB in media supplemented with the different JAK inhibitors (100 nM) for 24 hrs.

Figure 70. Effect of 100 nM ruxolitinib (ruxo), 100 nM baricitinib (bari) and 100 nM tofacitinib (tofa) on CD19-TCB-dependent killing of CTV labelled SUDLH-8 cells in the assay of Figure 69. The killing of CTV labelled SUDLH-8 cells was measured by flow cytometry at 24 hrs using a Live/Dead stain allowing for exclusion of dead cells. Mean of n=3 donors + SD.

5 **Figure 71.** Effect of 100 nM ruxolitinib (ruxo), 100 nM baricitinib (bari) and 100 nM tofacitinib (tofa) on CD19-TCB-dependent T cell activation in the assay of Figure 69. The expression of CD69 and CD25 on CD4+ (A, B) and CD8+ (C, D) T cells was measured by flow cytometry at 24 hrs. Mean of n=3 donors + SD.

Figure 72. Effect of 100 nM ruxolitinib (ruxo), 100 nM baricitinib (bari) and 100 nM tofacitinib
10 (tofa) on CD19-TCB-dependent cytokine release in the assay of Figure 69. The levels of IL-2 (A), IFN-γ (B), TNF-α (C), IL-6 (D) and GM-CSF (E) were measured in the supernatants by Luminex (24 hrs). 1 representative donor out of 3.

Figure 73. *In vitro* killing assay set-up. PBMCs were co-cultured with CTV labelled SUDLH-8 cells (E:T=10:1) in the presence of escalating concentrations of CD19-TCB in media
15 supplemented with the different kinase inhibitors (100 nM), dexamethasone (100 nM), 5 μg/mL anti-TNF-α antibody (aTNF-α) or 5 μg/mL anti-IL-6R antibody (aIL-6R) for 24 hrs.

Figure 74. Effect of 100 nM dexamethasone (dexa), 5 μg/mL anti-TNF-α antibody (aTNF-α) or 5 μg/mL anti-IL-6R antibody (aIL-6R) (A), or 100 nM dasatinib (dasa), 100 nM sirolimus (siro), 100 nM temsirolimus (temsi), 100 nM everolimus (evero) or 100 nM ruxolitinib (ruxo) (B), on
20 CD19-TCB-dependent killing of CTV labelled SUDLH-8 cells in the assay of Figure 73. The killing of CTV labelled SUDLH-8 cells was measured by flow cytometry at 24 hrs using a Live/Dead stain allowing for exclusion of dead cells. Mean of n=3 donors + SD with * $p \leq 0.0332$, ** $p \leq 0.0021$ by 1 way ANOVA (Friedman test).

Figure 75. Effect of 100 nM dexamethasone (dexa), 5 μg/mL anti-TNF-α antibody (aTNF-α) or 5
25 μg/mL anti-IL-6R antibody (aIL-6R) (A, B) or 100 nM dasatinib (dasa), 100 nM sirolimus (siro), 100 nM temsirolimus (temsi), 100 nM everolimus (evero) or 100 nM ruxolitinib (ruxo) (C, D) on CD19-TCB-dependent T cell activation in the assay of Figure 73. The expression of CD25 (A, C) and CD69 (B, D) on CD4+ T cells was measured by flow cytometry at 24 hrs. Mean of n=3 donors + SD with * $p \leq 0.0332$, ** $p \leq 0.0021$ by 1 way ANOVA (Friedman test).

Figure 76. Effect of 100 nM dexamethasone (dexa), 5 μg/mL anti-TNF-α antibody (aTNF-α) or 5 μg/mL anti-IL-6R antibody (aIL-6R) (A, B), or 100 nM dasatinib (dasa), 100 nM sirolimus (siro), 100 nM temsirolimus (temsi), 100 nM everolimus (evero) or 100 nM ruxolitinib (ruxo) (C, D), on CD19-TCB-dependent T cell activation in the assay of Figure 73. The expression of CD25 (A, C) and CD69 (B, D) on CD8+ T cells was measured by flow cytometry at 24 hrs. Mean of n=3 donors + SD with * $p \leq 0.0332$, ** $p \leq 0.0021$ by 1 way ANOVA (Friedman test).

Figure 77. Effect of 100 nM dexamethasone (dexa), 5 μg/mL anti-TNF-α antibody (aTNF-α) or 5 μg/mL anti-IL-6R antibody (aIL-6R) (A-D), or 100 nM dasatinib (dasa), 100 nM sirolimus (siro), 100 nM temsirolimus (temsi), 100 nM everolimus (evero) or 100 nM ruxolitinib (ruxo) (E-H), on CD19-TCB-dependent cytokine release in the assay of Figure 73. The levels of IFN-γ (A, E), IL-2 (B, F), TNF-α (C, G) and GM-CSF (D, H) were measured in the supernatants by Luminex (24 hrs). Mean of n=3 donors + standard error of mean (SEM).

Figure 78. *In vitro* killing assay set-up. PBMCs were co-cultured with CTV labelled NALM-6 cells (E:T=10:1) in the presence of escalating concentrations of CD19-TCB for 24 hrs. At 24 hrs, the culture medium was supplemented with 100 nM dasatinib, 100 nM sirolimus or 100 nM ruxolitinib.

Figure 79. Effect of 100 nM dasatinib (dasa), 100 nM sirolimus (siro), 100 nM ruxolitinib (ruxo) on CD19-TCB-induced tumor cell killing when added in the system after 24 hrs of activation in the assay of Figure 78. The killing of CTV labelled NALM-6 cells was measured by flow cytometry at 24 hrs and 48 hrs using a Live/Dead stain allowing exclusion of dead cells. 1 representative donor out of 2.

Figure 80. Effect of 100 nM dasatinib (dasa), 100 nM sirolimus (siro), 100 nM ruxolitinib (ruxo) on CD19-TCB-induced cytokine release when added in the system after 24 hrs of activation in the assay of Figure 78. The levels of IFN-γ (A), TNF-α (B), IL-2 (C) and IL-6 (D) were measured in the supernatants by Luminex (24 hrs and 48 hrs). 1 representative donor out of 2.

Figure 81. *In vivo* experiment timelines and dosing schedule. Humanized NSG mice were co-treated with 0.5 mg/kg CD19-TCB (i.v.) and (i) 6x 50 mg/kg dasatinib (p.o.), (ii) 6 x 30 mg/kg ruxolitinib (p.o), (iii) 4 x 5 mg/kg sirolimus (p.o.), (iv) 2 x 1 mg/kg, 1 x 0.5 mg/kg and 1x 0.25 mg/kg dexamethasone (p.o), or (v) 2 x 10 mg/kg , 1 x 5 mg/kg, 1 x 2.5 mg/kg methylprednisolone

(p.o.), or pre-treated with 30 mg/kg obinutuzumab (Gazyva®) (GpT) (i.v.) and then treated with 0.5 mg/kg CD19-TCB (i.v.).

Figure 82. Effect of obinutuzumab (Gazyva®) pre-treatment (GpT), ruxolitinib (ruxo), dasatinib (dasa), sirolimus (siro), dexamethasone (dexa) and methylprednisolone (MP) on CD19-TCB induced B cell depletion in the experiment described in Figure 81. CD20+ B cell count was measured by flow cytometry in blood collected 48 hrs (A) and 72 hrs (B) post-treatment with CD19-TCB. Mean of n= 4 mice or n= 3 mice (dexa, MP and GpT) +/-SEM with * $p \leq 0.0332$, ** $p \leq 0.0021$ by 1 way ANOVA (Kruskal wallis test).

Figure 83. Effect of obinutuzumab (Gazyva®) pre-treatment (GpT), ruxolitinib (ruxo), dasatinib (dasa), sirolimus (siro), dexamethasone (dexa) and methylprednisolone (MP) on CD19-TCB induced B cell depletion in the experiment described in Figure 81. CD20+ B cell count was measured by flow cytometry in spleen collected at termination, 72 hrs post-treatment with CD19-TCB. Mean of n= 4 mice or n= 3 mice (vehicle, dexa, MP and GpT) +/-SEM.

Figure 84. Effect of obinutuzumab (Gazyva®) pre-treatment (GpT), ruxolitinib (ruxo), dasatinib (dasa), sirolimus (siro), dexamethasone (dexa) and methylprednisolone (MP) on CD19-TCB induced cytokine release in the experiment described in Figure 81. The levels of human IFN-γ (hIFN-γ) (A), human IL-2 (hIL-2) (B), human TNF-α (hTNF-α) (C), human IL-6 (hIL-6) (D) were measured by Luminex in serum collected 6 hrs post-treatment with CD19-TCB. Mean of n= 4 mice or n= 3 mice (dexa, MP and GpT) +/-SEM.

Figure 85. Real time killing of MKN45 NucLightRed (NLR) cells by 10 nM CEA-TCB in the presence of escalating concentrations of fedratinib ranging from 0 nM to 1000 nM. MKN45 NLR target cells were co-cultured with PBMCs (E:T = 50 000 PBMCs : 5000 target cells) in medium supplemented with 10 nM CEA-TCB and fedratinib. The killing was followed using an Incucyte® (1 scan every 3 hours, zoom 10 x, phase and red 400 ms acquisition time). Killing [%] was measured by normalizing total red area with values at t = 0 hour and target cells + PBMCs + fedratinib control wells for each time point. Means of technical replicates +/- SEM for 1 donor.

Figure 86. Effect of escalating concentrations of fedratinib (0-1000 nM) on CD25 (B, D) and CD69 (A, C) expression on CD4+ (A, B) and CD8+ (C, D) T cells at 72 hours, after treatment with 10 nM CEA-TCB. Technical replicates were pooled and the expression of CD25 on CD4+ and CD8+ T cells was measured by flow cytometry at 72 hours. 1 donor.

Figure 87. Effect of escalating concentrations (0-1000 nM) of fedratinib on IFN-γ (A), IL-2 (B), TNF-α (C), IL-6 (D) and IL-8 (E) levels induced by 10 nM CEA-TCB. At 72 hours, the supernatants from technical replicates were pooled and the cytokine levels were analyzed by Luminex. 1 donor.

5 **Figure 88.** CD19-TCB kills lymphoma PDX cells *in vitro*. Lymphoma PDX cells were thawed on the day of the assay, labelled with the CTV dye and cultured with PBMCs (E:T=10:1) in the presence of CD19-TCB for 24 hrs. (**A**) Killing of CTV labelled PDX cells was measured by flow cytometry in pooled technical replicates, mean of n=3 PBMCs donors +/-SD. (**B-E**) The expression of CD69 (**B**, **D**) and CD25 (**C**, **E**) on CD4+ (**B**, **C**) and CD8+ (**D**, **E**) T cells was 10 measured by flow cytometry as a readout for T cell activation, pooled technical replicates, mean of n=3 PBMCs donors +/- SD.

Figure 89. *In vivo* experiment timelines and dosing schedule. Humanized NSG mice were engrafted with a lymphoma PDX (5 million cells, s.c.). When tumors reached 200 mm^3 in size, mice were randomized in groups of 8 or 7 based on their tumor size and treated weekly with vehicle 15 (i.v.), 0.5 mg/kg CD19-TCB (i.v.) alone, 0.5 mg/kg CD19-TCB (solid black arrows, i.v.) together with 20 mg/kg dasatinib ("Srci", dotted arrows, p.o), 5 mg/kg sirolimus ("mTORi", dashed arrows, p.o.), 30 mg/kg ruxolitinib ("JAKi", dotted arrows, p.o), 2 times 1 mg/kg, 0.5 mg/kg or 4 times 0.25 mg/kg dexamethasone ("dexa", dashed arrows, p.o), kinase inhibitors and dexamethasone alone, or pre-treated with 30 mg/kg obinutuzumab ("GpT", solid grey arrow, i.v.) 3 days before 20 the first treatment with CD19-TCB. The kinase inhibitors were given twice on the day of the first CD19-TCB administration (D16, once 1 hour before CD19-TCB, and once afterwards), and then once (sirolimus) or twice (dasatinib, ruxolitinib) on the two following days (D17 and D18), as well as once 1 hour before each subsequent CD19-TCB administration.

Figure 90. Tumor growth curves of sirolimus alone or combined with CD19-TCB in comparison 25 to vehicle, obinutuzumab pre-treatment (GpT) or CD19-TCB as a monotherapy. Tumor volumes were measured using a caliper two or three times per week, mean of n= 6-8 mice + SD with * $p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$ by 1 way ANOVA (Kruskal Wallis test).

Figure 91. Tumor growth curves of ruxolitinib alone or combined with CD19-TCB in comparison to vehicle, obinutuzumab pre-treatment (GpT) or CD19-TCB as a monotherapy. Tumor volumes 30 were measured using a caliper two or three times per week, mean of n= 6-8 mice + SD with * $p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$ by 1 way ANOVA (Kruskal Wallis test).

Figure 92. Tumor growth curves of dasatinib alone or combined with CD19-TCB in comparison to vehicle, obinutuzumab pre-treatment (GpT) or CD19-TCB as a monotherapy. Tumor volumes were measured using a caliper two or three times per week, mean of n= 6-8 mice + SD with * $p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$ by 1 way ANOVA (Kruskal Wallis test).

5 **Figure 93.** Tumor growth curves of dexamethasone alone or combined with CD19-TCB in comparison to vehicle, obinutuzumab pre-treatment (GpT) or CD19-TCB as a monotherapy. Tumor volumes were measured using a caliper two or three times per week, mean of n= 6-8 mice + SD with * $p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$ by 1 way ANOVA (Kruskal Wallis test).

Figure 94. Effect of sirolimus (mTOR inhibitor), ruxolitinib (JAK1/2 inhibitor), dasatinib (Src
10 inhibitor) and dexamethasone on CD19-TCB-mediated release of (A) IL-2, (B) IFN-γ, (C) TNF-α and (D) IL-6. Cytokine levels were measured by Luminex in serum collected 6 hrs post first infusion with CD19-TCB. Mean of n= 6-8 mice +SD with * $p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$ by 1 way ANOVA (Kruskal Wallis test).

Figure 95. *In vivo* experiment timelines and dosing schedule. Humanized NSG mice were treated
15 with vehicle or 0.15 mg/kg CD20-TCB (i.v.) alone or in combination with different doses of mTOR inhibitors (p.o.) (2, 5 or 10 mg/kg sirolimus, 10 mg/kg temsirolimus and 10 mg/kg everolimus), JAK inhibitor (p.o.) (30 or 60 mg/kg ruxolitinib) and Src inhibitor (p.o.) (10 or 50 mg/kg dasatinib) or pre-treated with 30 mg/kg obinutuzumab (Gazyva®) (GpT) (i.v.). n=4 mice per group.

20 **Figure 96.** Effect of obinutuzumab (Gazyva®) pre-treatment (GpT), ruxolitinib, dasatinib, sirolimus, everolimus, temsirolimus on CD20-TCB induced B cell depletion at 48 hrs (A) and 72 hrs (B) in the experiment described in Figure 95. The proportion of CD19+ B cells among human CD45+ (huCD45) cells was measured by flow cytometry in the blood collected 48 hrs and 72 hrs post-treatment with CD20-TCB. Mean of n=4 mice or n=3 mice (everolimus group) +/-SEM. The
25 statistical comparison to the vehicle group is summarized table (C) where the p values were calculated by Kruskal-Wallis test.

Figure 97. Levels of IFN-γ in the serum of the mice from the experiment described in Figure 95, 4 hrs (A) and 24 hrs (B) after treatment with CD20-TCB alone or in combination with mTOR inhibitors (sirolimus, temsirolimus and everolimus), JAK inhibitor (ruxolitinib), Src inhibitor

(dasatinib) or obinutuzumab (Gazyva®) pre-treatment (GpT). Mean of n=4 mice +/- SEM, or n=3 mice +/- SEM (everolimus group) at 24 hrs.

Figure 98. Levels of IL-2 in the serum of the mice from the experiment described in Figure 95, 4 hrs (A) and 24 hrs (B) after treatment with CD20-TCB alone or in combination with mTOR inhibitors (sirolimus, temsirolimus and everolimus), JAK inhibitor (ruxolitinib), Src inhibitor (dasatinib) or obinutuzumab (Gazyva®) pre-treatment (GpT). Mean of n=4 mice +/- SEM, or n=3 mice +/- SEM for (everolimus group) at 24 hrs.

Figure 99. Levels of TNF-α in the serum of the mice from the experiment described in Figure 95, 4 hrs (A) and 24 hrs (B) after treatment with CD20-TCB alone or in combination with mTOR inhibitors (sirolimus, temsirolimus and everolimus), JAK inhibitor (ruxolitinib), Src inhibitor (dasatinib) or obinutuzumab (Gazyva®) pre-treatment (GpT). Mean of n=4 mice +/- SEM, or n=3 mice +/- SEM (everolimus group) at 24 hrs.

Figure 100. Levels of IL-6 in the serum of the mice from the experiment described in Figure 95, 4 hrs (A) and 24 hrs (B) after treatment with CD20-TCB alone or in combination with mTOR inhibitors (sirolimus, temsirolimus and everolimus), JAK inhibitor (ruxolitinib), Src inhibitor (dasatinib) or obinutuzumab (Gazyva®) pre-treatment (GpT). Mean of n=4 mice +/- SEM, or n=3 mice +/- SEM (everolimus group) at 24 hrs.

Figure 101. Levels of IP-10 (CXCL10) in the serum of the mice from the experiment described in Figure 95, 4 hrs (A) and 24 hrs (B) after treatment with CD20-TCB alone or in combination with mTOR inhibitors (sirolimus, temsirolimus and everolimus), JAK inhibitor (ruxolitinib), Src inhibitor (dasatinib) or obinutuzumab (Gazyva®) pre-treatment (GpT). Mean of n=4 mice +/- SEM, or n=3 mice +/- SEM (everolimus group) at 24 hrs.

Figure 102. Levels of MCP-1 (CCL2) in the serum of the mice from the experiment described in Figure 95, 4 hrs (A) and 24 hrs (B) after treatment with CD20-TCB alone or in combination with mTOR inhibitors (sirolimus, temsirolimus and everolimus), JAK inhibitor (ruxolitinib), Src inhibitor (dasatinib) or obinutuzumab (Gazyva®) pre-treatment (GpT). Mean of n=4 mice +/- SEM, or n=3 mice +/- SEM (everolimus group) at 24 hrs.

Figure 103. Levels of IL-8 in the serum of the mice from the experiment described in Figure 95, 4 hrs (A) and 24 hrs (B) after treatment with CD20-TCB alone or in combination with mTOR inhibitors (sirolimus, temsirolimus and everolimus), JAK inhibitor (ruxolitinib), Src inhibitor

(dasatinib) or obinutuzumab (Gazyva®) pre-treatment (GpT). Mean of n=4 mice +/- SEM, or n=3 mice +/- SEM (everolimus group) at 24 hrs.

Figure 104. Levels of GM-CSF in the serum of the mice from the experiment described in Figure 95, 4 hrs (A) and 24 hrs (B) after treatment with CD20-TCB alone or in combination with mTOR inhibitors (sirolimus, temsirolimus and everolimus), JAK inhibitor (ruxolitinib), Src inhibitor (dasatinib) or obinutuzumab (Gazyva®) pre-treatment (GpT). Mean of n=4 mice +/- SEM, or n=3 mice +/- SEM (everolimus group) at 24 hrs.

Examples

The following are examples of methods and compositions of the invention. It is understood that various other aspects may be practiced, given the general description provided above.

Example 1. mTOR inhibitor sirolimus prevents TCB-mediated cytokine release with minimal impact on TCB-mediated target cell killing.

To assess the inhibitory effect of sirolimus on TCB-mediated target-cell killing, we conducted killing assays using peripheral blood mononuclear cells (PBMCs), MKN45 NucLight Red (NLR) target cells and 10 nM CEA-TCB (SEQ ID NOs 4-23) in media supplemented with escalating concentrations of sirolimus (**Figure 1**). The Incucyte® system (Essen Bioscience) was used to capture the loss of red fluorescent protein signal over time as a readout of target cell killing. Doses of sirolimus ranging from 1 µM (~915 ng/mL) to 12.5 nM (~11.4 ng/mL) only partially reduced MKN45 NLR target cell killing by 10 nM CEA-TCB (**Figure 2A** and **Figure 3A**).

At assay endpoint (72h), PBMCs were stained with a live/dead stain in order to verify the impact of sirolimus on PBMC viability. At concentrations ranging from 1 µM (~915 ng/mL) to 12.5 nM (~11.4 ng/mL), sirolimus did not have a direct effect on PBMC viability in samples treated with 10 nM CEA-TCB (**Figure 4B**). Expression of CD25 and CD69 on live CD4+ and CD8+ T cells was also measured by flow cytometry as a readout for T cell activation. Sirolimus did not affect expression of CD69 on CD8+ T cells, while it reduced it from ~45% to ~25% on CD4+ T cells at concentrations above 25 nM. At concentration above 25 nM, sirolimus reduced the expression of CD25 from ~45% to ~15% on CD4+ and from ~75% to 40% on CD8+ T cells (**Figure 6**).

Lastly, the levels of cytokines were measured by Luminex in the supernatants of the assay to determine the impact of sirolimus on CEA-TCB-induced cytokine release. In the presence of any concentration of sirolimus, the levels of IFN-γ, TNF-α, IL-2, IL-6, MCP-1, IL-8, IL-10, IL-4 and GM-CSF in samples treated with 10 nM CEA-TCB were found very low in comparison to samples that did not receive any sirolimus treatment (**Figure 8**). Sirolimus concentrations over the full range from 12.5 nM to 1 μM led to comparable impact on CEA-TCB induced cytokine release, showing that sirolimus strongly downregulated cytokine release.

Although sirolimus does not fully inhibit target cell killing and T cell activation triggered by the TCB, it strongly reduces cytokine release even at the lowest doses tested.

A similar experiment was conducted with another TCB. WSU DLCL2 cells were co-cultured together with PBMCs in the presence of 1 nM CD20-TCB (SEQ ID NOs 4-11, 24-35), and escalating sirolimus doses ranging from 0 nM to 1000 nM (**Figure 13**). The killing of CTV labelled WSU target cells (**Figure 14C**) as well as the expression of CD25 and CD69 on CD4+ and CD8+ T cells (**Figure 17**) was measured at 24h as a readout of the effect of sirolimus on TCB efficacy and T cell activation, respectively. Lastly, the levels of IFN-γ, IL-2, TNF-α, GM-CSF and IL-6 were measured by Luminex (**Figure 21**) to assess the impact of escalating sirolimus concentrations on CD20-TCB-induced cytokine release. In line with the findings with CEA-TCB, sirolimus did not fully inhibit CD20-TCB-mediated target cell killing and T cell activation, while it strongly reduced cytokine release induced by CD20-TCB at concentrations above 12.5 nM.

Example 2. mTOR inhibitor temsirolimus is prevents TCB-mediated cytokine release with minimal impact on TCB-mediated target cell killing.

To assess the inhibitory effect of temsirolimus on TCB-mediated target cell killing, we conducted killing assays using peripheral blood mononuclear cells (PBMCs), NucLight Red (NLR) target cells and 10 nM CEA-TCB in media supplemented with escalating concentrations of temsirolimus (**Figure 1**). The Incucyte® system (Essen Bioscience) was used to capture the loss of red fluorescent protein signal over time as a readout of target cell killing. Doses of temsirolimus ranging from 1 μM (~1031 ng/mL) to 12.5 nM (~12.9 ng/mL) only partially reduced MKN45 NLR target cell killing by 10 nM CEA-TCB (**Figure 2C** and **Figure 3C**).

At assay endpoint (72h), PBMCs were stained with a live/dead stain in order to verify the impact of temsirolimus on PBMC viability. At concentrations ranging from 1 μM (~1031 ng/mL) to 12.5 nM (~12.9 ng/mL), temsirolimus did not have a direct effect on PBMC viability in samples treated with 10 nM CEA-TCB (**Figure 4C**). Expression of CD25 and CD69 on live CD4+ and CD8+ T cells was also measured by flow cytometry as a readout for T cell activation. Temsirolimus did not affect expression of CD69 on CD8+ T cells, while it reduced it from ~45% to ~25% on CD4+ T cells at concentrations above 25 nM. At concentrations above 25 nM, temsirolimus reduced the expression of CD25 from ~45% to ~15% on CD4+ and from ~75% to 40% on CD8+ T cells (**Figure** 7).

The levels of cytokines were measured by Luminex in the supernatants of the assay to determine the impact of temsirolimus on CEA-TCB-induced cytokine release. In the presence of any concentration of temsirolimus, the levels of IFN-γ, TNF-α, IL-2, IL-6, MCP-1, IL-8, IL-10, IL-4 and GM-CSF in samples treated with 10 nM CEA-TCB were found very low in comparison to samples that did not receive any temsirolimus treatment (**Figure 8**). Temsirolimus concentrations over the full range from 12.5 nM to 1 μM led to comparable impact on CEA-TCB induced cytokine release, showing that temsirolimus strongly downregulated cytokine release.

Although temsirolimus does not fully inhibit target cell killing and T cells activation triggered by the TCB, it strongly reduces cytokine release even at the lowest doses tested.

A similar experiment was conducted with CD20-TCB. WSU DLCL2 cells were co-cultured together with PBMCs in the presence of 1 nM CD20-TCB, and escalating temsirolimus doses ranging from 0 nM to 1000 nM (**Figure 13**). The killing of CTV labelled WSU target cells (**Figure 14B**) as well as the expression of CD25 and CD69 on CD4+ and CD8+ (**Figure 16**) was measured at 24h as a readout of the effect of temsirolimus on TCB efficacy and T cell activation, respectively. Lastly, the levels of IFN-γ, IL-2, TNF-α, GM-CSF and IL-6 were measured by Luminex (**Figure 20**) to assess the impact of escalating temsirolimus concentrations on CD20-TCB-induced cytokine release. In line with the findings with CEA-TCB, temsirolimus did not fully inhibit CD20-TCB-mediated target cell killing and T cell activation, while it strongly reduced cytokine release induced by CD20-TCB at concentrations above 12.5 nM.

Example 3. mTOR inhibitor everolimus prevents TCB-mediated cytokine release with minimal impact on TCB-mediated target cell killing.

To assess the inhibitory effect of everolimus on TCB-mediated target cell killing, we conducted killing assays using peripheral blood mononuclear cells (PBMCs), NucLight Red (NLR) target cells and 10 nM CEA-TCB in media supplemented with escalating concentrations of everolimus (**Figure 1**). The Incucyte® system (Essen Bioscience) was used to capture the loss of red fluorescent protein signal over time as a readout of target cell killing. Doses of everolimus ranging from 1 µM (~ 959 ng/mL) to 12.5 nM (~12.0 ng/ mL) only partially reduced MKN45 NLR target cell killing by 10 nM CEA-TCB (**Figure 2B** and **Figure 3B**).

At assay endpoint (72h), PBMCs were stained with a live/dead stain in order to verify the impact of everolimus on PBMC viability. At concentrations ranging from 1 µM (~ 959 ng/mL) to 12.5 nM (~12.0 ng/ mL), everolimus did not have a direct effect on PBMC viability in samples treated with 10 nM CEA-TCB (**Figure 4A**). Expression of CD25 and CD69 on live CD4+ and CD8+ T cells was also measured by flow cytometry as a readout for T cell activation. Everolimus did not affect expression of CD69 on CD8+ T cells, while it reduced it from ~45% to ~25% on CD4+ T cells at concentrations above 25 nM. At concentrations above 25 nM, everolimus reduced the expression of CD25 from ~45% to ~15% on CD4+ and from ~70% to 40% on CD8+ T cells (**Figure 5**).

The levels of cytokines were measured by Luminex in the supernatants of the assay to determine the impact of everolimus on CEA-TCB-induced cytokine release. In the presence of any concentration of everolimus, the levels of IFN-γ, TNF-α, IL-2, IL-6, MCP-1, IL-8, IL-10, IL-4 and GM-CSF in samples treated with 10 nM CEA-TCB were found very low in comparison to samples that did not receive any everolimus treatment (**Figure 8**). Everolimus concentrations over the full range from 12.5 nM to 1 µM led to comparable impact on CEA-TCB induced cytokine release, showing that everolimus strongly downregulated cytokine release.

Although everolimus does not fully inhibit target cell killing and T cell activation triggered by the TCB, it strongly reduces cytokine release even at the lowest doses tested.

A similar experiment was conducted with CD20-TCB. WSU DLCL2 cells were co-cultured together with PBMCs in the presence of 1 nM CD20-TCB, and escalating everolimus doses ranging from 0 nM to 1000 nM (**Figure 13**). The killing of CTV labelled WSU target cells (**Figure**

14D) as well as the expression of CD25 and CD69 on CD4+ and CD8+ (**Figure 18**) was measured at 24h as a readout of the effect of everolimus on TCB efficacy and T cell activation, respectively. Lastly, the levels of IFN-γ, IL-2, TNF-α, GM-CSF and IL-6 were measured by Luminex (**Figure 22**) to assess the impact of escalating everolimus concentrations on CD20-TCB-induced cytokine release. In line with the findings with CEA-TCB, everolimus did not fully inhibit CD20-TCB-mediated target cell killing and T cell activation, while it strongly reduced cytokine release induced by CD20-TCB for concentrations above 12.5 nM.

Example 4. JAK1/2 inhibitor ruxolitinib prevents TCB-mediated cytokine release with minimal impact on TCB-mediated target cell killing.

To assess the inhibitory effect of ruxolitinib on TCB-mediated target cell killing, we conducted killing assays using peripheral blood mononuclear cells (PBMCs), MKN45 NucLight Red (NLR) target cells and 10 nM CEA-TCB in media supplemented with escalating concentrations of ruxolitinib (**Figure 1**). The Incucyte® system (Essen Bioscience) was used to capture the loss of red fluorescent protein signal over time as a readout of target cell killing. Doses of ruxolitinib ranging from 100 nM (~30.7 ng/mL) to 6.25 nM (~1.9 ng/mL) only partially reduced MKN45 NLR target cell killing by 10 nM CEA-TCB (**Figure 9A** and **B**).

At assay endpoint (69h), PBMCs were stained with a live/dead stain in order to verify the impact of ruxolitinib on PBMC viability. At concentration ranging from 100 nM (~30.7 ng/mL) to 6.25 nM (~1.9 ng/mL), ruxolitinib did not have a direct effect on PBMC viability in samples treated with 10 nM CEA-TCB (**Figure 10**). Expression of CD25 and CD69 on live CD4+ and CD8+ T cells was also measured by flow cytometry as a readout for T cell activation. Ruxolitinib dose-dependently influenced the expression of CD25 and CD69 on both CD4+ and CD8+ T cells (**Figure 11**). The effect of escalating doses of ruxolitinib on CD69 expression on CD4+ and CD8+ T cells was less pronounced than the effect on CD25 expression on CD4+ and CD8+ T cells.

The levels of cytokines were measured by Luminex in the supernatants of the assay to determine the impact of ruxolitinib on CEA-TCB-induced cytokine release. In the presence of escalating doses of ruxolitinib, the levels of IFN-γ, TNF-α, IL-2, IL-6, MCP-1, IL-8, IL-10, IL-4 and GM-CSF in samples treated with 10 nM CEA-TCB were found very low in comparison to samples that

did not receive any ruxolitinib treatment (**Figure 12**). Ruxolitinib strongly downregulated overall CEA-TCB induced cytokine release.

Although ruxolitinib does not fully inhibit target cell killing of and T cell activation triggered by the TCB, it strongly reduces cytokine release even at the lowest doses tested.

5 A similar experiment was conducted with CD20-TCB. WSU DLCL2 cells were co-cultured together with PBMCs in the presence of 1 nM CD20-TCB, and escalating ruxolitinib doses ranging from 0 nM to 1000 nM (**Figure 13**). The killing of CTV labelled WSU target cells (**Figure 14A**) as well as the expression of CD25 amd CD69 on CD4+ and CD8+ (**Figure 15**) was measured at 24h as a readout of the effect of ruxolitinib on TCB efficacy and T cell activation, respectively.
10 Lastly, the levels of IFN-γ, IL-2, TNF-α, GM-CSF and IL-6 were measured by Luminex (**Figure 19**) to assess the impact of escalating sirolimus concentrations on CD20-TCB-induced cytokine release. Ruxolitinib did not fully inhibit CD20-TCB-mediated target cell killing and T cell activation while it strongly reduced IL-6 and IFN-γ release induced by CD20-TCB for concentrations above 25 nM nM. Unlike mTOR inhibitors, ruxolitinib did not have a strong effect
15 on the release of TNF-α, IL-2 and GM-CSF.

Example 5. Effect of mTOR inhibitors (temsirolimus, sirolimus and everolimus) and JAK inhibitors (ruxolitinib) vs. anti-TNF-α antibodies, anti-IL-6R antibodies, dexamethasone and dasatinib on TCB-mediated target killing, T cell activation and cytokine release.

20 To assess the impact of mTOR inhibitors (temsirolimus, sirolimus and everolimus) and JAK inhibitors (ruxolitinib) as compared to anti-TNF-α antibodies, anti-IL-6R antibodies, dexamethasone and dasatinib on TCB-mediated target cell killing, we conducted killing assays using peripheral blood mononuclear cells (PBMCs), MKN45 NucLight Red (NLR) target cells and 10 nM CEA-TCB in media supplemented with the different compounds (**Figure 1**). The
25 Incucyte® system (Essen Bioscience) was used to capture the loss of red fluorescent protein signal over time as a readout of target-cell killing. Neutralizing anti-TNF-α and anti-IL-6R antibodies (5 μg/ml) did not impact kinetics of target cell killing, nor maximal target cell killing in comparison the TCB alone. 50 nM JAK inhibitor (ruxolitinib), 50 nM mTOR inhibitors (temsirolimus, sirolimus and everolimus) had similar impact on target cell killing like 1 μM and 0.1 μM

dexamethasone, and the addition of 50 nM dasatinib fully switched off TCB-induced target cell killing. (**Figure 23**).

At assay endpoint (66h), expression of CD25 and CD69 on CD4+ and CD8+ T cells was measured by flow cytometry to assess the impact of the different compounds on T cell activation. While the mTOR inhibitors (temsirolimus, sirolimus and everolimus) and the JAK inhibitor (ruxolitinib) had a milder impact on CD25 and CD69 expression on CD4+ and CD8+ T cells than dexamethasone or dasatinib, the anti-TNF-α and anti-IL-6R antibodies did not affect T cell activation (**Figure 24** and **Figure 25**).

The levels of cytokines were measured by Luminex in the supernatants of the assay to determine the impact of the different compounds on CEA-TCB-induced cytokine release. The use of kinase inhibitors and dexamethasone led to an overall decrease of the TCB-induced cytokine release in comparison to neutralizing antibodies toward specific cytokines like TNF-α and IL-6 (**Figure 26**). Dasatinib fully prevented CEA-TCB-induced cytokine release as well as T cell activation and target cell killing, while the mTOR inhibitors (temsirolimus, sirolimus and everolimus) and the JAK inhibitor (ruxolitinib) independently inhibited cytokine release with milder effect on T cell activation and target cell killing. The JAK1/2 inhibitor ruxolitinib reduced IL-2 levels less strongly that the mTOR inhibitors (temsirolimus, sirolimus and everolimus) (**Figure 26B**).

The effect of the mTOR inhibitors (temsirolimus, sirolimus and everolimus) and the JAK inhibitor (ruxolitinib) on CEA-TCB-induced cytokine release is comparable to dexamethasone and dasatinib, and stronger as compared to anti-TNF-α and anti-IL-6R antibodies. On the other hand, the mTOR and JAK inhibitors have lower impacts on killing efficacy and T cell activation as compared to dasatinib.

A similar experiment was conducted with another TCB. WSU cells were co-cultured together with PBMCs in the presence of escalating doses of CD20-TCB, and mTOR inhibitors (temsirolimus, sirolimus, everolimus), JAK inhibitor (ruxolitinib), dasatinib, dexamethasone, anti-TNF-α antibody or anti-IL-6R antibody. The killing of B cells was measured as a readout for the impact of the various compounds on TCB efficacy at 24h (**Figure 27** and **28**). The expression of CD25 and CD69 on CD4+ and CD8+ T cells was measured by flow cytometry as a readout for T cell activation at 24h (**Figure 29**, **30** and **31**). Lastly, the levels of cytokines (TNF-α, IFN-γ, IL-2, IL-6, IL-4, IL-10, GM-CSF and IL-1β) were analyzed by Luminex at assay endpoint to assess the impact of the different treatments on TCB-induced cytokine release (**Figure 32** and **33**). As seen

with CEA-TCB, mTOR (temsirolimus, sirolimus and everolimus) and JAK (ruxolitinib) inhibitors have a comparable effect to dexamethasone and dasatinib on CD20-TCB-induced cytokine release and a stronger effect than anti-TNF-α and anti-IL-6R antibodies in reducing overall cytokine release. Unlike dasatinib, mTOR (temsirolimus, sirolimus and everolimus) and JAK (ruxolitinib) inhibitors did neither prevent the killing of B cells, nor switch off T cell activation induced by CD20-TCB, suggesting that they do not strongly influence the efficacy of the TCB.

In summary, mTOR and JAK inhibitors have lower impacts on killing efficacy and T cell activation than dasatinib (a Src inhibitor) or dexamethasone. Instead, their impact on killing efficacy and T cell activation is rather comparable impact to anti-TNF-α or anti-IL-6R antibodies. On the other hand, mTOR and JAK inhibitors, like dexamethasone and dasatinib, decrease cytokine release more potently than anti-TNF-α or anti-IL-6R antibodies. The differential activities of mTOR and JAK inhibitors show an uncoupling of TCB-induced cytokine release and cytotoxicity, suggesting these compounds may be attractive alternatives or complements to steroids or IL-6/IL-6R blockade for the mitigation of CRS.

Example 6. Effect of sirolimus, temsirolimus, everolimus and ruxolitinib on CD20-TCB-induced cytokine release from pre-activated effector cells

To assess whether mTOR inhibitors (temsirolimus, sirolimus and everolimus) and JAK inhibitor (ruxolitinib) can prevent further release of cytokine induced by the treatment of CD20-TCB, they were added in an *in vitro* killing assay after 18 hours of activation. In this assay, CTV labelled WSU DLCL2 tumors cells were co-cultured with PBMCs in the presence of escalating doses of CD20-TCB for 18 hours. At 18 hours, 100 nM ruxolitinib, 100 nM temsirolimus, 100 nM sirolimus or100 nM everolimus were added in the system (**Figure 34**). To verify if T cells were activated before the addition of 100 nM ruxolitinib, 100 nM temsirolimus, 100 nM sirolimus or100 nM everolimus, tumor cell killing, T cell activation and cytokine release were measured at 18h. To assess the impact of the addition 100 nM ruxolitinib, 100 nM temsirolimus, 100 nM sirolimus or 100 nM everolimus on TCB-induced cytokine release with respect to their impact on TCB efficacy; cytokine release, T cell activation and tumor cell killing were measured at 44 hours.

At 18 hours, treatment with CD20-TCB resulted in killing of CTV labelled WSU DLCL2 tumor cells (**Figure 35**) as well as upregulation of CD25 on both CD4+ and CD8+ T cells (**Figure 36**),

indicating that T cells were activated before the addition of the different inhibitors in the assay system. The addition of 100 nM ruxolitinib, 100 nM temsirolimus, 100 nM sirolimus or 100 nM everolimus did not impact CTV labelled WSU DLCL2 tumor cell killing (**Figure 37**) and T cell activation (**Figure 38**) measured at 44 hours. However, the addition of mTOR inhibitors 5 (temsirolimus, sirolimus and everolimus) stopped IL-2, IFN-γ, IL-6 and GM-CSF release and, to a lower extent, the release of TNF-α and IL-1β between 18 hours and 44 hours (**Figure 39**). The addition of JAK inhibitor (ruxolitinib) prevented further production of IFN-γ, IL-6 and and, to a lower extent, production of GM-CSF and TNF-α but did not prevent further production of IL-2 between 18 hours and 44 hours (**Figure 39**).

10 Furthermore, these results were confirmed for three donors at a fixed CD20-TCB concentration of 1 nM. The addition of 100 nM ruxolitinib, 100 nM temsirolimus, 100 nM sirolimus or 100 nM everolimus did not impact further CTV labelled WSU DLCL2 tumor cell killing (**Figure 40**) and T cell activation (**Figure 41** and **42**) between 18 hours and 44 hours. However, the addition of 100 nM temsirolimus, 100 nM sirolimus or 100 nM everolimus prevented further release of IFN-γ, IL-15 2, IL-6 and to a lower extent TNF-α and IL-1β release between 18 hours and 44 hours (**Figure 43**). The addition of 100 nM ruxolitinib prevented further release of IFN-γ and IL-6 and to a lower extent TNF-α and IL-1β but did not prevent IL-2 release (**Figure 43**).

Overall, these data suggest that mTOR (temsirolimus, sirolimus and everolimus) as well as JAK (ruxolitinib) inhibitors can rapidly switch off CD20-TCB-induced cytokine release from pre-20 activated effector cells while not strongly affecting CD20-TCB efficacy.

Example 7. JAK1/2 inhibitor ruxolitinib prevents TCB-mediated cytokine release with minimal impact on TCB-mediated target cell killing.

The effect of ruxolitinib on TCB-induced tumor cell killing and cytokine release was assessed 25 using an additional TCB, the MAGEA4-TCB (SEQ ID NOs 37-56). Similarly to Example 4, a killing assay was conducted using peripheral blood mononuclear cells (PBMCs), A375 NucLight Red (NLR) target-cells and 8 nM MAGEA4-TCB in media supplemented with escalating concentrations of ruxolitinib ranging from 0 nM to 100 nM. The Incucyte® system (Essen Bioscience) was used to capture the loss of red fluorescent protein signal over time as a readout of 30 killing, allowing to evaluate the effect of ruxolitinib on MAGEA4-TCB-induced target-cell killing.

Lastly, the supernatants were collected at assay endpoint (72 hours) and cytokines were measured by Luminex to assess the effect of escalating concentrations of ruxolitinib on MAGEA4-TCB-induced cytokine release.

While ruxolitinib did not prevent MAGEA4-TCB induced tumor cell killing (**Figure 44**), the levels of IFN-γ (**Figure 45A**), TNF-α (**Figure 45C**), IL-8 (**Figure 45G**), IL-6 (**Figure 45E**), MCP-1 (**Figure 45H**), IL-10 (**Figure 45I**) and IL-1β (**Figure 45F**) were reduced with increasing concentrations of ruxolitinib. The levels of GM-CSF (**Figure 45D**) and IL-2 (**Figure 45B**) were not impacted by ruxolitinib, in line with the data generated with CEA-TCB and CD20-TCB. Hence, the JAK1/2 inhibitor ruxolitinib could represent an attractive approach to mitigate MAGEA4-TCB-induced cytokine release while not affecting its efficacy, nor IL-2 and GM-CSF levels.

Example 8. mTOR inhibitors sirolimus, temsirolimus and everolimus prevent TCB-mediated cytokine release with minimal impact on TCB-mediated target cell killing.

The effect of sirolimus, temsirolimus and everolimus on TCB-induced tumor cell killing and cytokine release was assessed using an additional TCB, the MAGEA4-TCB. Similarly to Examples 1-3, a killing assay was conducted using peripheral blood mononuclear cells (PBMCs), A375 NucLight Red (NLR) target-cells and 8 nM MAGEA4-TCB in media supplemented with escalating concentrations of sirolimus, temsirolimus and everolimus ranging from 0 nM to 100 nM. The Incucyte® system (Essen Bioscience) was used to capture the loss of red fluorescent protein signal over time as a readout of target-cell killing. Lastly, the supernatants were collected at assay endpoint (72 hours) and cytokines were measured by Luminex to assess the effect of escalating concentrations of sirolimus, temsirolimus and everolimus on MAGEA4-TCB-induced cytokine release.

While sirolimus, temsirolimus and everolimus did not prevent MAGEA4-TCB induced tumor cell killing (**Figure 46A, B, C**), the levels of most of the tested cytokines, including IFN-γ (**Figure 47A**), IL-2 (**Figure 47B**), TNF-α (**Figure 47C**), IL-8 (**Figure 47G**), IL-6 (**Figure 47E**), MCP-1(**Figure 47H**) and IL-10 (**Figure 47I**), were reduced with increasing concentrations of sirolimus, temsirolimus and everolimus. In line with the data generated with CEA-TCB and CD20-TCB, the mTOR inhibitors sirolimus, temsirolimus and everolimus could represent an attractive approach to mitigate MAGEA4-TCB-induced cytokine release while not impacting its efficacy.

Example 9. The JAK inhibitor baricitinib can efficiently prevent TCB-induced cytokine release while not affecting its efficacy.

To assess the inhibitory effect of the JAK1/2 inhibitor baricitinib on CD20-TCB-mediated target-cell killing and cytokine release, peripheral blood mononuclear cells (PBMCs) were co-cultured with CTV labelled WSU target-cells and CD20-TCB in media supplemented with escalating concentrations of baricitinib. At 24 hours, tumor cell killing was measured by flow cytometry by exclusion of dead CTV cells. The supernatants were collected and cytokines were measured by Luminex. In addition, the expression of CD69 and CD25 on CD4+ and CD8+ T cells was measured by flow cytometry to assess the impact of baricitinib on T cell activation.

As a result, baricitinib concentrations ranging from 0 nM to 100 nM did not impair CD20-TCB-induced tumor cell killing (**Figure 48** and **49**) and T cell activation, as shown by the expression of CD25 and CD69 on CD4+ (**Figure 50A**, **C** and **51**) and CD8+ (**Figure 50B**, **D** and **52**) T cells. At a higher concentration of 1 μM, baricitinib slightly reduced CD20-TCB induced tumor cell killing. (**Figure 48** and **49**) and T cell activation (**Figure 50A-D**, **51** and **52**). Similarly to the other JAK inhibitor ruxolitinib, escalating doses of baricitinib reduced the levels of IFN-γ, TNF-α, GM-CSF, IL-6 and IL-8 (**Figure 53A**, **C**, **D**, **E** and **F**, **Figure 54A**, **C**, **D**, **E** and **F**) but not IL-2 (**Figure 53B**, **Figure 54B**).

While baricitinib did not prevent the killing of CTV WSU tumor cells and T cell activation triggered by CD20-TCB, it strongly reduced CD20-TCB-induced cytokine release for doses ranging from 12.5 nM to 100 nM. Hence, the JAK inhibitor baricitinib, further to ruxolitinib, could be used to mitigate CD20-TCB-induced cytokine release while not affecting its efficacy.

Example 10. The effect of the JAK inhibitor baricitinib on TCB-induced tumor cell killing, T cell activation and cytokine release is comparable to the JAK inhibitor ruxolitinib.

To verify whether the effect of baricitinib on TCB-induced cytokine release, T cell activation and tumor cell killing is comparable to the effect of ruxolitinib, we conducted killing assays using peripheral blood mononuclear cells (PBMCs), MKN45 NucLight Red (NLR) target-cells and 10 and 1 nM CEA-TCB in media supplemented with escalating concentrations of ruxolitinib and baricitinib. The Incucyte® system (Essen Bioscience) was used to capture the loss of red fluorescent protein signal over time as a readout of target cell killing. At 72 hours, the expression of CD25 on CD4+ and CD8+ T cells was measured by flow cytometry to assess the impact of

baricitinib and ruxolitinib on CEA-TCB-induced T cell activation. Lastly, the supernatants were collected at assay endpoint (72 hours) and cytokines were measured by Luminex to assess the effect of baricitinib vs. ruxolitinib on CEA-TCB-induced cytokine release. The percentage of cytokine inhibition was calculated as a percentage of cytokines found in the absence of kinase
5 inhibitors and allowed to compare the effect of baricitinib and ruxolitinib on CEA-TCB-induced cytokine release.

Both baricitinib and ruxolitinib did not prevent the killing of MKN45 NLR tumor cell induced by 1 nM CEA-TCB (**Figure 55A** and **B**) for escalating concentrations ranging between 0 nM and 100 nM. At a higher concentration of 1 μM, both baricitinib and ruxolitinib partially prevented the
10 killing of MKN45 NLR tumor cells (**Figure 55A** and **B**). Comparably to ruxolitinib, escalating concentrations of baricitinib reduced the expression of CD25 on CD4+ (**Figure 56A**) and CD8+ (**Figure 56B**) T cells, indicating that the effect of baricitinib on CEA-TCB-induced T cell activation is comparable to ruxolitinib. The degree of inhibition of IFN-γ, TNF-α, GM-CSF, IL-6 and IL-8 levels (**Figure 57A**, **C**, **D**, **E** and **F**) was the similar for escalating concentrations of
15 ruxolitinib and baricitinib. In line with what was observed with ruxolitinib, escalating concentrations of baricitinib did not reduce IL-2 (**Figure 57B**).

The comparison of baricitinib to ruxolitinib was done using another TCB, the MAGEA4-TCB. Similarly to CEA-TCB, killing assays were conducted using peripheral blood mononuclear cells (PBMCs), A375 NucLight Red (NLR) target-cells and 25 nM MAGEA4-TCB in media
20 supplemented with escalating concentrations of ruxolitinib and baricitinib ranging from 0 nM to 100 nM. The Incucyte® system (Essen Bioscience) was used to capture the loss of red fluorescent protein signal over time as a readout of target-cell killing. Cytokines were measured by Luminex in the supernatants collected at assay endpoint (72 hours) to assess the effect of baricitinib vs. ruxolitinib on MAGEA4-TCB-induced cytokine release.

25 In line with the data generated with CEA-TCB, the effect of baricitinib (**Figure 58A**) on MAGEA4-TCB-induced tumor cell killing was comparable to ruxolitinib (**Figure 58B**) for escalating concentrations ranging from 0 nM to 100 nM. In addition, the effect of baricitinib (**Figure 59A-F**) on MAGEA4-TCB-induced GM-CSF, IL-2, IFN-γ, IL-2, TNF-α, IL-1β and IL-6 release is also comparable to ruxolitinib (**Figure 60A-F**) for concentrations ranging from 0 nM to
30 100 nM.

In summary, the effect of baricitinib on CEA-TCB and MAGEA4-TCB-induced tumor cell killing, T cell activation and cytokine release is comparable to the effect of ruxolitinib. Thus, further to

ruxolitinib, baricitinib represents an attractive approach for the mitigation of TCB-induced cytokine release.

Example 11. JAK inhibitor ruxolitinib and mTOR inhibitor sirolimus prevent CAR-T cell-induced cytokine release.

To assess the impact of JAK inhibitor ruxolitinib and mTOR inhibitor sirolimus on cytokine release induced by CAR-T cells, we conducted a killing assay where PGLALA and CD16 universal CAR-T cells (CAR-T cells with a CAR comprising an anti-P329G-Fc scFv (binding to PGLALA Fc) or CD16 (binding to wild-type Fc)) were co-cultured with CTV WSU tumor cells in the presence of escalating concentrations of PGLALA Fc and wild-type Fc anti-CD20 IgG in medium supplemented with 100 nM ruxolitinib or 100 nM sirolimus. To verify whether ruxolitinib and sirolimus interfered with tumor cell killing by CAR-T cells, we measured killing of CTV WSU tumor cells by flow cytometry at 24 hours. To verify whether ruxolitinib and sirolimus reduced the cytokine release induced by CAR-T cells, the cytokines were measured by Luminex in the supernatants of the assay at 72 hours.
As a result, neither sirolimus nor ruxolitinib prevented tumor cell killing by PGLALA (**Figure 61A**) and CD16 (**Figure 61B**) CAR-T cells. For both PGLALA (**Figure 62**) and CD16 (**Figure 63**) CAR-T cells, ruxolitinib reduced the release of IFN-γ (**Figure 62B** and **63B**), TNF-α (**Figure 62D** and **63D**) and GM-CSF (**Figure 62A** and **63A**), but not IL-2 (**Figure 62C** and **63C**), in line with findings with TCBs. Finally, sirolimus strongly reduced the release of all cytokines tested, namely IFN-γ (**Figure 62B** and **63B**), TNF-α (**Figure 62D** and **63D**), GM-CSF (**Figure 62A** and **63A**) and IL-2 (**Figure 62C** and **63C**).
Overall, this data suggest that JAK1/2 inhibitor ruxolitinib and mTOR inhibitor sirolimus could be an attractive approach to prevent cytokine release induced by CAR-T cells while not affecting CAR-T cell efficacy.

Example 12. mTOR inhibitors sirolimus, temsirolimus and everolimus and JAK inhibitor ruxolitinib do not prevent CD19-TCB dependent killing and T cell activation while strongly reducing cytokine release.

The effect of the mTOR inhibitors sirolimus, everolimus and temsirolimus and the JAK1/2 inhibitor ruxolitinib on TCB-induced T cell cytotoxicity, T cell activation and cytokine release was assessed using another TCB, CD19-TCB (SEQ ID NOs 5, 7-9, 11, 64-74, 76-78, 80). PBMCs were co-cultured together with CellTrace™ Violet (CTV) labelled SUDLH-8 tumor cells and
5 escalating concentrations of CD19-TCB in the presence of the different kinase inhibitors, including also the Src inhibitor dasatinib (**Figure 64**). At assay endpoint (24 hrs), the killing of CTV labelled SUDLH-8 cells was measured by flow cytometry by exclusion of the dead SUDLH-8 cells with a Live/Dead stain. The expression of CD25 and CD69 on CD4+ and CD8+ T cells was also measured by flow cytometry as a readout for T cell activation. Lastly, the levels of
10 cytokines were measured in the supernatants of the assay to evaluate the effect of mTOR, JAK and Src inhibitors on CD19-TCB-induced cytokine release.

As a result, unlike the Src inhibitor dasatinib, the mTOR and JAK inhibitors did not prevent CD19-TCB-dependent SUDLH-8 killing (**Figure 65A**, **Figure 66**). In line with the effect on killing, mTOR and JAK inhibitors did not block the expression of CD25 and CD69 on CD4+ and CD8+
15 T cells, as opposed to the Src inhibitor dasatinib (**Figure 65B-E**, **Figure 67**). Finally, the JAK1/2 inhibitor ruxolitinib prevented CD19-TCB-induced IFN-γ, TNF-α, IL-6 and GM-CSF release and, to a lower extent, IL-2 release while the mTOR imhibitors strongly reduced the release of all four cytokines (**Figure 68**).

Overall, the differential activities of the mTOR, JAK and Src inhibitors revealed the uncoupling
20 of CD19-TCB-induced T cell cytotoxicity and cytokine release. In addition, these data suggest that the mTOR inhibitors sirolimus, temsirolimus and everolimus as well as the JAK1/2 inhibitor ruxolitinib could mitigate CD19-TCB induced cytokine release while not preventing tumor cell killing and T cell activation. The Src inhibitor dasatinib would rather stand as an antidote for off-tumor toxicities or high grade CRS where a switch-off in T cell functionality would be required to
25 block both cytokine release and killing.

Example 13. JAK inhibitors baricitinib and tofacitinib have comparable effect to ruxolitinib on CD19-TCB induced tumor cell killing, T cell activation and cytokine release.

To compare the effect of the JAK inhibitors baricitinib and tofacitinib to ruxolitinib on CD19-
30 TCB-induced T cell cytoxicity, T cell activation and cytokine release, PBMCs were co-cultured together with CTV labelled SUDLH-8 tumor cells and escalating concentrations of CD19-TCB in the presence of 100 nM ruxolitinib, 100 nM baricitinib and 100 nM tofacitinib (**Figure 69**). At

assay endpoint (24 hrs), the killing of CTV labelled SUDLH-8 cells was measured by flow cytometry by exclusion of the dead SUDLH-8 cells with a Live/Dead stain. The expression of CD25 and CD69 on CD4+ and CD8+ T cells was also measured by flow cytometry as a readout for T cell activation. Lastly, the levels of cytokines were measured in the supernatants of the assay to assess the effect of the JAK inhibitors on CD19-TCB-induced cytokine release.

Similarly to ruxolitinib, baricitinib and tofacitinib did not prevent CD19-TCB dependent killing of CTV labelled SUDLH-8 tumor cells (**Figure 70**), nor did they prevent the expression of CD25 and CD69 on CD4+ (**Figure 71A** and **B**) and CD8+ (**Figure 71C** and **D**) T cells. In addition, the JAK inhibitors baricitinib and tofacitinib prevented CD19-TCB-induced IFN-γ, TNF-α, IL-6 and GM-CSF release and, to a lower extent, IL-2 release (**Figure 72**), comparably to ruxolitinib. Baricitinib and tofacitinib appeared to be as potent as ruxolitinib in preventing CD19-TCB-induced cytokine release while not blocking T cell activation and T cell cytotoxicity *in vitro*.

Finally, this experiment suggests that baricitinib and tofacitinib could represent two additional JAK inhibitors for the mitigation of CD19-TCB-induced cytokine release as alternatives to ruxolitinib.

Example 14. Comparison of JAK inhibitor ruxolitinib, mTOR inhibitors sirolimus, temsirolimus and everolimus, and Src inhibitor dasatinib, to current CRS mitigation approaches.

To verify whether the use of JAK and mTOR inhibitors would be comparable to current approaches used for the mitigation of TCB-induced cytokine release, we compared the effect of the kinase inhibitors to the corticosteroid dexamethasone and to the use of anti-TNF-α and anti-IL-6R antibodies *in vitro*. Therefore, PBMCs were co-cultured together with CTV labelled SUDLH-8 tumor cells and escalating concentrations of CD19-TCB in the presence of the mTOR, JAK and Src inhibitors, as well as dexamethasone, anti-TNF-α antibody (aTNF-α; Biolegend #502922 (antibody Mab11)) and anti-IL-6R antibody (aIL-6R; Roche in-house) (**Figure 73**). At assay endpoint (24 hrs), the killing of CTV labelled SUDLH-8 cells was measured by flow cytometry by exclusion of the dead SUDLH-8 cells with a Live/Dead stain to evaluate the effect of the different mitigation approaches on CD19-TCB-dependent killing. Then, the expression of CD25 and CD69 on CD4+ and CD8+ T cells was also measured by flow cytometry as a readout for the effect on T cell activation. Lastly, the levels of cytokines were measured in the supernatants of the assay to address the effect of the JAK inhibitors on CD19-TCB-induced cytokine release.

Comparably to dexamethasone, anti-TNF-α antibody and anti-IL-6R antibody, the mTOR inhibitors sirolimus, temsirolimus and everolimus, as well as the JAK1/2 inhibitor ruxolitinib, did not prevent CD19-TCB-dependent killing of CTV SUDLH-8 cells – unlike the Src inhibitor dasatinib (**Figure 74**). In addition, the mTOR and JAK inhibitors, as well as dexamethasone, anti-TNF-α and anti-IL-6R, did not prevent the expression of CD25 (**Figure 75A** and **C, Figure 76A** and **C**) and CD69 (**Figure 75B** and **D, Figure 76B** and **D**) on CD4+ and CD8+ T cells, in contrast to the Src inhibitor dasatinib which fully blocked T cell activation. Lastly, the effect of the mTOR inhibitors sirolimus, temsirolimus and everolimus and the JAK1/2 inhibitor ruxolitinib on CD19-TCB-induced IFN-γ and TNF-α was comparable to the effect of dexamethasone (**Figure** 77). The effect of the mTOR inhibitors on CD19-TCB-induced IL-2 and GM-CSF was comparable to the effect of dexamethasone, yet the effect of the JAK1/2 inhibitor ruxolitinib on these two cytokines was weaker (**Figure** 77). The effect of the JAK and mTOR inhibitors in reducing CD19-TCB-induced IFN-γ, IL-2, TNF-α and GM-CSF was stronger than the effect of anti-IL-6R antibody, which only slightly decreased IFN-γ, IL-2, TNF-α and GM-CSF levels, or the effect of anti-TNF-α antibody which specifically reduced TNF-α and to a lower extent IFN-γ, IL-2 and GM-CSF. Finally, the Src inhibitor dasatinib shows complete inhibition of CD19-TCB-induced cytokine release (**Figure** 77), correlating with the inhibition of killing and T cell activation.

In summary, this experiment suggests that the effect of the mTOR inhibitors sirolimus, temsirolimus and everolimus, as well as the JAK1/2 inhibitor ruxolitinib, is comparable to the effect of the corticosteroid dexamethasone to reduce CD19-TCB-dependent cytokine release, while not preventing T cell cytotoxicity and T cell activation. In addition, the effect of the mTOR inhibitors sirolimus, temsirolimus and everolimus and the JAK1/2 inhibitor ruxolitinib on CD19-TCB-induced cytokine release was shown to be stronger than the blockade of IL-6R and TNF-α. Taken together, this data highlights that the JAK and mTOR inhibitors could represent alternative approaches for the mitigation of CD19-TCB-induced cytokine release to the use of anti-IL-6R or anti-TNF-α antibodies or even corticosteroids.

Example 15. Effect of sirolimus (as an exemplary mTOR inhibitor), ruxolitinib (as an exemplary JAK inhibitor) and dasatinib (as an exemplary Src inhibitor) on CD19-TCB-induced cytokine release from pre-activated effector cells.

To assess whether the mTOR inhibitor sirolimus, the JAK1/2 inhibitor ruxolitinib and the Src inhibitor dasatinib can prevent further release of cytokines induced by the treatment of CD19-TCB,

they were added in an *in vitro* killing assay after 24 hrs of activation (**Figure 78**). In this assay CTV labelled NALM-6 tumors cells were co-cultured with PBMCs in the presence of escalating doses of CD19-TCB for 24 hrs. At 24 hrs, 100 nM ruxolitinib, 100 nM sirolimus or 100 nM dasatinib were added in the system. To verify if T cells were activated before the addition of the different kinase inhibitors, tumor cell killing and cytokine release were measured at 24 hrs (**Figure 79** and **80**). Lastly, cytokine release and tumor cell killing were measured at 48 hrs to assess the effect of the addition of 100 nM ruxolitinib, 100 nM sirolimus or 100 nM dasatinib on CD19-TCB-induced cytokine release as compared to the effect on killing.

At 24 hrs, the treatment with CD19-TCB resulted in killing of CTV labelled NALM-6 tumor cells (**Figure 79**) and in the release of IFN-γ (**Figure 80A**), TNF-α (**Figure 80B**), IL-2 (**Figure 80C**) and IL-6 (**Figure 80D**), indicating that T cells were activated before the addition of the kinase inhibitors. The addition of 100 nM ruxolitinib or 100 nM sirolimus did not prevent CTV labelled NALM-6 tumor cell killing (**Figure 79)** measured at 48 hrs, whereas the addition of 100 nM dasatinib moderately reduced the killing of NALM-6 tumor cells. However, the addition of 100 nM sirolimus or 100 nM dasatinib prevented further release of IFN-γ, TNF-α, IL-2 and IL-6 between 24 hrs and 48 hrs (**Figure 80**), while the addition of 100 nM ruxolitinib only prevented further release of IFN-γ, TNF-α and IL-6 and, to a lower extent, IL-2 (**Figure 80**).

Overall, this experiment suggests that the mTOR inhibitor sirolimus as well as the JAK1/2 inhibitor ruxolitinib can rapidly stop CD19-TCB-induced cytokine release from pre-activated effector cells, while not affecting CD19-TCB efficacy. On the other hand, the Src inhibitor dasatinib can rapidly switch off CD19-TCB-induced cytokine release from pre-activated effector cells, while reducing also CD19-TCB-induced T cell cytotoxicity.

Example 16. The effect of JAK inhibitor ruxolitinib, Src inhibitor dasatinib and mTOR inhibitor sirolimus in reducing CD19-TCB induced cytokine release in humanized NSG mice is comparable to the effect of the corticosteroids dexamethasone and methylprednisolone and to the pre-treatment with obinutuzumab.

The effect of the JAK1/2 inhibitor ruxolitinib, the mTOR inhibitor sirolimus, the Src inhibitor dasatinib, the pre-treatment with obinutuzumab (Gazyva®), and the corticosteroids dexamethasone and methylprednisolone on CD19-TCB-induced cytokine release vs. B cell depletion was evaluated *in vivo*. Therefore, humanized NSG mice were either pre-treated with obinutuzumab (Gazyva®) and then treated with 0.5 mg/kg CD19-TCB, or co-treated with 0.5

mg/kg CD19-TCB and (i) 4 x 5 mg/kg sirolimus, (ii) 6 x 30 mg/kg ruxolitinib, (iii) 6 x 50 mg/kg dasatinib, (iv) 2 x 1 mg/kg, 1 x 0.5 mg/kg and 1 x 0.25 mg/kg dexamethasone, or (v) 2 x 10 mg/kg, 1 x 5 mg/kg and 1 x 2.5 mg/kg methylprednisolone (**Figure 81**). To best reproduce the pharmacodynamic profile of ruxolitinib, dasatinib and sirolimus in the clinic and to verify whether the resulting exposure would be sufficient to prevent CD19-TCB-induced cytokine release, dasatinib and ruxolitinib were given per os twice per day, and sirolimus was given per os twice per day on day 1 and then once per day.

At 48 hrs and 72 hrs, blood was collected by tail-vein bleeding and the CD20+ B cell count was measured by flow cytometry (**Figure 82A** and **B**) to assess the effect of the different treatments on CD19-TCB-induced B cell depletion. At 48 hrs and 72 hrs, the effect of the obinituzumab (Gazyva®) pre-treatment (GpT) followed by CD19-TCB treatment lead to a complete depletion of B cells, similarly to the treatment with CD19-TCB alone. On the other hand, ruxolitinib (ruxo) and sirolimus (siro), similarly to dexamethasone (dexa) and methylprednisolone (MP), slightly prevented B cell depletion, with a milder inhibitory effect than the Src inhibitor dasatinib (dasa), not fully preventing CD19-TCB efficacy (**Figure 82A**). At 72 hrs, the effect of ruxolitinib and sirolimus was comparable to the effect dexamethasone but appeared stronger than methylprednisolone in preventing B cell depletion (**Figure 82B**), indicating a small inhibitory effect on the activity of CD19-TCB. At 72 hrs, dasatinib did no longer fully block B cell depletion. Its half-life being around 6-7 hrs and dasatinib being given only twice per day, a lack of exposure could likely explain that CD19-TCB was partially active, resulting in B cell depletion. At experiment termination (72 hrs), spleens were collected and the B cell count was measured by flow-cytometry (**Figure 83**). In line with the observations in the blood, ruxolitinib and sirolimus only partially prevented CD19-TCB-induced CD20+ B cell depletion, comparably to dexamethasone. However, their inhibitory effect was stronger than methylprednisolone (**Figure 83**). At 72 hrs, dasatinib did not prevent CD20+ B cell depletion in the spleen, probably due to a lack of exposure (**Figure 83**). As expected, the pre-treatment with obinutuzumab (Gazyva®) lead to a complete depletion of CD20+ B cells due to the dual activity of the two depleting antibodies (**Figure 83**).

Finally, serum was collected from blood 6 hrs post treatment with CD19-TCB and the cytokine levels were measured by Luminex to assess the effect of the different treatments on CD19-TCB-induced cytokine release (**Figure 84**). The effect of sirolimus, ruxolitinib, dexamethasone and methylprednisolone on CD19-TCB-induced IFN-γ (**Figure 84A**) and IL-6 release (**Figure 84D**) was comparable to the pre-treatement with obinutuzumab (Gazyva®). The effect of sirolimus,

dexamethasone and methylprednisolone on CD19-TCB-induced IL-2 (**Figure 84B**) and TNF-α (**Figure 84C**) was comparable to the pre-treatement with Gazyva. However, the effect of ruxolitinib appeared to be slightly weaker in reducing IL-2 and TNF-α. Overall, the effect of the mTOR and JAK inhibitors was found to be comparable to the effect the corticosteroids dexamethasone and methylprednisolone and to the pre-treatment with obinutuzumab (Gazyva®) in reducing CD19-TCB induced cytokine release with a milder effect of ruxolinib on IL-2 and TNF-α release.

In line with the *in vitro* findings, the co-treatment with ruxolitinib or sirolimus and CD19-TCB allowed to control CD19-TCB-induced cytokine release in humanized NSG mice while not fully preventing B cell depletion, similarly to dexamethasone and methylprednisolone. In addition, the effect of ruxolitinib, sirolimus, dexamethasone and methylprednisolone in preventing CD19-TCB-induced cytokine release was comparable to the pre-treatment with obinutuzumab (Gazyva®), although the latter induced a stronger B cell depletion.

Example 17. The JAK inhibitor fedratinib prevents CEA-TCB-mediated cytokine release with minimal impact on TCB-mediated target cell killing.

To assess the effect of another JAK inhibitor, fedratinib, on TCB-mediated target cell killing, T cell activation and cytokine release, we conducted a killing assay using peripheral blood mononuclear cells (PBMCs), NucLight Red (NLR) MKN45 tumor cells and 10 nM CEA-TCB in media supplemented with escalating concentrations of fedratinib. The Incucyte® system (Essen Bioscience) was used to capture the loss of red fluorescent protein signal over time as a readout of target cell killing. Doses of fedratinib ranging from 12.5 nM to 1 μM only partially reduced MKN45 NLR target cell killing by 10 nM CEA-TCB, with doses below 1 μM having only a minor effect (**Figure 85**).

At assay endpoint (72 hrs), the expression of CD25 and CD69 on live CD4+ and CD8+ T cells was measured by flow cytometry as a readout for T cell activation. Fedratinib did not affect expression of CD69 and CD25 on CD4+ T cells (**Figure 86A, B**) and CD8+ T cells (**Figure 86C, D**) at concentrations below 1 μM.

The levels of cytokines were measured by Luminex in the supernatants of the assay to determine the effect of fedratinib on CEA-TCB-induced cytokine release. In the presence of concentrations of fedratinib ranging from 50 nM to 1 μM, the levels of IFN-γ, IL-2, TNF-α, IL-6 and IL-8 were reduced (**Figure 87**).

Overall, this data suggests that fedratinib reduces CEA-TCB-induced cytokine release while not preventing T cell cytotoxicity and T cell activation.

Exemple 18. mTOR and JAK inhibitors do not suppress CD19-TCB anti-tumor activity in lymphoma PDX bearing mice.

To evalute the impact of mTOR and JAK inhibition in comparison to Src inhibition, dexamethasone or pre-treatment with obinutuzumab (GpT) on CD19-TCB anti-tumor activity, we used a lymphoma patient derived xenograft (PDX) model in humanized NSG mice. First, we verified if the PDX cells were killed upon treatment with CD19-TCB *in vitro*, using a killing assay. The lymphoma PDX cells were co-cultured together with PBMCs (E:T=10:1) in the presence of CD19-TCB. The lymphoma PDX cells were efficiently depleted by CD19-TCB *in vitro* (**Figure 88A**), resulting in T cell activation as shown by the expression of CD25 and CD69 on CD4+ and CD8+ T cells (**Figure 88 B-E**). Then, PDX-bearing mice were treated with either vehicle, sirolimus (5 mg/kg), ruxolitinib (30 mg/kg), dasatinib (20 mg/kg), dexamethasone (2 times 1 mg/kg, 0.5 mg/kg, 4 times 0.25 mg/kg) alone or in combination with CD19-TCB (0.5 mg/kg), CD19-TCB (0.5 mg/kg) as a monotherapy or in combination with obinutuzumab pre-treatment (GpT) (30 mg/kg). The different kinase inhibitors and dexamethasone were given one hour prior to the first treatment with CD19-TCB and then once or twice per day for three days to suppress cytokine release, predominantly occurring upon the first infusion (**Figure 89**). Moreover, they were also administered one hour before each subsequent treatment to prevent residual cytokine secretion (**Figure 89**). Both, dexamethasone and sirolimus, given as a single agent, induced a reduction in tumor growth, yet not significant (**Figure 90** and **93**).When combined with CD19-TCB, the resulting anti-tumor activity was comparable to CD19-TCB alone, however sirolimus and dexamethasone suppressed IL-2, IFN-γ, TNF-α and IL-6 release upon the first infusion (**Figure 90**, **93** and **94**). Similarly, co-treatment with ruxolitinib minimally interfered with CD19-TCB anti-tumor activity and decreased IL-6 and to a lower extent IFN-γ, TNF-α and IL-2 release (**Figure 91** and **94**). The effects of ruxolitinib, sirolimus and dexamethasone on cytokine levels appeared stronger than the effect of obinutuzumab pre-treatment, while their effects on anti-tumor efficacy are similar (**Figure 90**, **91**, **93** and **94**). Also dasatinib did not significantly suppress CD19-TCB anti-tumor efficacy while strongly reducing T-cell derived cytokines (IFN-γ, TNF-α, IL-2 and IL-6) upon the first infusion. This indicates that the transient use of dasatinib in the present

experiment did not continuously block CD19-TCB-induced T cell cytotoxicity, as the inhibitory effect of dasatinib is reversible (**Figure 92** and **94**).

Taken together, this data shows that transient use of the JAK inhibitor ruxolitinib and the mTOR inhibitor sirolimus did not impair anti-tumor efficacy while suppressing T-cell mediated cytokine release upon first infusion of CD19-TCB, supporting the use of these compounds for the mitigation of TCB-related CRS.

Example 19. Effect of ruxolitinib (JAK1/2 inhibitor), mTOR inhibitors (sirolimus, everolimus, temsirolimus) and dasatinib (Src inhibitor) on CD20-TCB-induced cytokine release and B cell depletion in non-tumor bearing humanized NSG mice.

In this experiment, we verified if the *in vivo* combination of short PK/PD properties of small molecule kinase inhibitors with long PK/PD properties of CD20-TCB efficiently switches-off cytokine release in humanized NSG mice. Additionally, we also assessed the effect of JAK and mTOR inhibitors on CD20-TCB-mediated B cell depletion in comparison to the Src inhibitor dasatinib, by measuring CD19+ B cells in the peripheral blood.

Humanized NSG mice were treated with 0.15 mg/kg CD20-TCB alone or in combination with mTOR inhibitors (sirolimus, temsirolimus, everolimus), JAK inhibitor (ruxolitinib), Src inhibitor (dasatinib) or pre-treated with obinutuzumab (Gazyva®), as described **Figure 95**. Different doses of mTOR, JAK and Src kinase inhibitors were tested to determine the optimal dose that reduces CD20-TCB-induced cytokine release while minimally interfering with B cell depletion. The doses of 2, 5 and 10 mg/kg were tested for sirolimus and only the highest dose of 10 mg/kg was used for the other mTOR inhibitors temsirolimus and everolimus. The doses of 30 and 60 mg/kg were tested for ruxolitinib and the doses of 10 and 50 mg/kg were tested for dasatinib. To reproduce the clinical route of administration, the different kinase inhibitors were given orally (p.o.) once or twice per day as depicted in **Figure 95**. Mice were bled 4 hours and 24 hours after the treatment with CD20-TCB (**Figure 95**) to collect serum for cytokine level measurements by Luminex. Additionally, blood was collected 48 hours and 72 hours (termination) post treatment with CD20-TCB to measure the percentage of CD19+ B cells among human CD45+ cells by flow cytometry (**Figure 95**).

As a result, the co-treatment with 2, 5, 10 mg/kg sirolimus, 10 mg/kg everolimus or 10 mg/kg temsirolimus (mTOR inhibitors) did not interfere with B cell depletion induced by CD20-TCB, as indicated by the percentage of CD19+ B cells among human CD45+ cells in the peripheral blood (**Figure 96A-C**). However, mTOR inhibitors durably reduced CD20-TCB-induced cytokine 5 release, as shown by the levels of IFN-γ (**Figure 97 A, B**), IL-2 (**Figure 98 A, B**), TNF-α (**Figure 99 A, B**), IL-6 (**Figure 100 A, B**), IP-10 (**Figure 101 A, B**), MCP-1 (**Figure 102 A, B**), IL-8 (**Figure 103 A, B**) and GM-CSF (**Figure 104 A, B**). Furthermore, the reduction of cytokine release by mTOR inhibitors was comparable to dasatinib and obinutuzumab (Gazyva®) pre-treatment in this model (**Figure 97-104**). Overall, mTOR inhibitors strongly reduced CD20-TCB-mediated 10 cytokine release while retaining B cell depletion at doses ranging from 2 to 10 mg/kg, unlike the Src inhibitor dasatinib that switched-off TCB activity up to 48 hours.

The co-treatment with 30 and 50 mg/kg of ruxolitinib (JAK1/2 inhibitor) slightly interfered with B cell depletion induced by CD20-TCB, as indicated by the percentage of CD19+ B cells among human CD45+ cells in the peripheral blood (**Figure 96A-C**). The inhibitory effect on B cell 15 depletion appears to be dependent on the dose of ruxolitinib. Besides, ruxolitinib durably reduced CD20-TCB-mediated cytokine release, except for IL-2 and GM-CSF release, as shown by the levels of IFN-γ (**Figure 97 A, B**), IL-2 (**Figure 98 A, B**), TNF-α (**Figure 99 A, B**), IL-6 (**Figure 100 A, B**), IP-10 (**Figure 101 A, B**), MCP-1 (**Figure 102 A, B**), IL-8 (**Figure 103 A, B**) and GM-CSF (**Figure 104 A, B**). Overall, the co-treatment with the JAK1/2 inhibitor ruxolitinib reduced 20 CD20-TCB-mediated cytokine release, with the exception of IL-2 and GM-CSF, while minimally preventing B cell depletion at doses ranging from 30 to 60 mg/kg, unlike the Src inhibitor dasatinib that fully switched off TCB activity up to 48 hours.

In summary, this data show that the combination of mTOR and JAK inhibitors with CD20-TCB reduced cytokine release in humanized NSG, in line with the *in vitro* observations. The JAK 25 inhibitor (ruxolitinib) and the mTOR inhibitors (sirolimus, everolimus and temsirolimus) minimally interfered with CD20-TCB-mediated B cell depletion as opposed to the Src inhibitor dasatinib. This indicates that they could represent a promising approach to prevent cytokine release upon the first infusion with CD20-TCB while retaining anti-tumor efficacy.

30 * * *

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

5

Claims

1. A T cell engaging agent for use in the treatment of a disease in an individual, wherein said treatment comprises
(a) the administration of the T cell engaging agent to the individual, and
5 (b) the administration of an inhibitor of Janus kinase (JAK) and/or mammalian target of rapamycin (mTOR) signaling to the individual.

2. Use of a T cell engaging agent in the manufacture of a medicament for the treatment of a disease in an individual, wherein said treatment comprises
(a) the administration of the T cell engaging agent to the individual, and
10 (b) the administration of an inhibitor of JAK and/or mTOR signaling to the individual.

3. A method for treatment of a disease in an individual, wherein said method comprises
(a) the administration of a T cell engaging agent to the individual, and
(b) the administration of an inhibitor of JAK and/or mTOR signaling to the individual.

4. The T cell engaging agent, use or method of any one of claims 1 to 3, wherein the administration
15 of the inhibitor of JAK and/or mTOR signaling is for the prevention or mitigation of an adverse effect related to the administration of the T cell engaging agent.

5. An inhibitor of JAK and/or mTOR signaling for use in the prevention or mitigation of an adverse effect related to the administration of a T cell engaging agent to an individual.

6. Use of an inhibitor of JAK and/or mTOR signaling in the manufacture of a medicament for the
20 prevention or mitigation of an adverse effect related to the administration of a T cell engaging agent.

7. A method for preventing or mitigating an adverse effect related to the administration of a T cell engaging agent to an individual, comprising the administration of an inhibitor of JAK and/or mTOR signaling to the individual.

25 8. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of the preceding claims, wherein the inhibitor of JAK and/or mTOR signaling is an mTOR inhibitor, optionally selected from the group consisting of sirolimus, temsirolimus and everolimus.
9. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of the preceding claims, wherein the inhibitor of JAK and/or mTOR signaling is a JAK inhibitor,

optionally a JAK1 and/or JAK2 inhibitor, optionally ruxolitinib, baricitinib, tofacitinib or fedratinib.

10. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of the preceding claims, wherein (administration of) the inhibitor of JAK and/or mTOR signaling causes inhibition of an adverse effect related to the administration of the T cell engaging agent.

11. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of the preceding claims, wherein (administration of) the inhibitor of JAK and/or mTOR signaling does not cause inhibition of a desired effect related to the administration of the T cell engaging agent.

12. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 10 or 11, wherein the inhibition is a complete inhibition, or a clinically meaningful and/or statistically significant inhibition.

13. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 4 to 12, wherein the adverse effect is
(i) cytokine release syndrome (CRS);
(ii) fever, hypotension and/or hypoxia; and/or
(iii) an elevated serum level of one of more cytokine, particularly one or more cytokine selected from the group consisting of IL-6, IFN-γ, IL-10, TNF-α, GM-CSF, MCP-1 and IL-1β.

14. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 4 to 13, wherein administration of the inhibitor of JAK and/or mTOR signaling is upon (clinical) manifestation of the adverse effect (in the individual)

15. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of the preceding claims, wherein administration of the inhibitor of JAK and/or mTOR signaling is
(i) before, concurrent to, or after the administration of the T cell engaging agent;
(ii) intermittently or continuously; and/or
(iii) oral or parenteral, particularly intravenous.

16. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of the preceding claims, wherein administration of the inhibitor of JAK and/or mTOR signaling is

associated with the first administration of the T cell engaging agent, and optionally is prior, concurrent or subsequent to the first administration of the T cell engaging agent.

17. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of the preceding claims, wherein the administration of the T cell engaging agent is
5 (i) at an effective dose;
(ii) parenteral, particularly intravenous; and/or
(iii) the first administration of the T cell engaging agent to the individual.

18. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of the preceding claims, wherein the T cell engaging agent is a T cell bispecific antibody or a
10 CAR-T cell.

19. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 18, wherein the T cell bispecific antibody binds to CD3 and a target cell antigen.

20. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 18 or 19, wherein the T cell bispecific antibody comprises an antigen binding moiety that binds to
15 CD3 and an antigen binding moiety that binds to a target cell antigen.

21. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method any of claim 19 or 20, wherein the target cell antigen is carcinoembryonic antigen (CEA).

22. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 21, wherein the T cell bispecific antibody comprises
20 (i) a first antigen binding moiety that binds to CD3 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 4, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 6; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9; and (ii) a second antigen binding moiety that binds to CEA and comprises a heavy chain variable region
25 comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 12, the HCDR2 of SEQ ID NO: 13, and the HCDR3 of SEQ ID NO: 14; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 15, the LCDR2 of SEQ ID NO: 16 and the LCDR3 of SEQ ID NO: 17.

23. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 21 or 22, wherein the T cell bispecific antibody comprises a third antigen binding moiety that binds to CEA and/or an Fc domain composed of a first and a second subunit.

24. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 21 to 23, wherein the T cell bispecific antibody comprises
(i) a first antigen binding moiety that binds to CD3, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 4, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 6; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9, wherein the first antigen binding moiety is a crossover Fab molecule wherein either the variable or the constant regions of the Fab light chain and the Fab heavy chain are exchanged;
(ii) a second and a third antigen binding moiety that bind to CEA, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 12, the HCDR2 of SEQ ID NO: 13, and the HCDR3 of SEQ ID NO: 14; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 15, the LCDR2 of SEQ ID NO: 16 and the LCDR3 of SEQ ID NO: 17; wherein the second and third antigen binding moiety are each a Fab molecule, particularly a conventional Fab molecule;
(iii) an Fc domain composed of a first and a second subunit,
wherein the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety, and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

25. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 21 to 24, wherein the first antigen binding moiety of the T cell bispecific antibody comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 10 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 11, and/or the second and (where present) third antigen binding moiety of the T cell bispecific antibody comprise a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:

18 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 19.

26. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 23 to 25, wherein the Fc domain of the T cell bispecific antibody comprises a modification promoting the association of the first and the second subunit of the Fc domain, and/or the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

27. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 18 to 26, wherein the T cell bispecific antibody is cibisatamab.

28. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 19 or 20, wherein the target cell antigen is CD20.

29. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 28, wherein the T cell bispecific antibody comprises
(i) a first antigen binding moiety that binds to CD3 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 4, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 6; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9; and
(ii) a second antigen binding moiety that binds to CD20 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 24, the HCDR2 of SEQ ID NO: 25, and the HCDR3 of SEQ ID NO: 26; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 27, the LCDR2 of SEQ ID NO: 28 and the LCDR3 of SEQ ID NO: 29.

30. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 28 or 29, wherein the T cell bispecific antibody comprises a third antigen binding moiety that binds to CD20 and/or an Fc domain composed of a first and a second subunit.

31. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 28 to 30, wherein the T cell bispecific antibody comprises
(i) a first antigen binding moiety that binds to CD3, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 4, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 6; and a light chain variable region comprising the light chain CDR

(LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9, wherein the first antigen binding moiety is a crossover Fab molecule wherein either the variable or the constant regions of the Fab light chain and the Fab heavy chain are exchanged;
(ii) a second and a third antigen binding moiety that bind to CD20, comprising a heavy chain
5 variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 24, the HCDR2 of SEQ ID NO: 25, and the HCDR3 of SEQ ID NO: 26; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 27, the LCDR2 of SEQ ID NO: 28 and the LCDR3 of SEQ ID NO: 29, wherein the second and third antigen binding moiety are each a Fab molecule, particularly a conventional Fab molecule;
10 (iii) an Fc domain composed of a first and a second subunit,
wherein the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety, and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third antigen binding moiety is fused at the C-terminus
15 of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

32. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 28 to 31, wherein the first antigen binding moiety of the T cell bispecific antibody comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 10 and a light chain variable region
20 sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 11, and/or the second and (where present) third antigen binding moiety of the T cell bispecific antibody comprise a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 30 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or
25 100% identical to the amino acid sequence of SEQ ID NO: 31.

33. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 28 to 32, wherein the first antigen binding moiety of the T cell bispecific antibody is a crossover Fab molecule wherein the variable regions of the Fab light chain and the Fab heavy chain are exchanged, and wherein the second and (where present) third antigen binding moiety of
30 the T cell bispecific antibody is a conventional Fab molecule wherein in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted

independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and in the constant domain CH1 the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering 5 according to Kabat EU index).

34. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 30 to 33, wherein the Fc domain of the T cell bispecific antibody comprises a modification promoting the association of the first and the second subunit of the Fc domain, and/or the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc 10 receptor and/or effector function.

35. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 18 to 20 and 28 to 34, wherein the T cell bispecific antibody is glofitamab.

36. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 19 or 20, wherein the target cell antigen is HLA-A2/MAGE-A4.

15 37. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 36, wherein the T cell bispecific antibody comprises
(i) a first antigen binding moiety that binds to CD3 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 37, the HCDR2 of SEQ ID NO: 38, and the HCDR3 of SEQ ID NO: 39; and a light chain variable region comprising the light chain 20 CDR (LCDR) 1 of SEQ ID NO: 40, the LCDR2 of SEQ ID NO: 41 and the LCDR3 of SEQ ID NO: 42; and
(ii) a second antigen binding moiety that binds to HLA-A2/MAGE-A4 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 45, the HCDR2 of SEQ ID NO: 46, and the HCDR3 of SEQ ID NO: 47; and a light chain variable region 25 comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 48, the LCDR2 of SEQ ID NO: 49 and the LCDR3 of SEQ ID NO: 50.

38. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 36 or 37, wherein the T cell bispecific antibody comprises a third antigen binding moiety that binds to HLA-A2/MAGE-A4 and/or an Fc domain composed of a first and a second subunit.

39. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 36 to 38, wherein the T cell bispecific antibody comprises
(i) a first antigen binding moiety that binds to CD3, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 37, the HCDR2 of SEQ ID NO: 38, and the HCDR3 of SEQ ID NO: 39; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 40, the LCDR2 of SEQ ID NO: 41 and the LCDR3 of SEQ ID NO: 42, wherein the first antigen binding moiety is a crossover Fab molecule wherein either the variable or the constant regions of the Fab light chain and the Fab heavy chain are exchanged;
(ii) a second and a third antigen binding moiety that bind to HLA-A2/MAGE-A4, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 45, the HCDR2 of SEQ ID NO: 46, and the HCDR3 of SEQ ID NO: 47; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 48, the LCDR2 of SEQ ID NO: 49 and the LCDR3 of SEQ ID NO: 50, wherein the second and third antigen binding moiety are each a Fab molecule, particularly a conventional Fab molecule;
(iii) an Fc domain composed of a first and a second subunit,
wherein the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety, and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

40. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 36 to 39, wherein the first antigen binding moiety of the T cell bispecific antibody comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 43 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 44, and/or the second and (where present) third antigen binding moiety of the T cell bispecific antibody comprise a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 51 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 52.

41. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 36 to 40, wherein the first antigen binding moiety of the T cell bispecific antibody is a

crossover Fab molecule wherein the variable regions of the Fab light chain and the Fab heavy chain are exchanged, and wherein the second and (where present) third antigen binding moiety of the T cell bispecific antibody is a conventional Fab molecule wherein in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and in the constant domain CH1 the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

42. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 38 to 41, wherein the Fc domain of the T cell bispecific antibody comprises a modification promoting the association of the first and the second subunit of the Fc domain, and/or the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

43. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 19 or 20, wherein the target cell antigen is CD19.

44. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 43, wherein the T cell bispecific antibody comprises
(i) a first antigen binding moiety that binds to CD3 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 61, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 62, or a heavy chain variable region comprising the HCDR1 of SEQ ID NO: 64, the HCDR2 of SEQ ID NO: 5 and the HCDR3 of SEQ ID NO: 65; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9; and
(ii) a second antigen binding moiety that binds to CD19 and comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 67, the HCDR2 of SEQ ID NO: 68, and the HCDR3 of SEQ ID NO: 69; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 70, the LCDR2 of SEQ ID NO: 71 and the LCDR3 of SEQ ID NO: 72.

45. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 43 or 44, wherein the T cell bispecific antibody comprises a third antigen binding moiety that binds to CD19 and/or an Fc domain composed of a first and a second subunit.

46. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 43 to 45, wherein the T cell bispecific antibody comprises
(i) a first antigen binding moiety that binds to CD3, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 61, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 62, or a heavy chain variable region comprising the HCDR1 of SEQ ID NO: 64, the HCDR2 of SEQ ID NO: 5, and the HCDR3 of SEQ ID NO: 65; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 8 and the LCDR3 of SEQ ID NO: 9, wherein the first antigen binding moiety is a crossover Fab molecule wherein either the variable or the constant regions of the Fab light chain and the Fab heavy chain are exchanged;
(ii) a second and a third antigen binding moiety that bind to CD19, comprising a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 67, the HCDR2 of SEQ ID NO: 68, and the HCDR3 of SEQ ID NO: 69; and a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 70, the LCDR2 of SEQ ID NO: 71 and the LCDR3 of SEQ ID NO: 72, wherein the second and third antigen binding moiety are each a Fab molecule, particularly a conventional Fab molecule;
(iii) an Fc domain composed of a first and a second subunit,
wherein the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety, and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

47. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 43 to 46, wherein the first antigen binding moiety of the T cell bispecific antibody comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 63 or a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 66, and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 11; and/or

the second and (where present) third antigen binding moiety of the T cell bispecific antibody comprise a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 73, and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 74.

48. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 43 to 47, wherein the first antigen binding moiety of the T cell bispecific antibody is a crossover Fab molecule wherein the variable regions of the Fab light chain and the Fab heavy chain are exchanged, and wherein the second and (where present) third antigen binding moiety of the T cell bispecific antibody is a conventional Fab molecule wherein in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and in the constant domain CH1 the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

49. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 45 to 48, wherein the Fc domain of the T cell bispecific antibody comprises a modification promoting the association of the first and the second subunit of the Fc domain, and/or the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

50. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of the preceding claims, wherein the disease (to be treated by the T cell engaging agent) is cancer, particularly a cancer expressing the target cell antigen of the T cell engaging agent.

51. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 50, wherein the cancer is

(i) a carcinoembryonic antigen (CEA)-expressing cancer, and/or

(ii) selected from the group consisting of colorectal cancer, lung cancer, pancreatic cancer, breast cancer, and gastric cancer.

52. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 50, wherein the cancer is
(i) a CD20-expressing cancer,
(ii) a B-cell cancer, and/or
5 (ii) selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL) and marginal zone lymphoma (MZL).

53. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 50, wherein the cancer is a MAGE-A4 expressing cancer.

10 54. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of claim 50, wherein the cancer is
(i) a CD19-expressing cancer,
(ii) a B-cell cancer, and/or
(ii) selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic
15 leukemia (ALL) and chronic lymphocytic leukemia (CLL).

55. The T cell engaging agent, inhibitor of JAK and/or mTOR signaling, use or method of any one of claims 43 to 49, wherein the disease (to be treated by the T cell engaging agent) is an autoimmune disease, particularly lupus, more particularly systemic lupus erythematosus (SLE) or lupus nephritis (LN).

20 56. The invention as described hereinbefore.

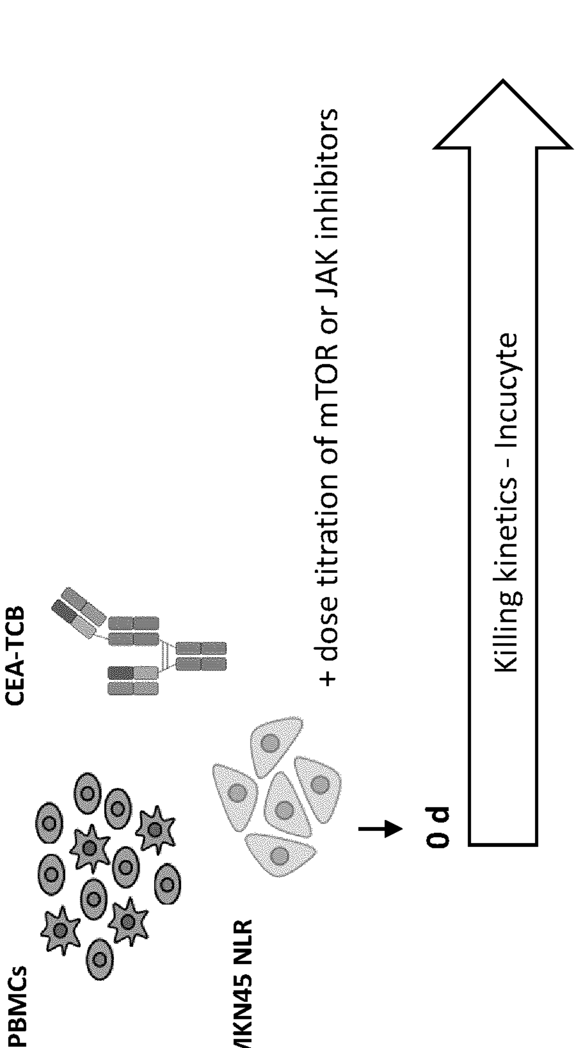


FIG. 1

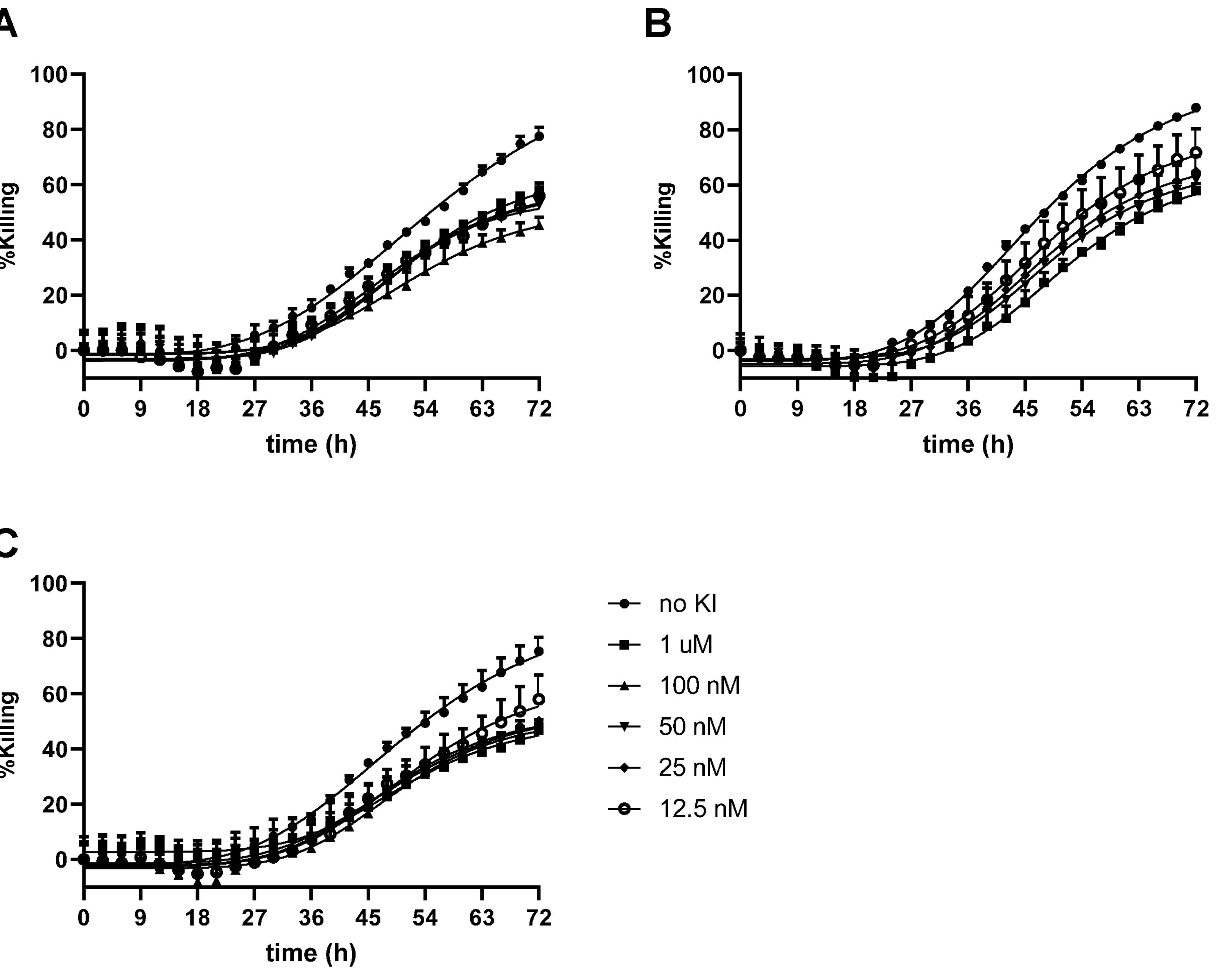
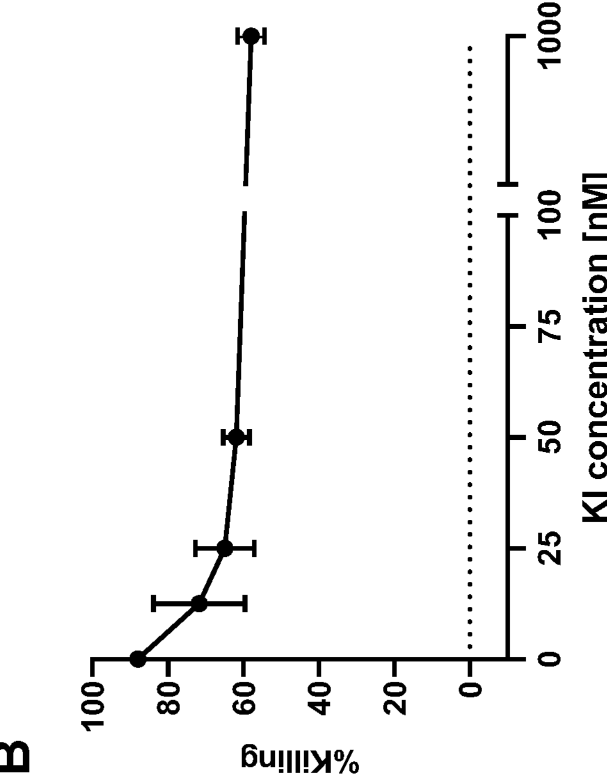


FIG. 2



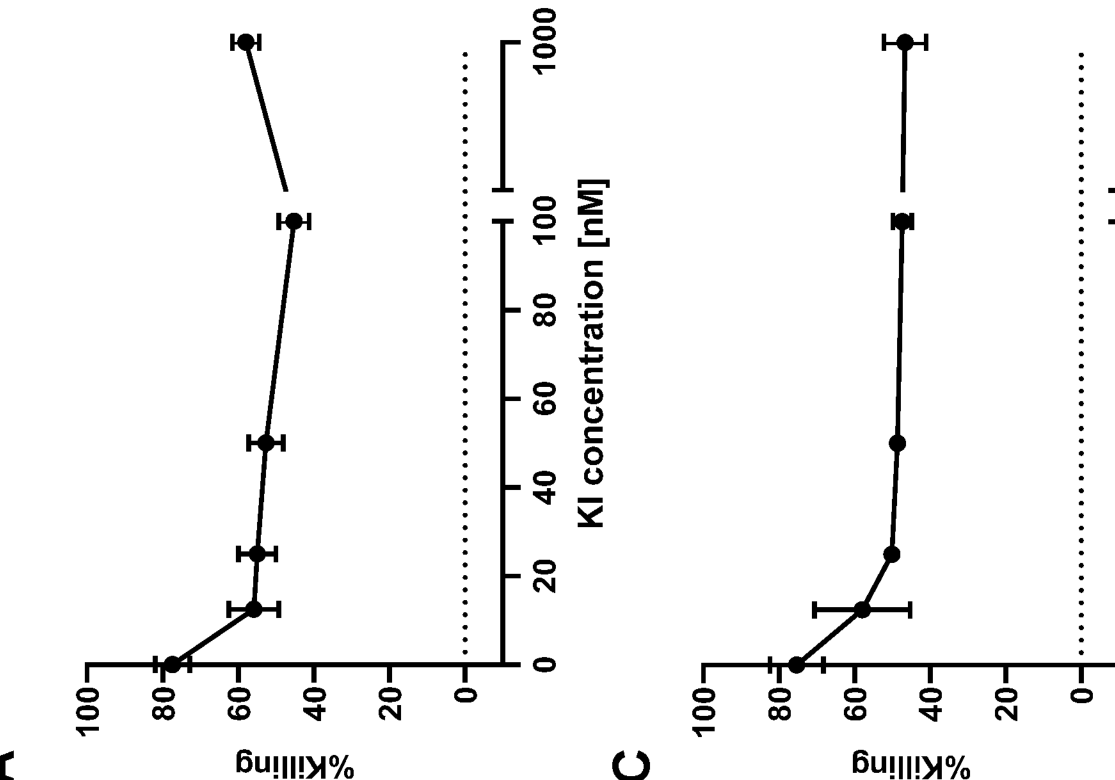
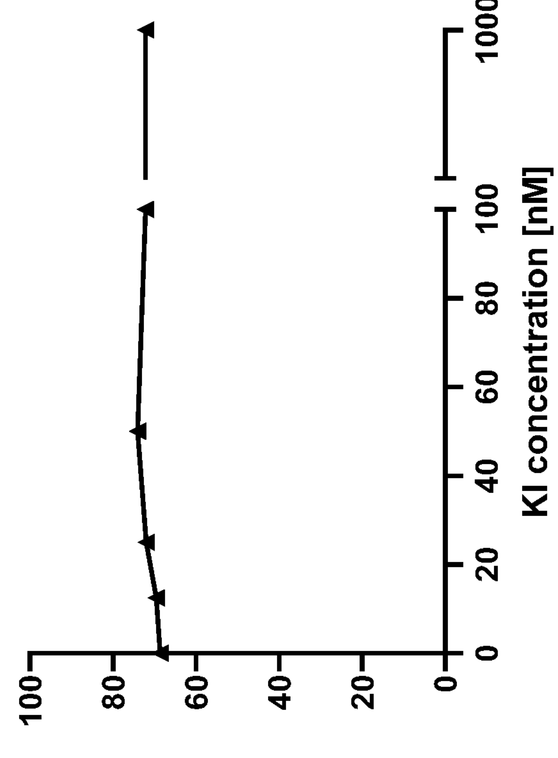
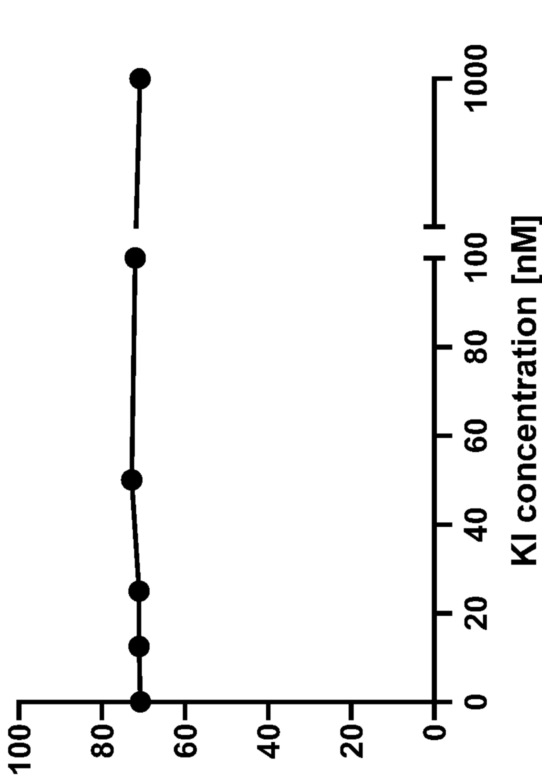


FIG. 3


A
%Live PBMCs
100
80
60
40
20
0
0
20
40
60
80
100
1000
KI concentration [nM]


100
80
60
40
20
0
0
20
40
60
80
100
1000
KI concentration [nM]

B
%Live PBMCs

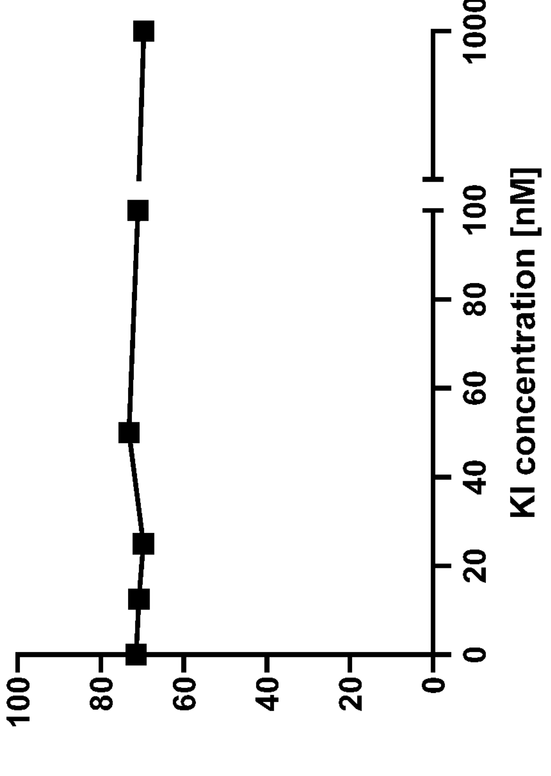
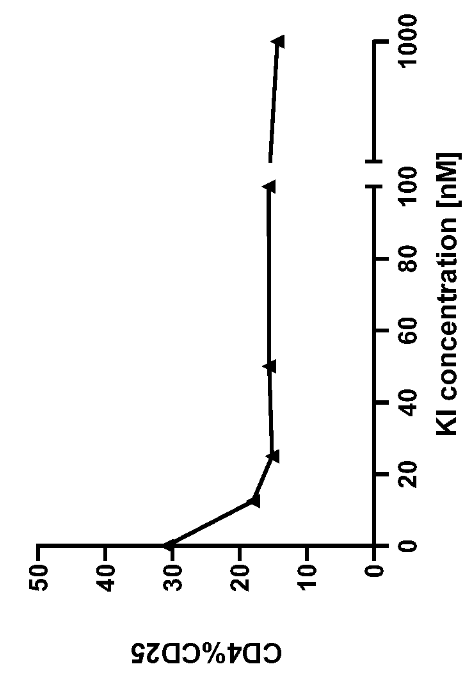

C
%Live PBMCs
100
80
60
40
20
0
0
20
40
60
80
100
1000
KI concentration [nM]

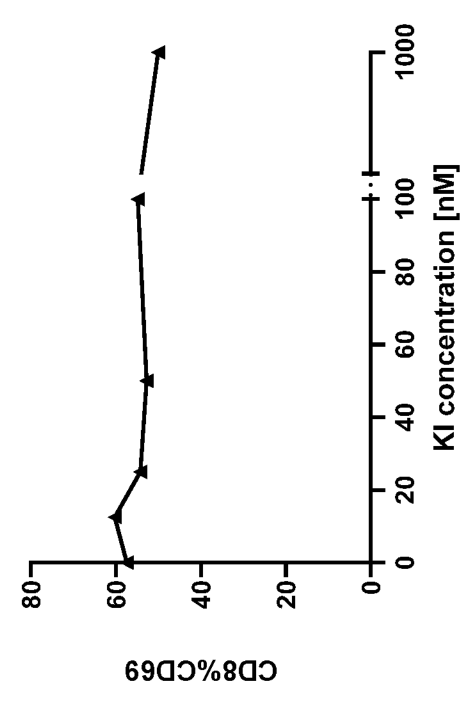
FIG. 4

A

CD4%CD69

KI concentration [nM]





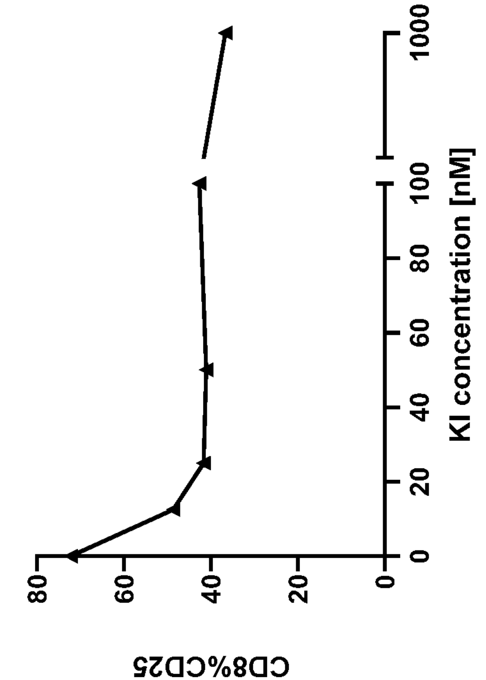
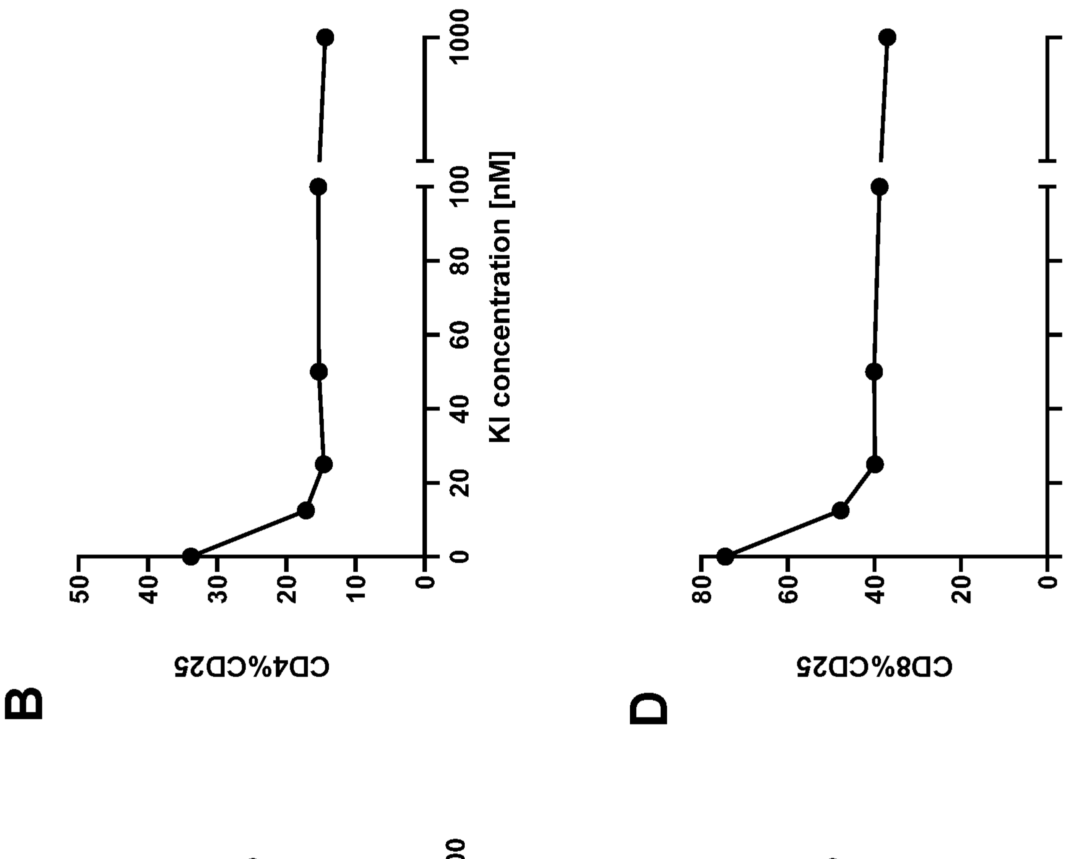
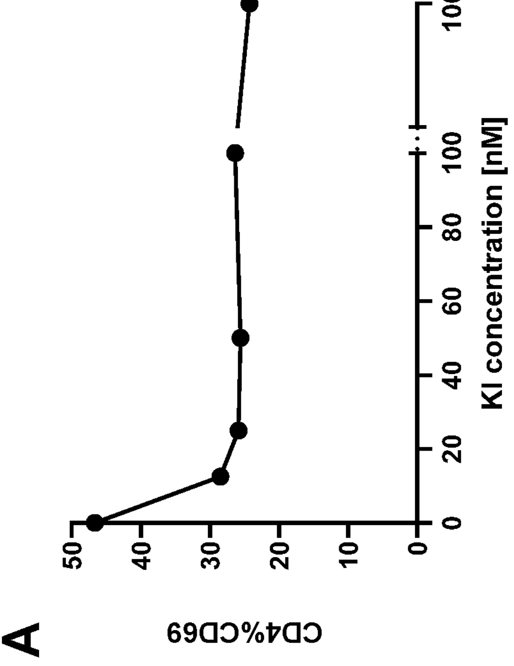


FIG. 5





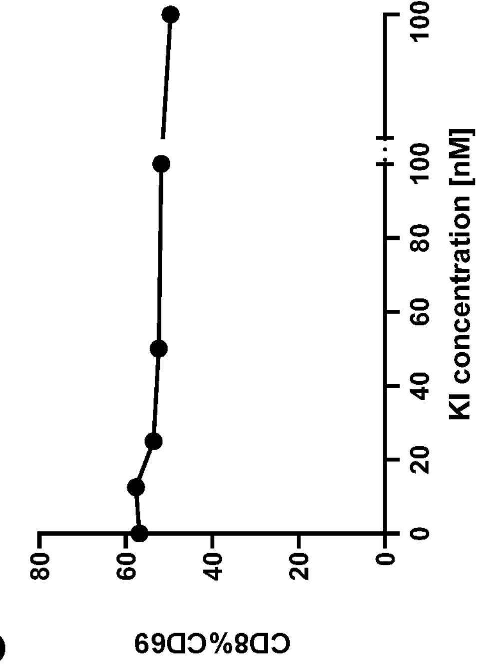


FIG. 6

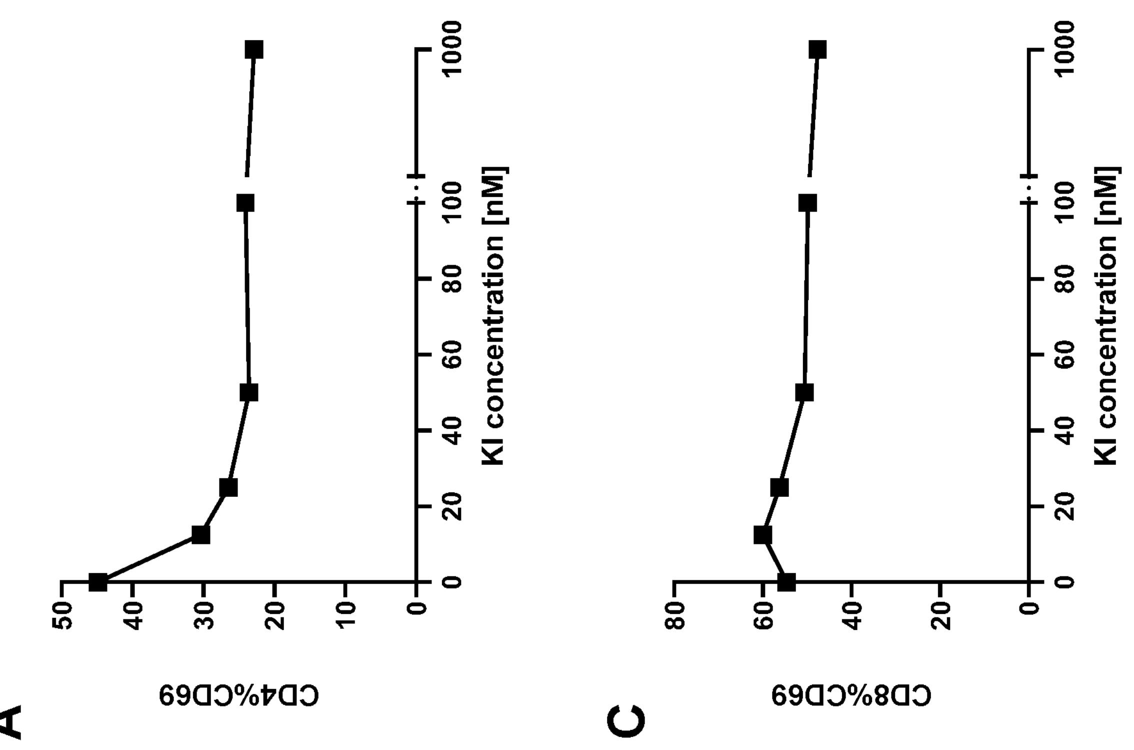


FIG. 7

FIG. 8

Everolimus
Sirolimus
Temsirolimus

A
IFN-γ (pg/mL)
2000 1500 1000 500 0
0 20 40 60 80 100 1000
KI concentration [nM]

B
IL-2(pg/mL)
400 300 200 100 0
0 20 40 60 80 100 1000
KI concentration [nM]

C
TNF-α(pg/mL)
10000 8000 6000 4000 2000 0
0 20 40 60 80 100 1000
KI concentration [nM]

D
IL-6 (pg/mL)
40 30 20 10 0
0 20 40 60 80 100 1000
KI concentration [nM]

E
GM-CSF (pg/mL)
500 400 300 200 100 0
0 20 40 60 80 100 1000
KI concentration [nM]

F
IL-8 (pg/mL)
10000 8000 6000 4000 2000 0
0 20 40 60 80 100 1000
KI concentration [nM]

G
IL-4 (pg/mL)
40 30 20 10 0
0 20 40 60 80 100 1000
KI concentration [nM]

H
IL-10 (pg/mL)
60 40 20 0
0 20 40 60 80 100 1000
KI concentration [nM]

I
MCP-1 (pg/mL)
400 300 200 100 0
0 20 40 60 80 100 1000
KI concentration [nM]

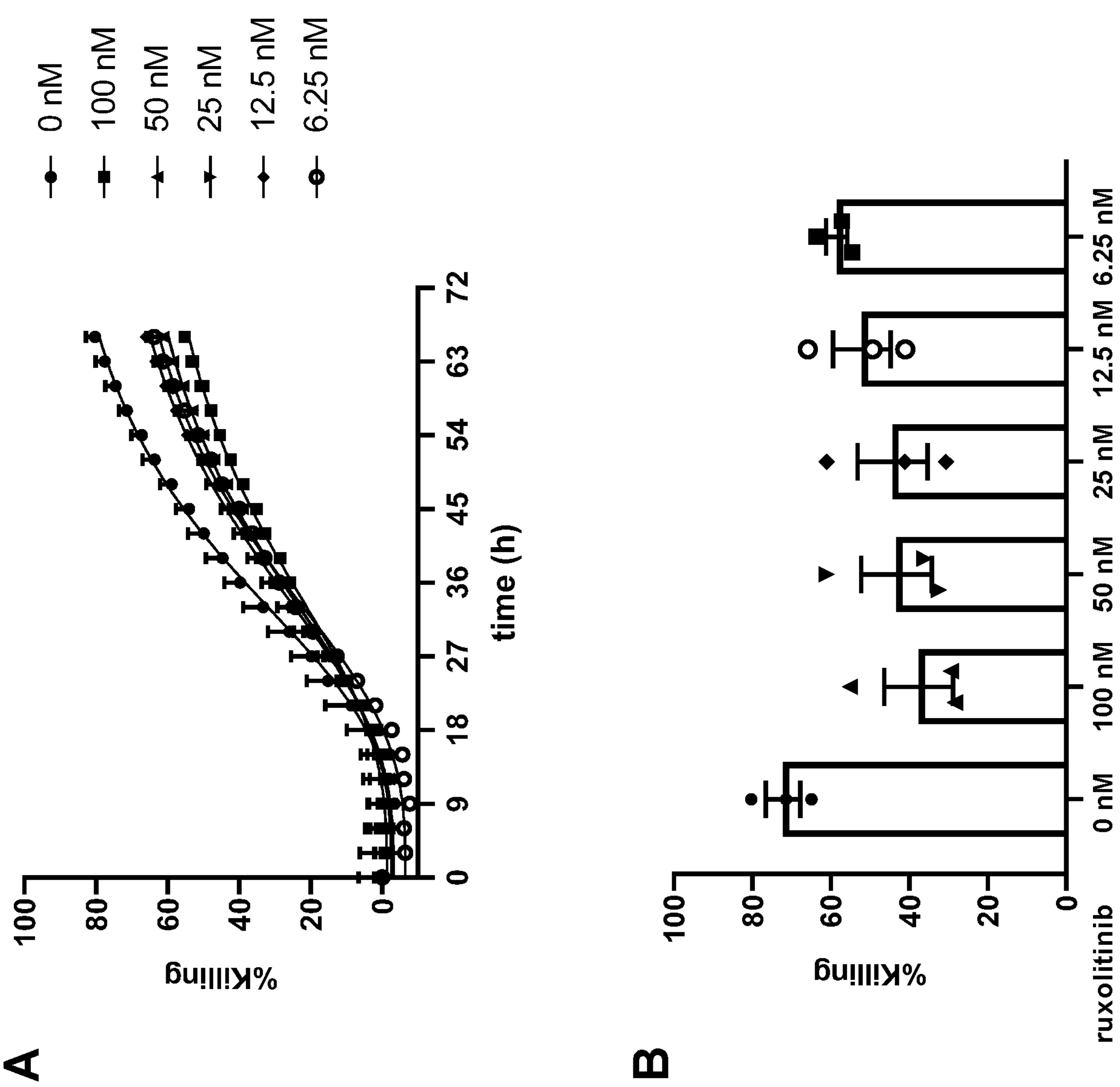


FIG. 9

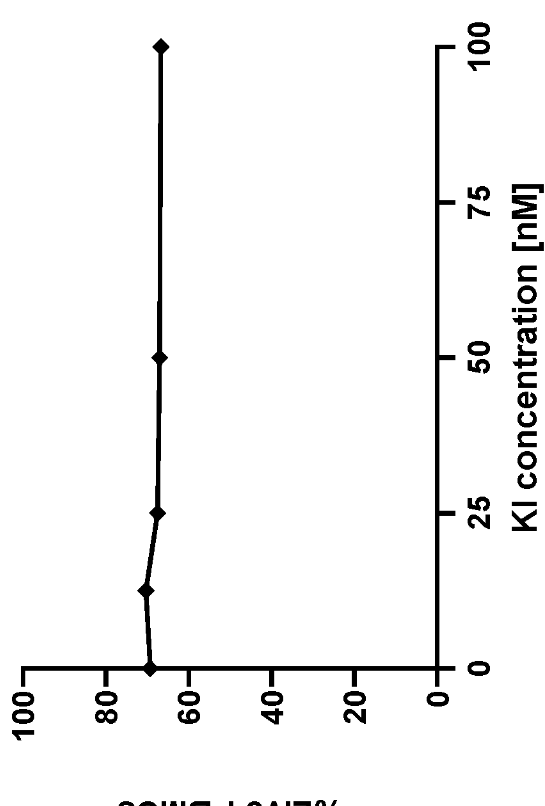


FIG. 10

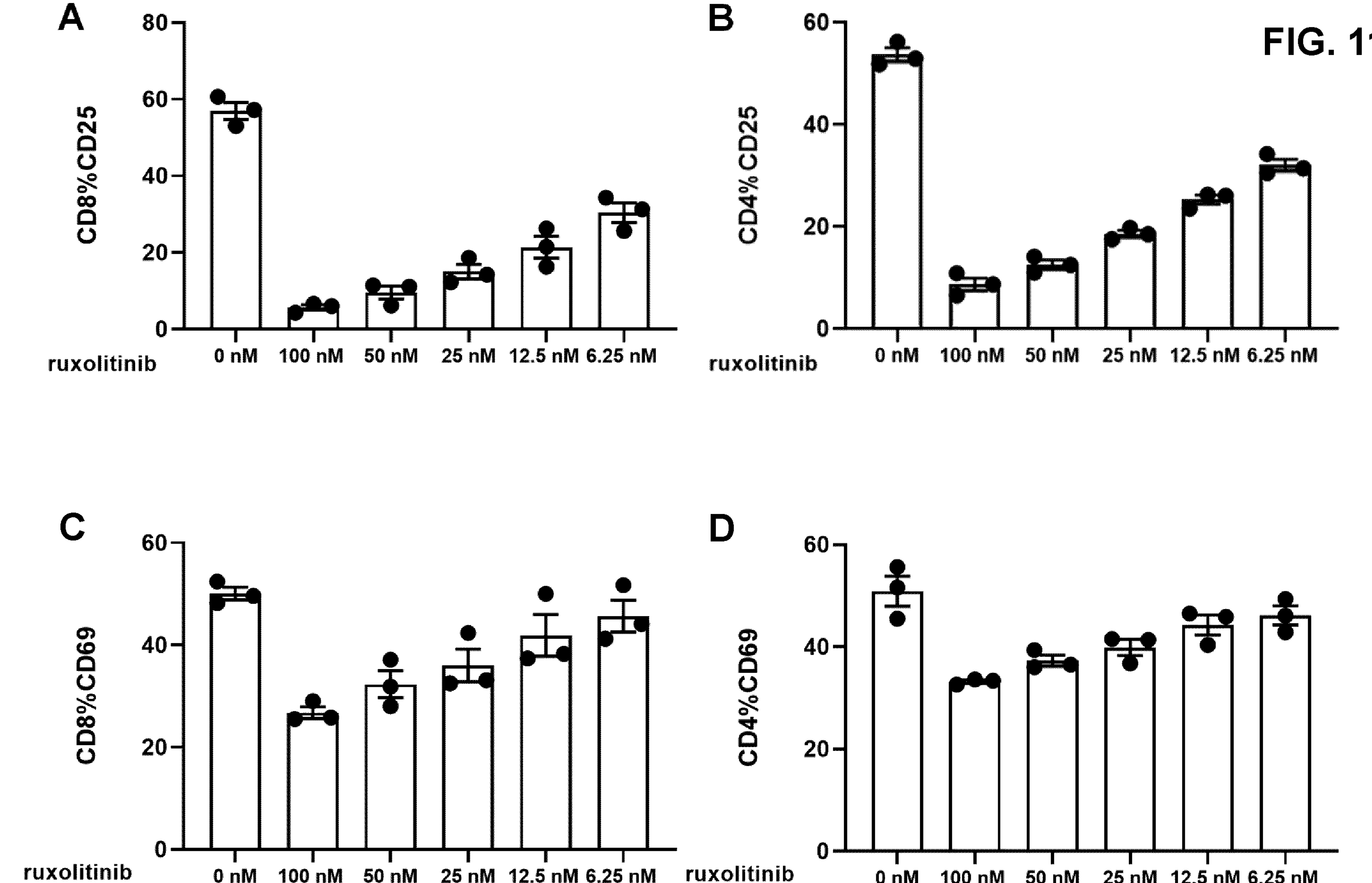


FIG. 11
A
CD8%CD25
0
20
40
60
80
ruxolitinib
0 nM
100 nM
50 nM
25 nM
12.5 nM
6.25 nM
B
CD4% CD25
0
20
40
60
ruxolitinib
0 nM
100 nM
50 nM
25 nM
12.5 nM
6.25 nM
C
CD8%CD69
0
20
40
60
ruxolitinib
0 nM
100 nM
50 nM
25 nM
12.5 nM
6.25 nM
D
CD4%CD69
0
20
40
60
ruxolitinib
0 nM
100 nM
50 nM
25 nM
12.5 nM
6.25 nM

FIG. 12

A
IFN-γ (pg/mL)
1500 1000 500 0
0 100 50 25 12.5 6.25
ruxolitinib [nM]

B
IL-2 (pg/mL)
250 200 150 100 50 0
0 100 50 25 12.5 6.25
ruxolitinib [nM]

C
TNF-α (pg/mL)
3000 2000 1000 0
0 100 50 25 12.5 6.25
ruxolitinib [nM]

D
IL-6 (pg/mL)
40 30 20 10 0
0 100 50 25 12.5 6.25
ruxolitinib [nM]

E
GM-CSF (pg/mL)
150 100 50 0
0 100 50 25 12.5 6.25
ruxolitinib [nM]

F
IL-8 (pg/mL)
25000 20000 15000 10000 5000 0
0 100 50 25 12.5 6.25
ruxolitinib [nM]

G
IL-4 (pg/mL)
25 20 15 10 5 0
0 100 50 25 12.5 6.25
ruxolitinib [nM]

H
IL-10 (pg/mL)
400 300 200 100 0
0 100 50 25 12.5 6.25
ruxolitinib [nM]

I
MCP-1 (pg/mL)
2000 1500 1000 500 0
0 100 50 25 12.5 6.25
ruxolitinib [nM]

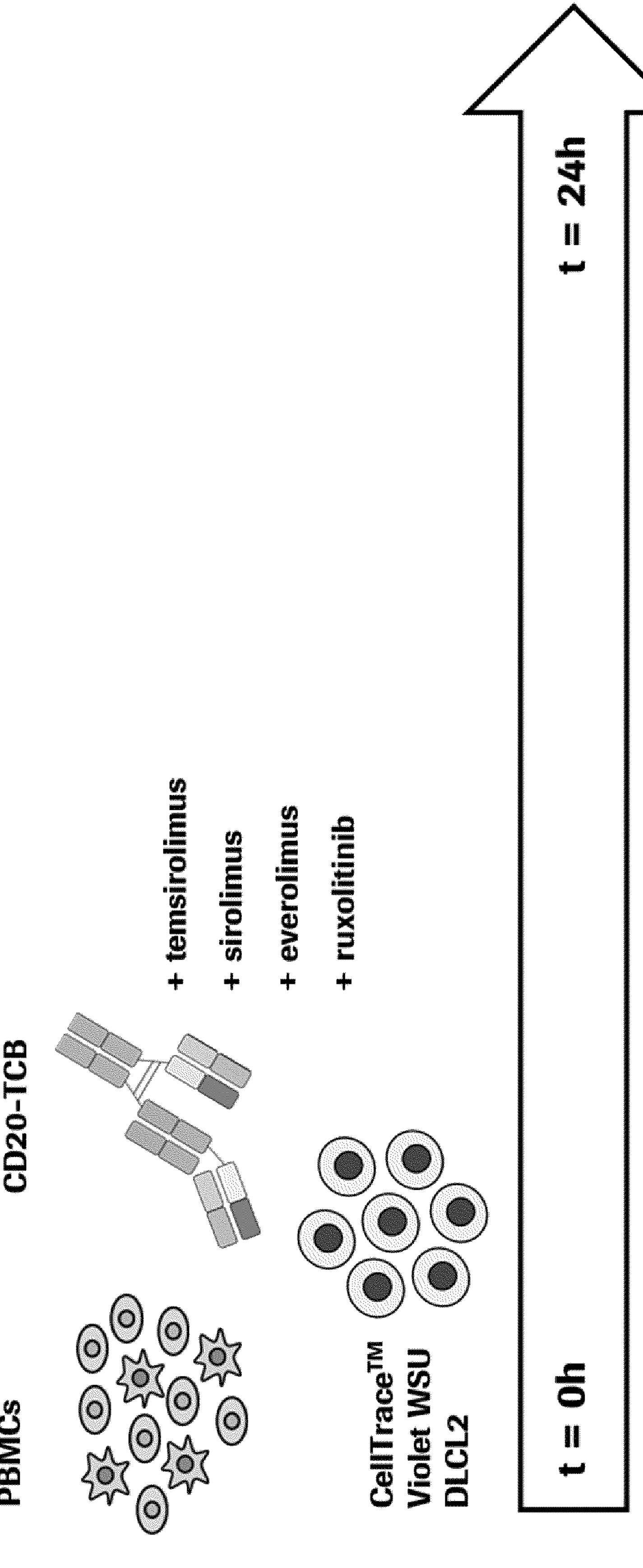
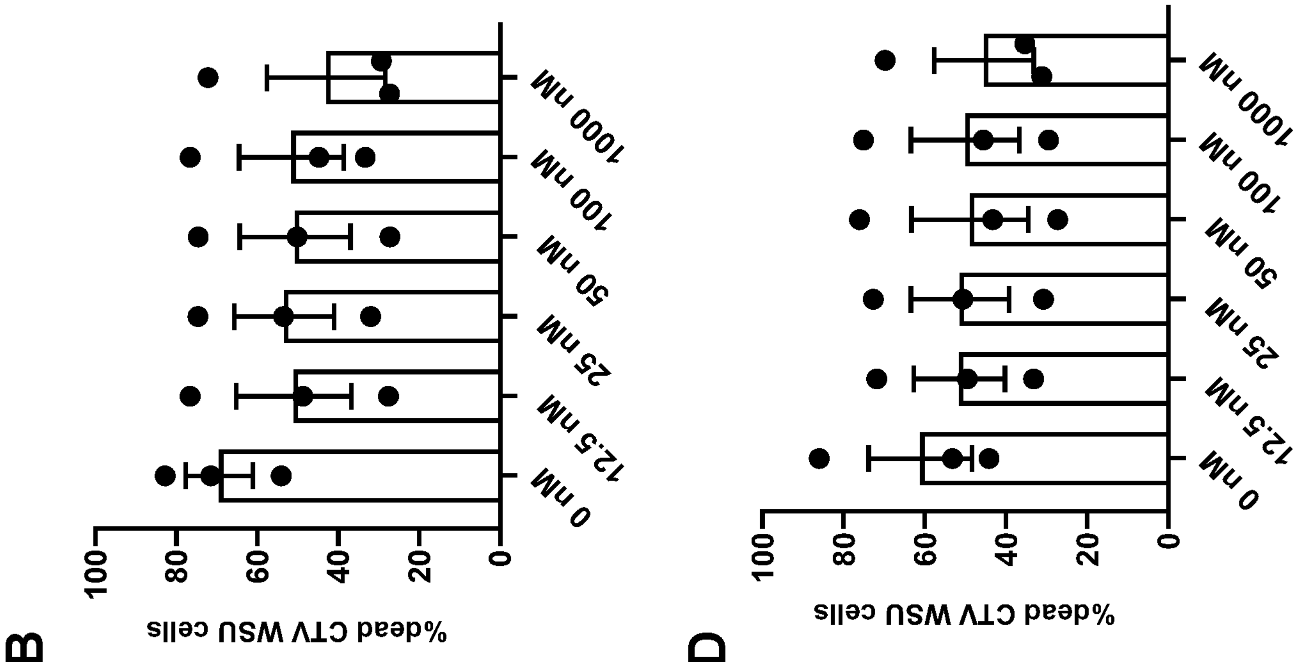
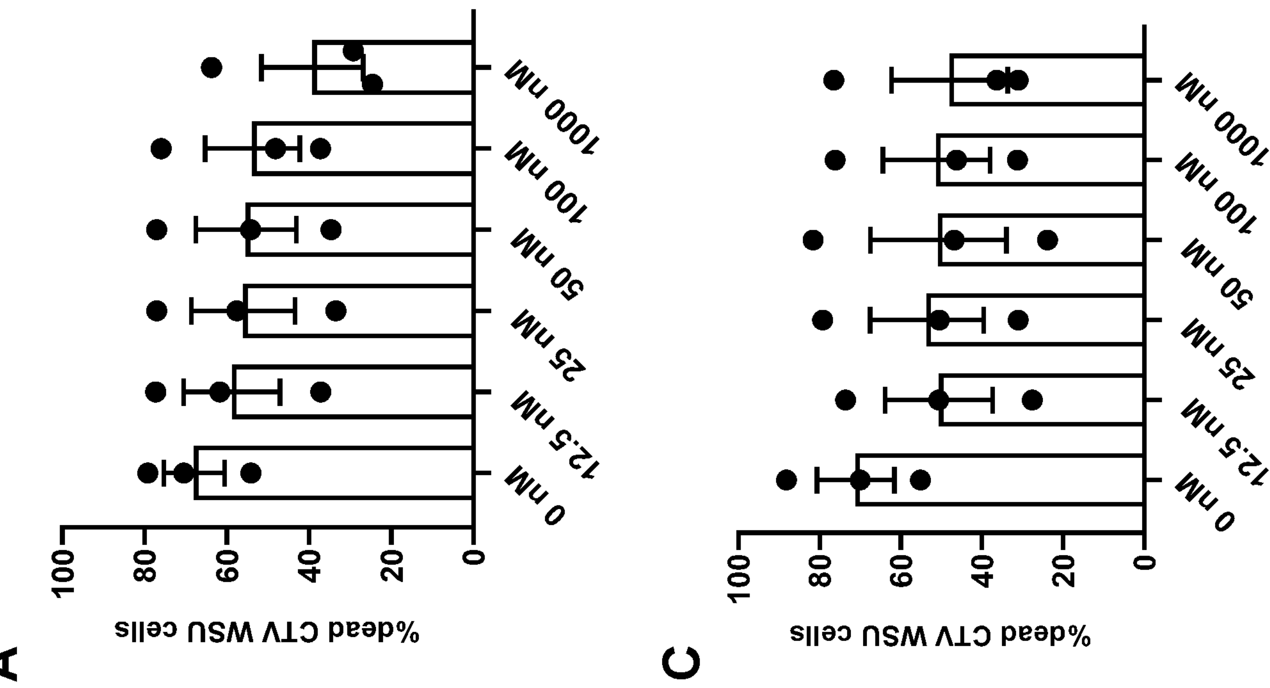
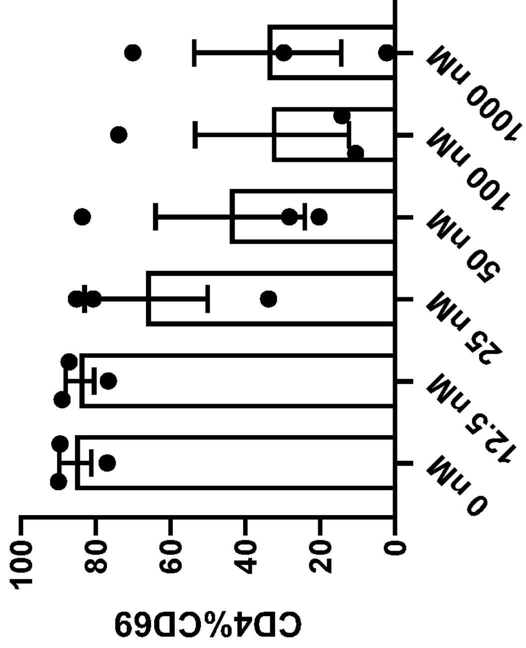


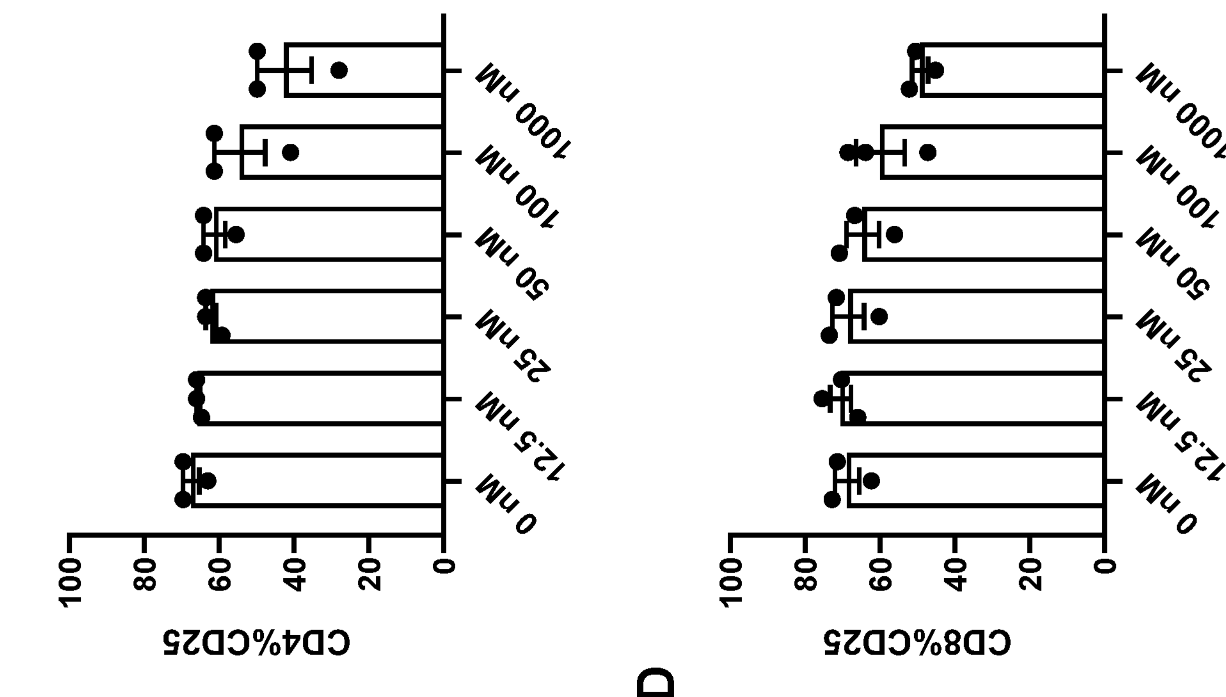
FIG. 13

FIG. 14









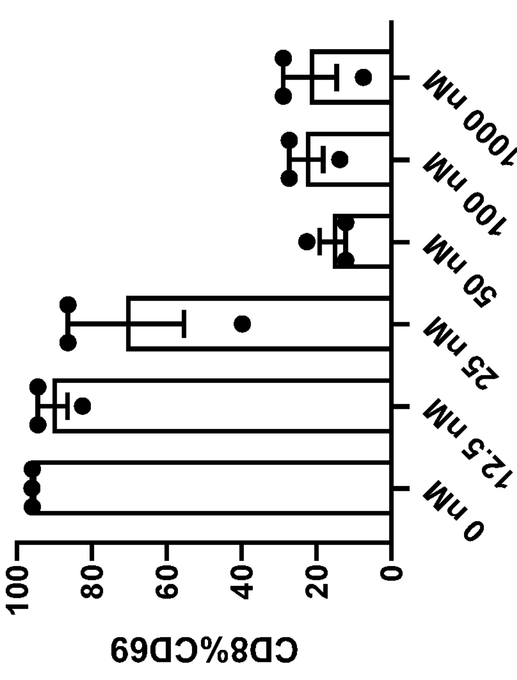
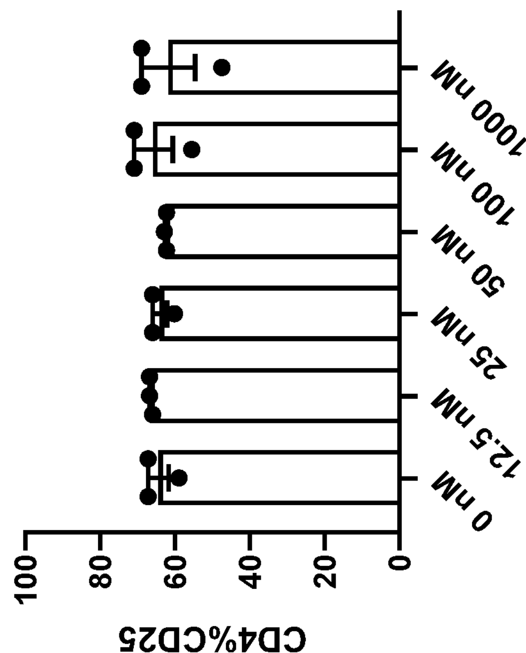


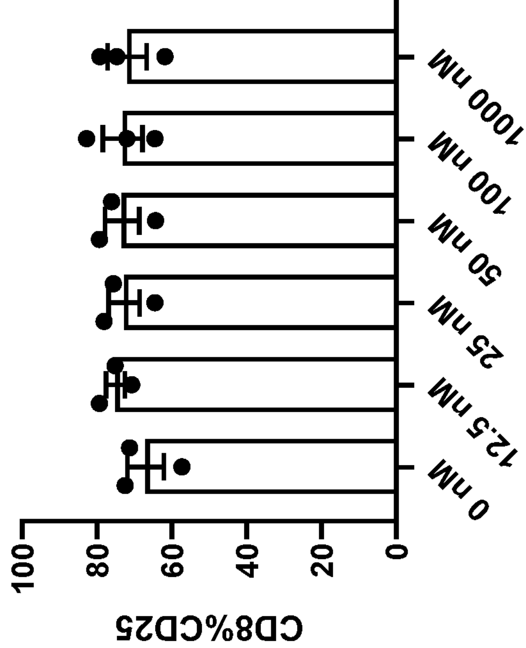
FIG. 15

FIG. 16

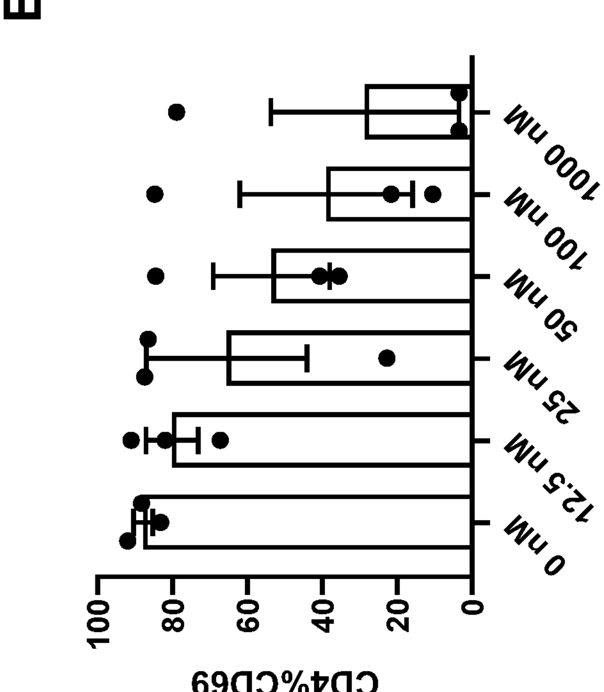
A



B



C



D

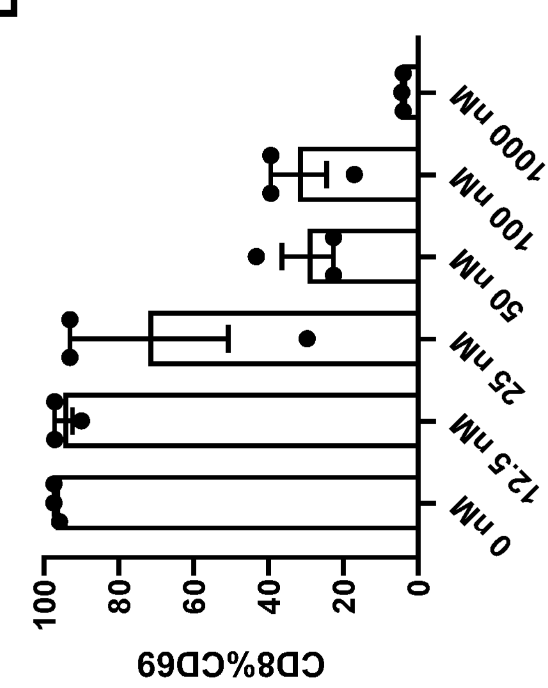


FIG. 17

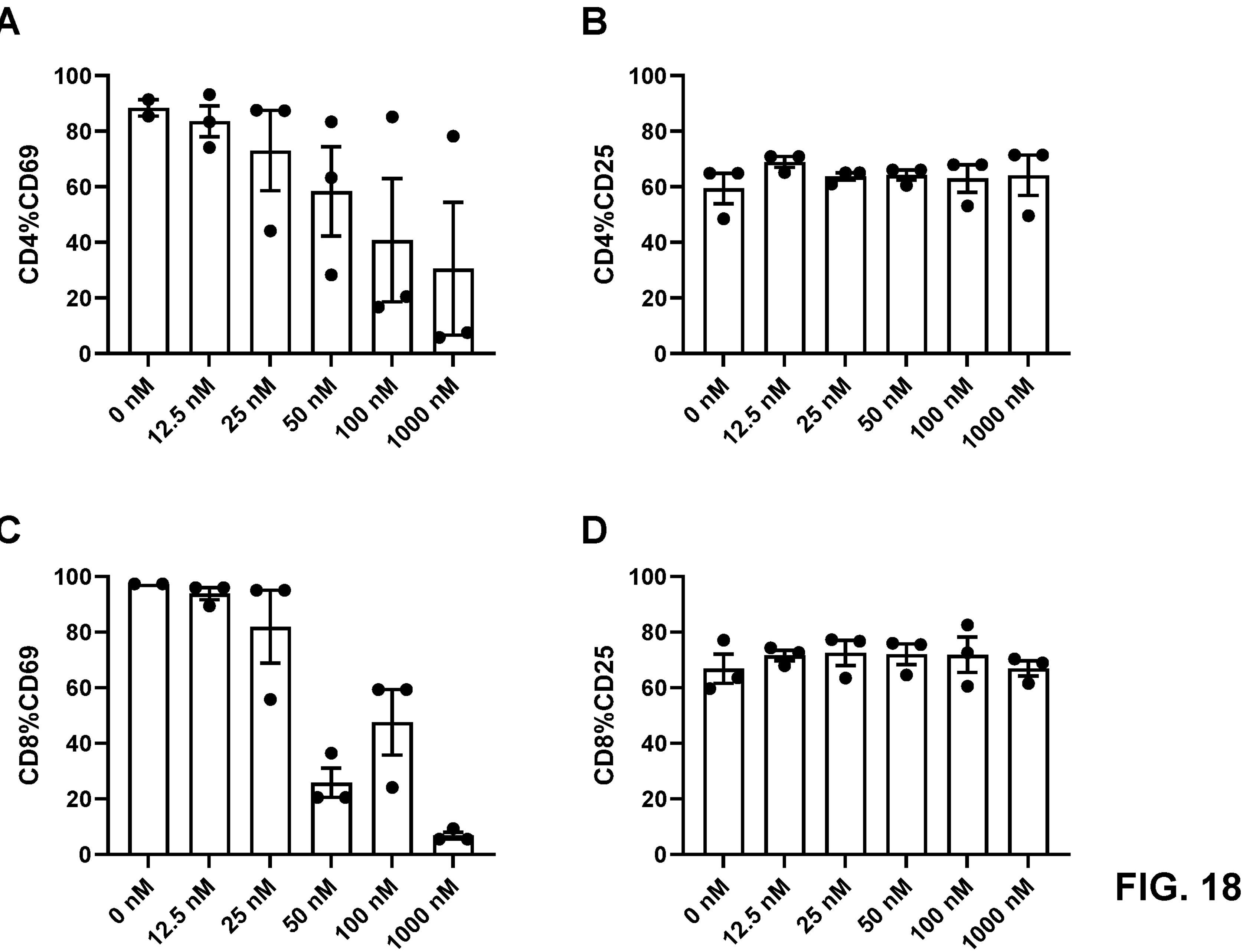


FIG. 18

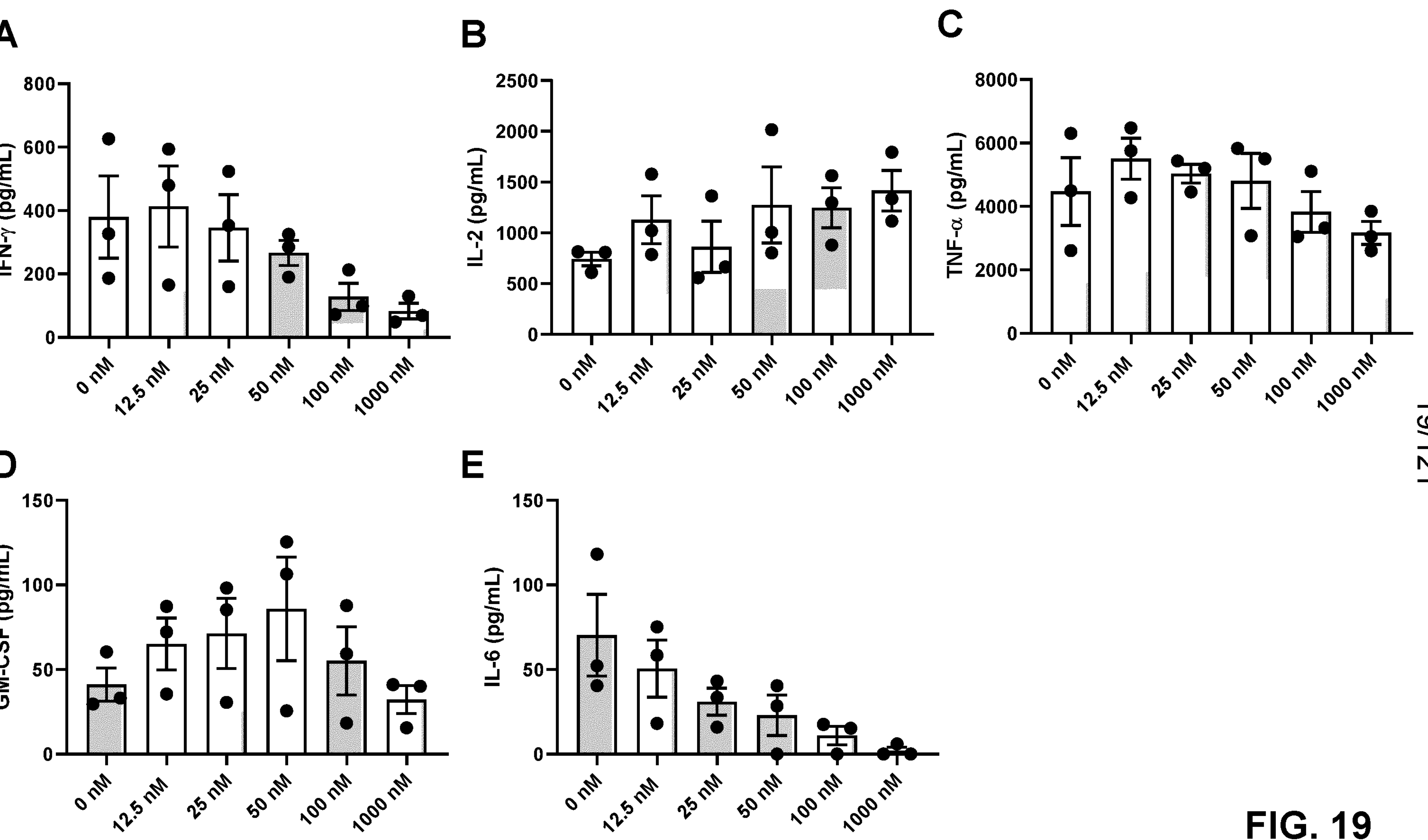


FIG. 19

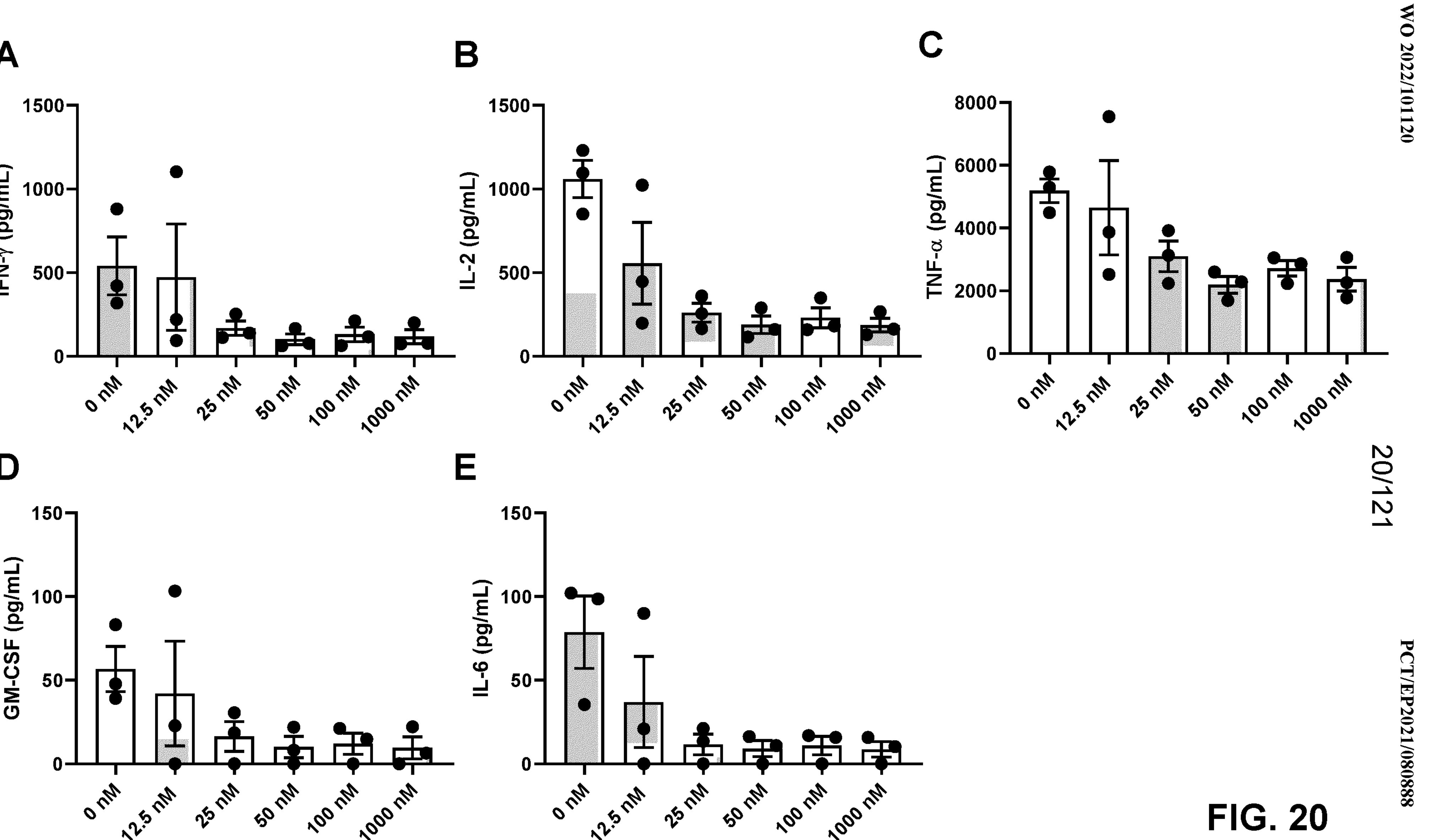


FIG. 20

A

IFN-γ (pg/mL)

0 200 400 600 800 1000

0 nM 12.5 nM 25 nM 50 nM 100 nM 1000 nM

B

IL-2 (pg/mL)

0 500 1000 1500

0 nM 12.5 nM 25 nM 50 nM 100 nM 1000 nM

C

TNF-α (pg/mL)

0 2000 4000 6000 8000

0 nM 12.5 nM 25 nM 50 nM 100 nM 1000 nM

D

GM-CSF (pg/mL)

0 20 40 60 80 100

0 nM 12.5 nM 25 nM 50 nM 100 nM 1000 nM

E

IL-6 (pg/mL)

0 50 100 150

0 nM 12.5 nM 25 nM 50 nM 100 nM 1000 nM

FIG. 21

A

IFN-γ (pg/mL)

0 200 400 600 800 1000

0 nM 12.5 nM 25 nM 50 nM 100 nM 1000 nM

B

IL-2 (pg/mL)

0 200 400 600 800 1000

0 nM 12.5 nM 25 nM 50 nM 100 nM 1000 nM

C

TNF-α (pg/mL)

0 2000 4000 6000 8000

0 nM 12.5 nM 25 nM 50 nM 100 nM 1000 nM

D

GM-CSF (pg/mL)

0 20 40 60 80 100

0 nM 12.5 nM 25 nM 50 nM 100 nM 1000 nM

E

IL-6 (pg/mL)

0 50 100 150

0 nM 12.5 nM 25 nM 50 nM 100 nM 1000 nM

FIG. 22

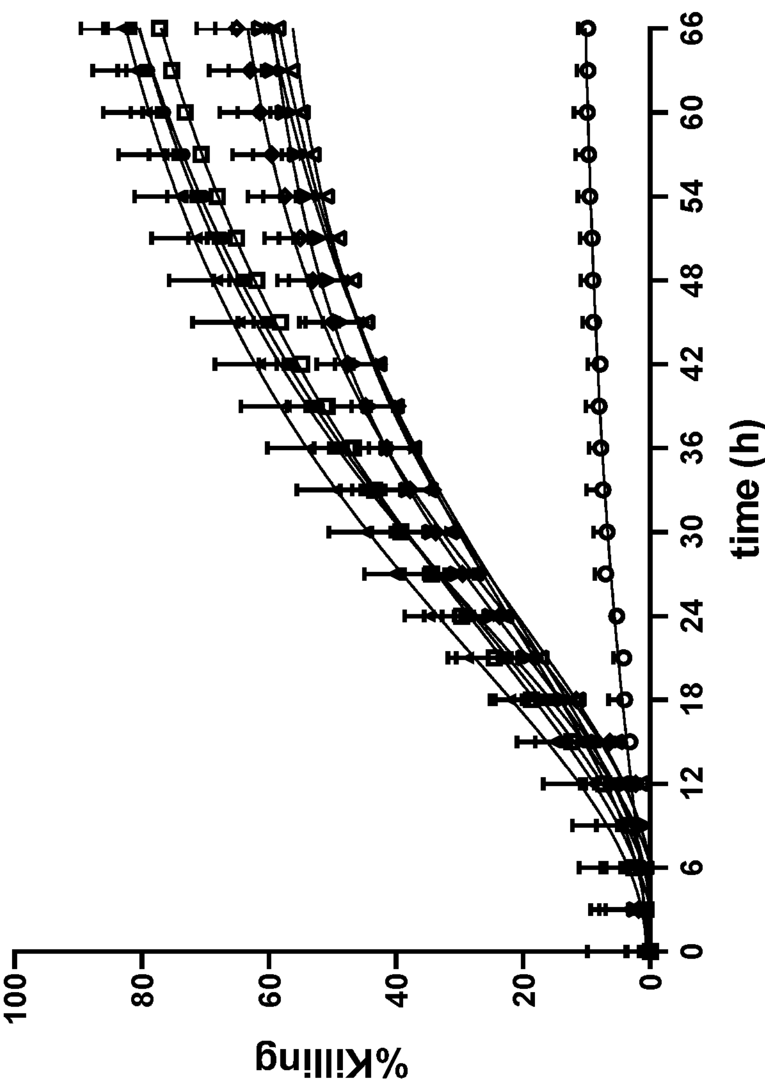


FIG. 23

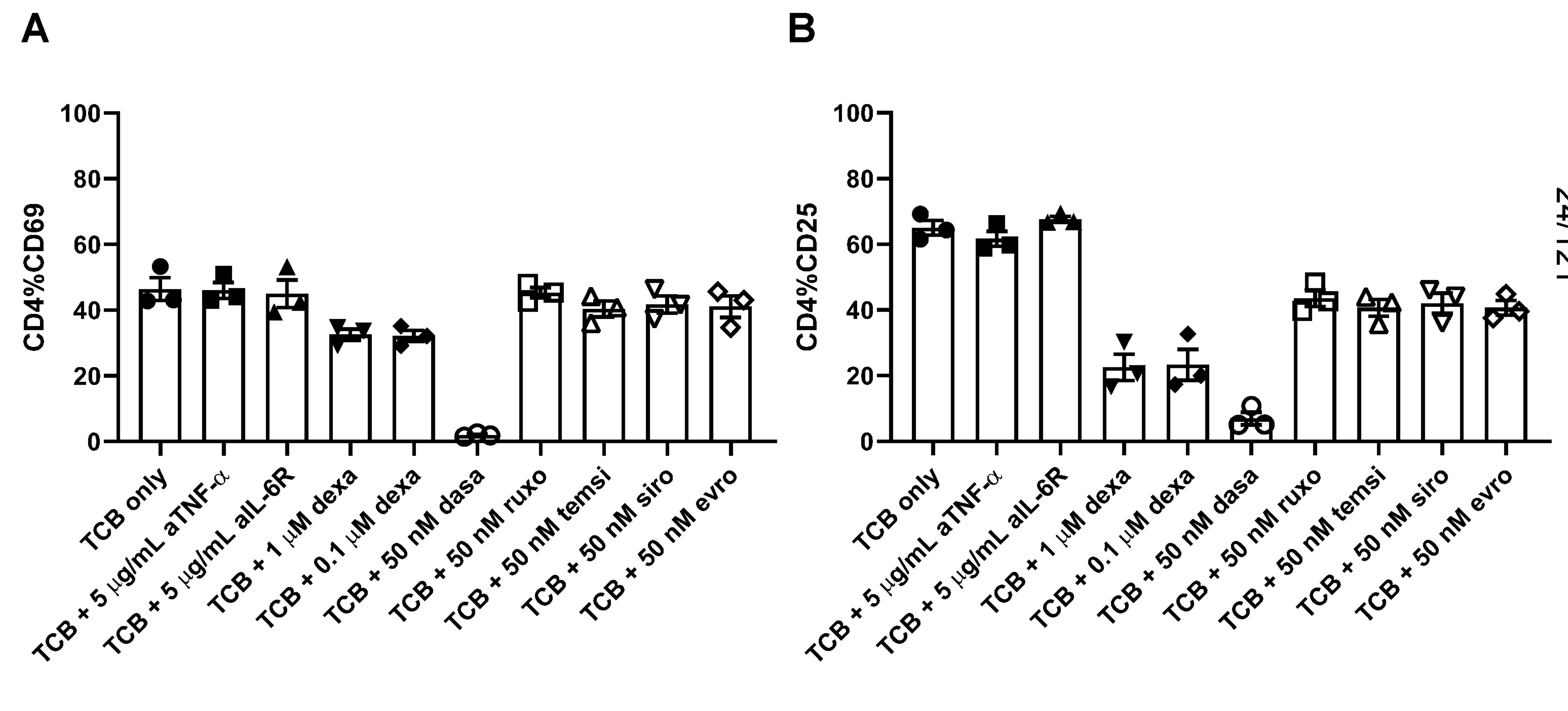


FIG. 24

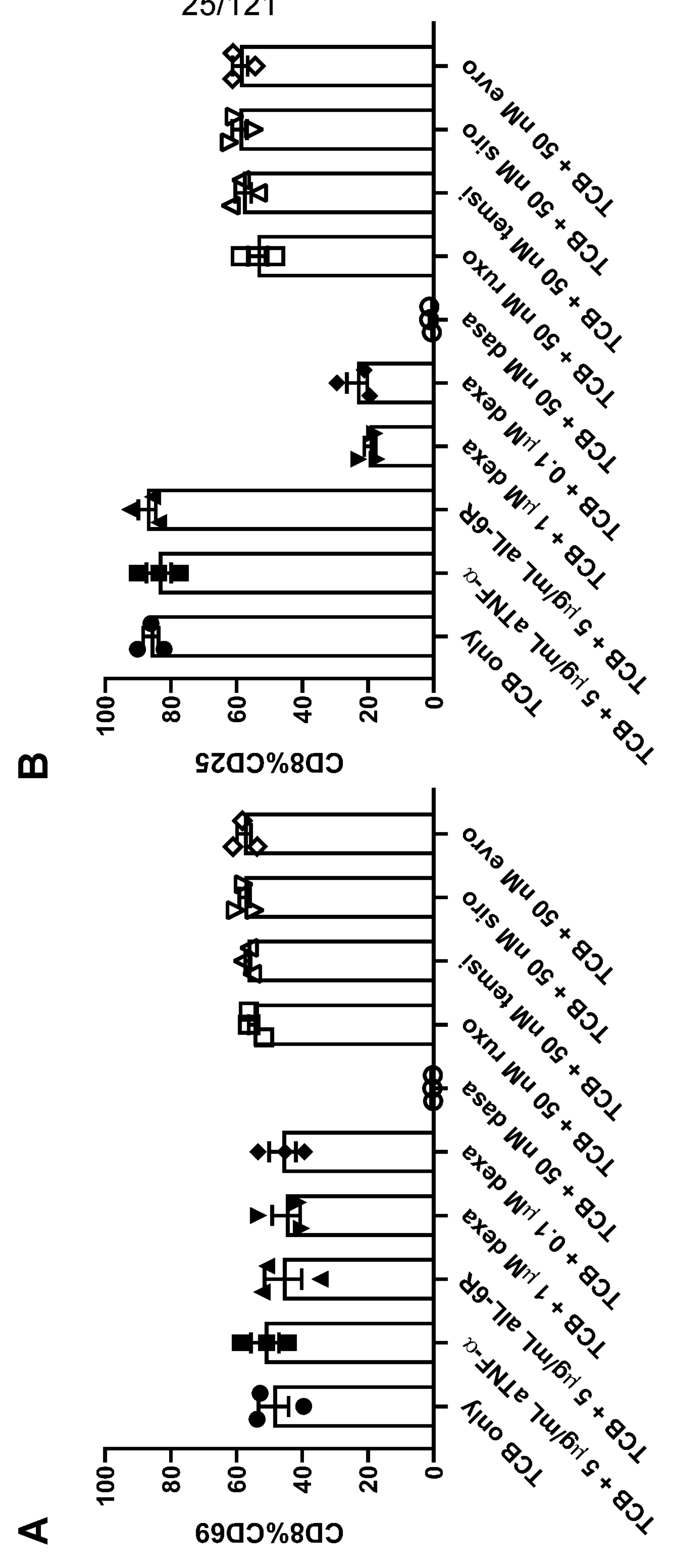


FIG. 25

A IFN-γ (pg/mL) 0 1000 2000 3000 4000 5000

B IL-2 (pg/mL) 0 50 100 150 200

C TNF-α (pg/mL) 0 2000 4000 6000 8000

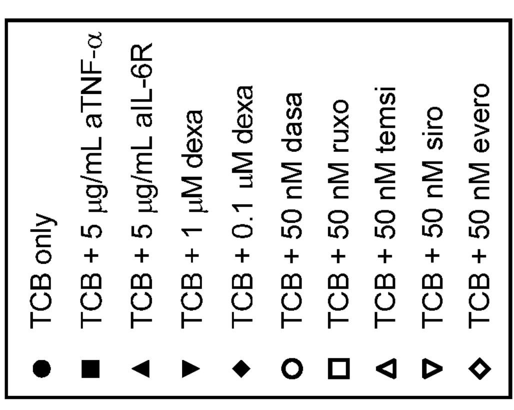
D IL-4 (pg/mL) 0 10 20 30 40

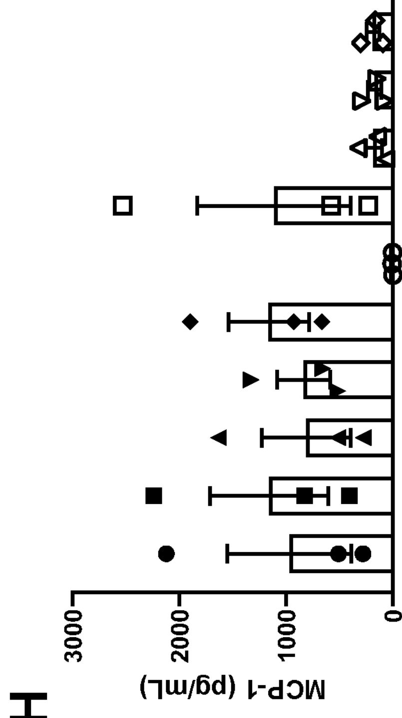
E IL-8 (pg/mL) 0 5000 10000 15000 20000

F IL-10 (pg/mL) 0 500 1000 1500

● TCB only
■ TCB + 5 μg/mL aTNF-α
▲ TCB + 5 μg/mL aIL-6R
▼ TCB + 1 μM dexa
◆ TCB + 0.1 μM dexa
○ TCB + 50 nM dasa
□ TCB + 50 nM ruxo
△ TCB + 50 nM temsi
▽ TCB + 50 nM siro
◇ TCB + 50 nM evero

FIG. 26





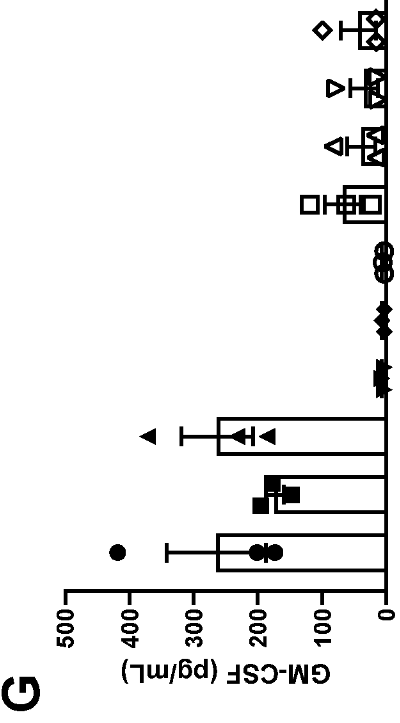
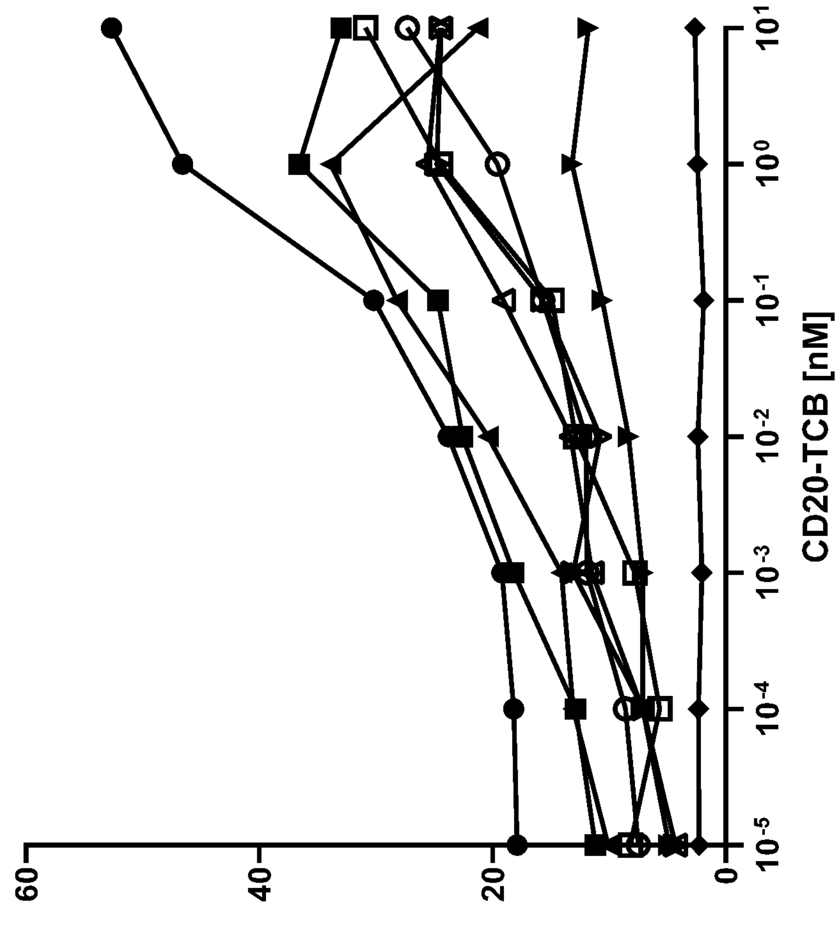


FIG. 26



- TCB only
- TCB + 5 μg/mL aTNF-α
- TCB + 5 μg/mL aIL-6R
- TCB + 100 nM dexa
- TCB + 100 nM dasa
- TCB + 100 nM ruxo
- TCB + 100 nM temsi
- TCB + 100 nM siro
- TCB + 100 nM evero

FIG. 27

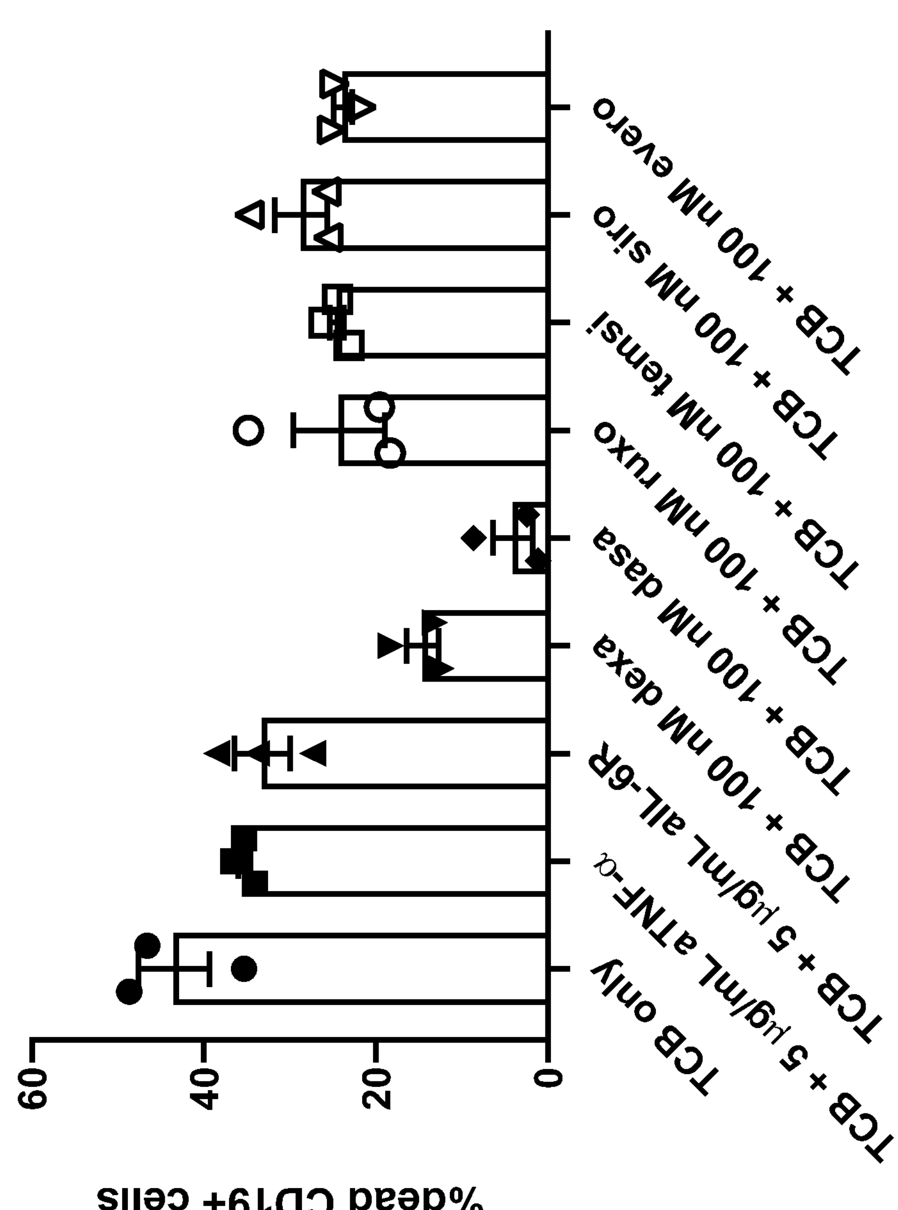
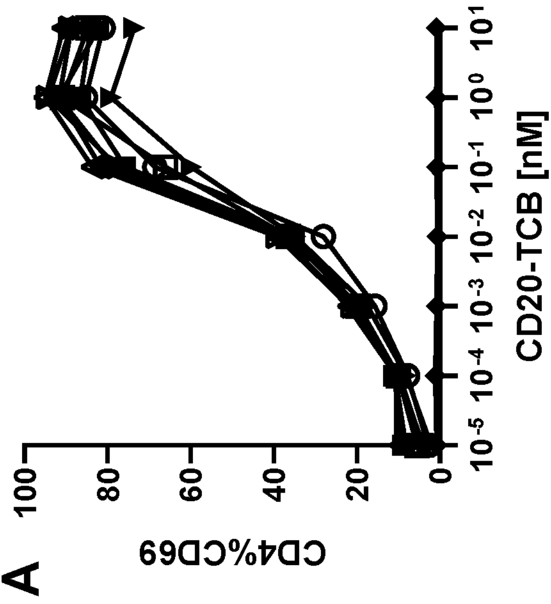
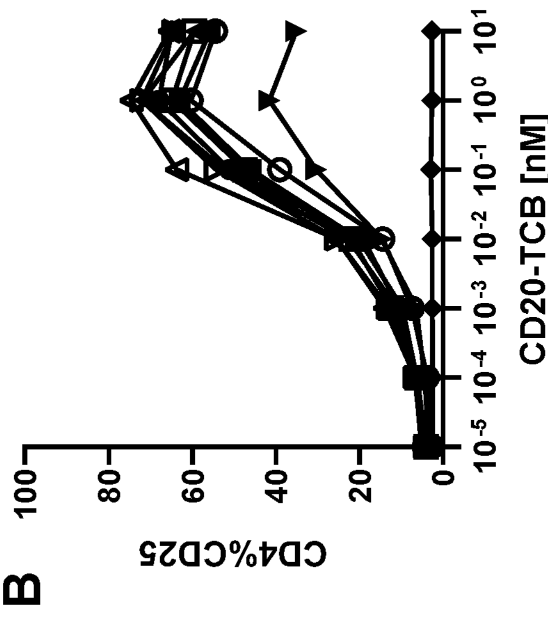
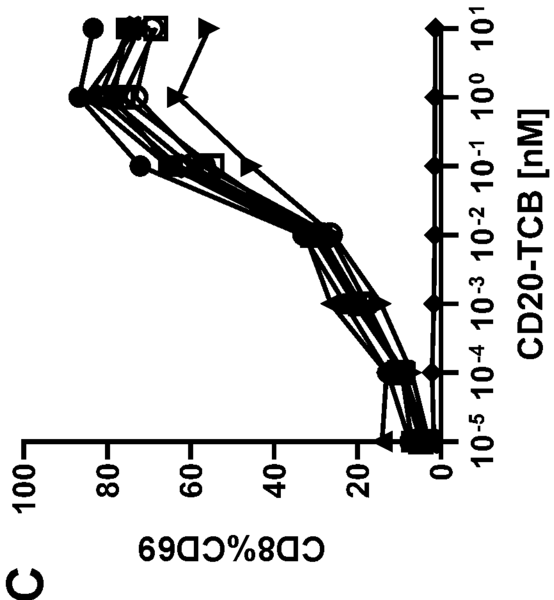
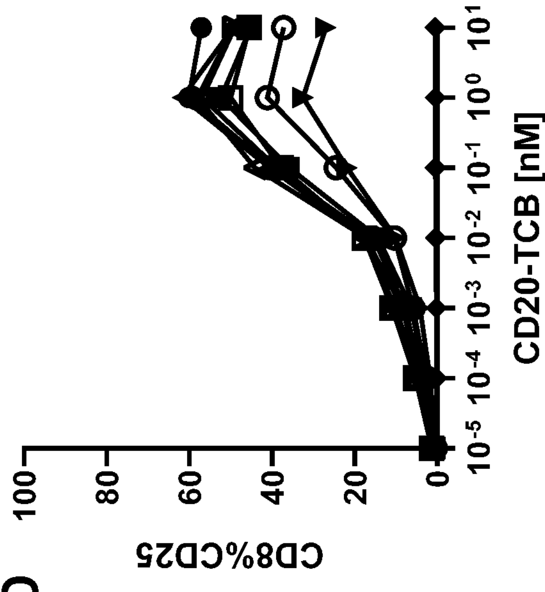


FIG. 28









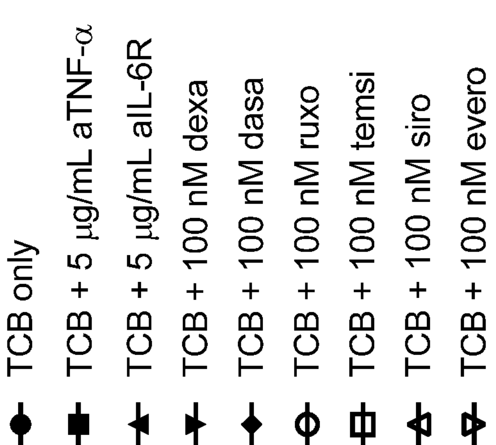
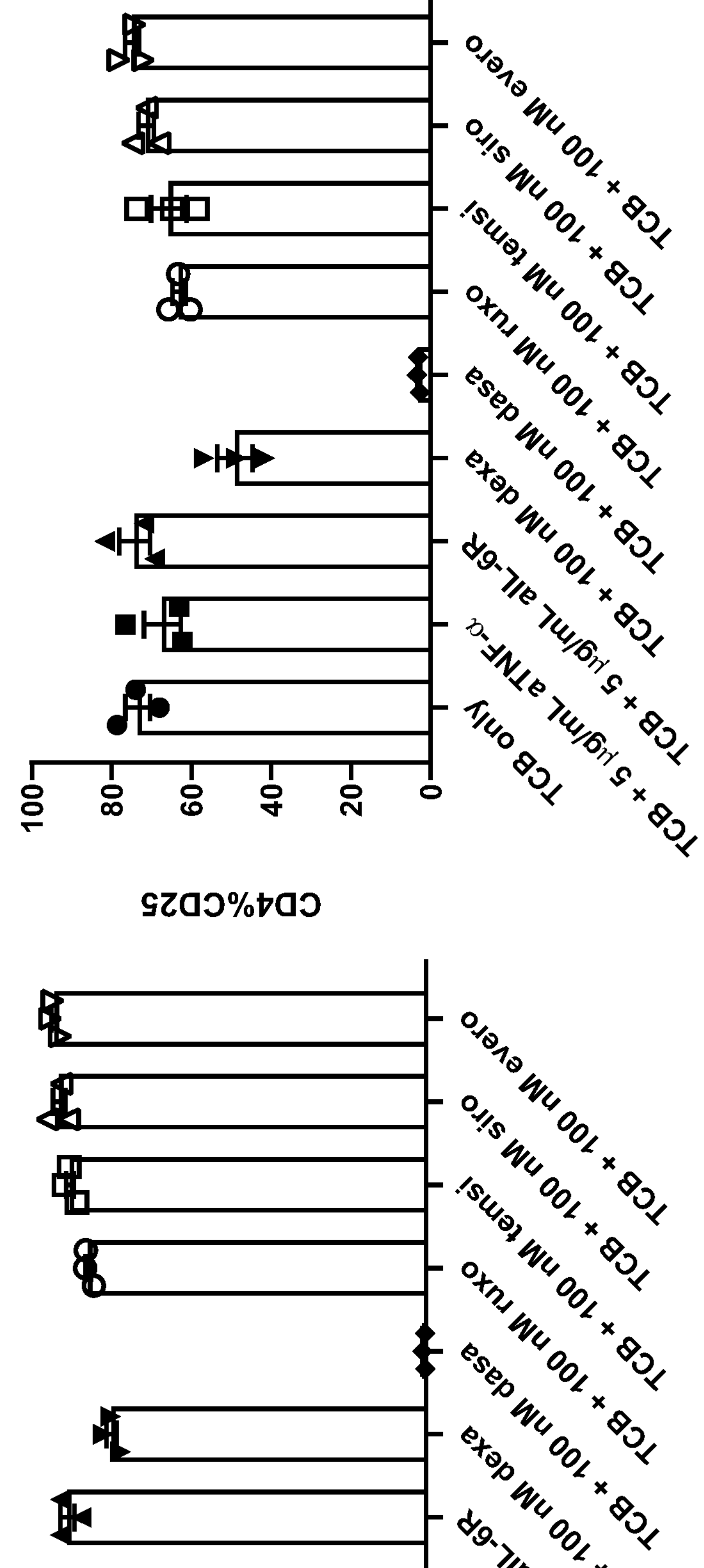
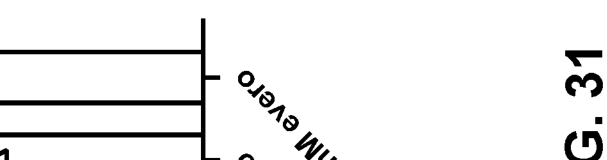


FIG. 29

FIG. 30


A
CD4%CD69
B
CD4%CD25
0
20
40
60
80
100
TCB only
TCB + 5 μg/mL aTNF-α
TCB + 5 μg/mL aIL-6R
TCB + 100 nM dexa
TCB + 100 nM dasa
TCB + 100 nM ruxo
TCB + 100 nM temsi
TCB + 100 nM siro
TCB + 100 nM evero



A

CD8%CD69

0
20
40
60
80
100

TCB only
TCB + 5 µg/mL aTNF-α
TCB + 5 µg/mL aIL-6R
TCB + 100 nM dexa
TCB + 100 nM dasa
TCB + 100 nM ruxo
TCB + 100 nM temsi
TCB + 100 nM siro
TCB + 100 nM evero

B

CD8%CD25

0
20
40
60
80
100

TCB only
TCB + 5 µg/mL aTNF-α
TCB + 5 µg/mL aIL-6R
TCB + 100 nM dexa
TCB + 100 nM dasa
TCB + 100 nM ruxo
TCB + 100 nM temsi
TCB + 100 nM siro
TCB + 100 nM evero

FIG. 31

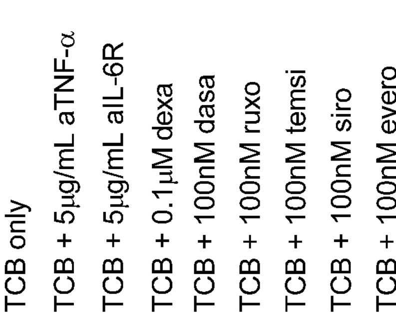
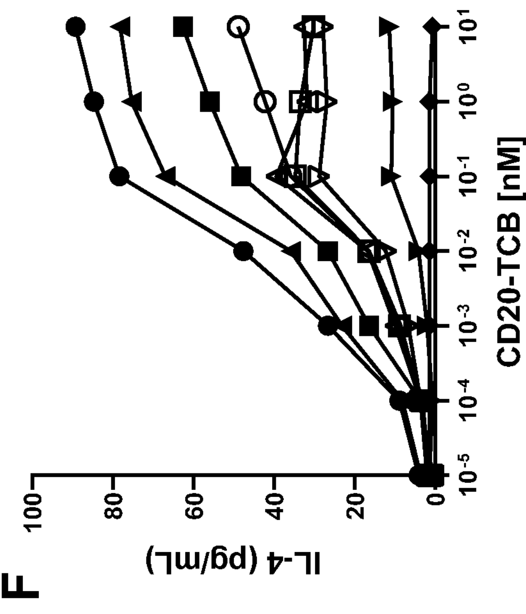
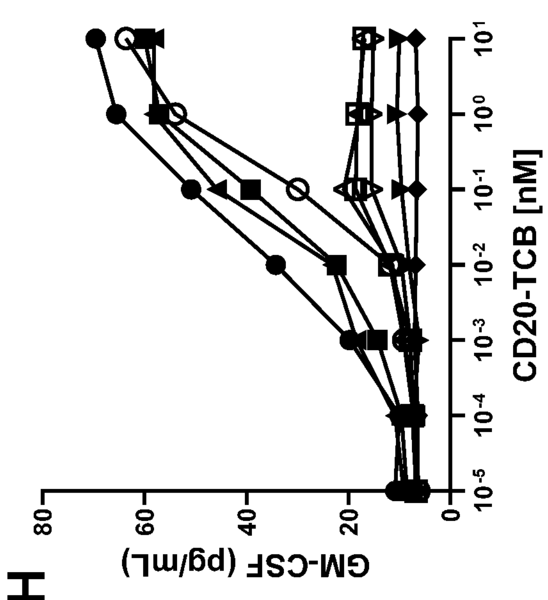
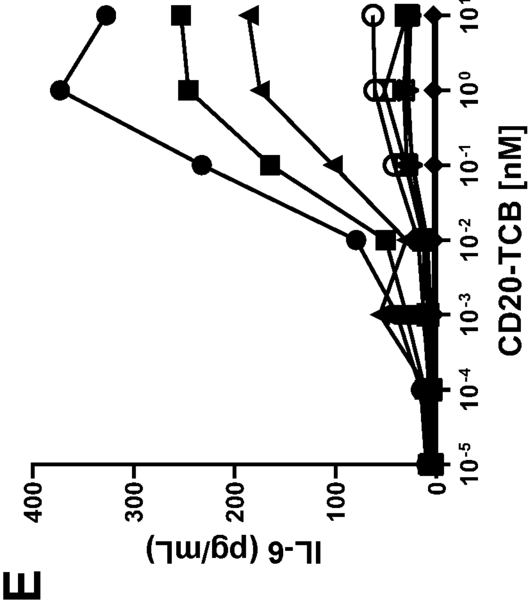
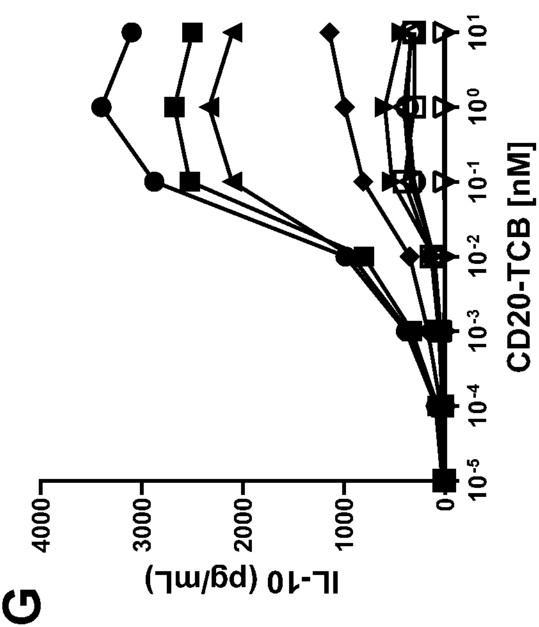


FIG. 32









- TCB only
- TCB + 5μg/mL aTNF-α
- TCB + 5μg/mL aIL-6R
- TCB + 0.1μM dexa
- TCB + 100nM dasa
- TCB + 100nM ruxo
- TCB + 100nM temsi
- TCB + 100nM siro
- TCB + 100nM evero

FIG. 32

A

TNF-α (pg/mL)

30000
20000
10000
0

B

IFN-γ (pg/mL)

5000
4000
3000
2000
1000
0

C

IL-2 (pg/mL)

8000
6000
4000
2000
0

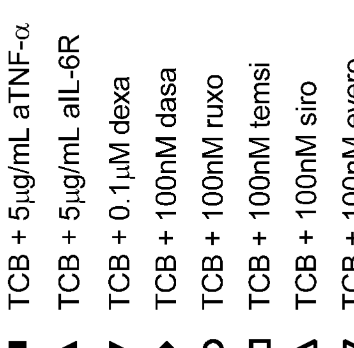
D

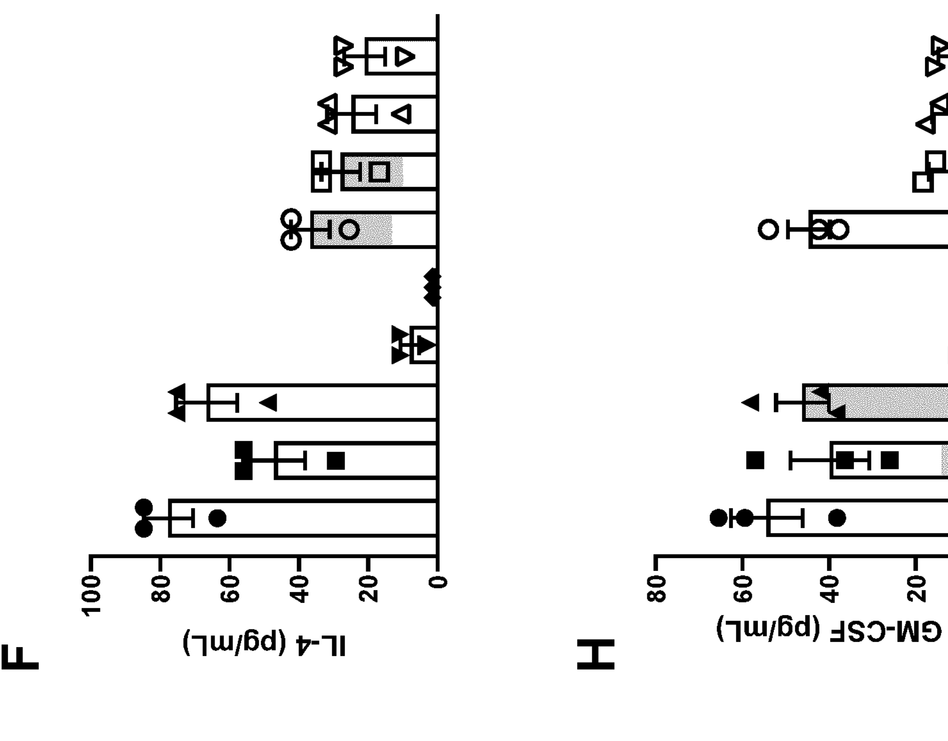
IL-1β (pg/mL)

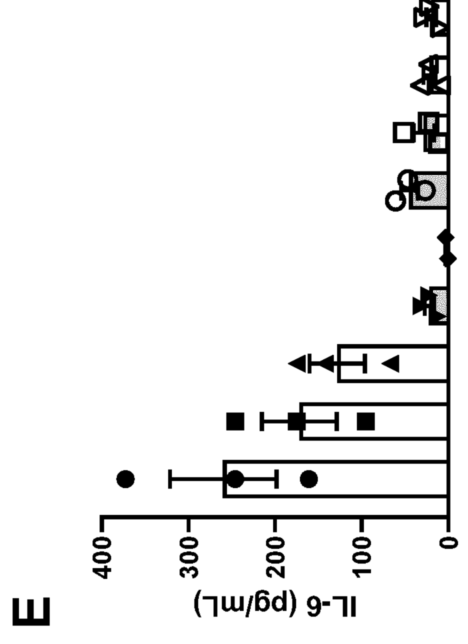
50
40
30
20
10
0

■ TCB + 5μg/mL aTNF-α
▲ TCB + 5μg/mL aIL-6R
▼ TCB + 0.1μM dexa
◆ TCB + 100nM dasa
○ TCB + 100nM ruxo
□ TCB + 100nM temsi
△ TCB + 100nM siro
▽ TCB + 100nM evero

FIG. 33







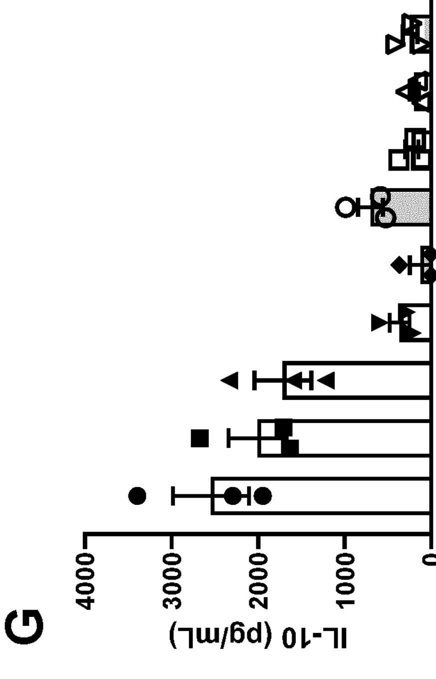


FIG. 33

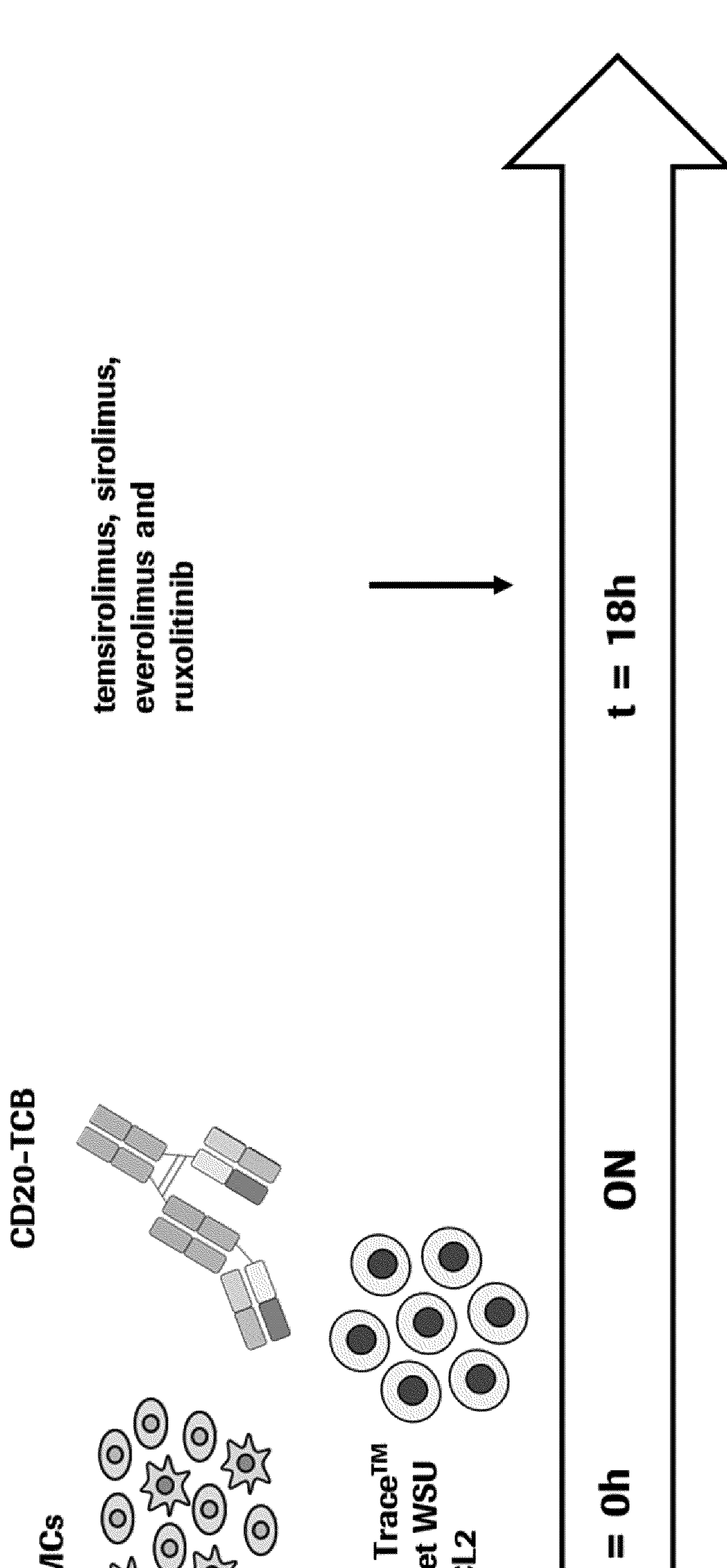
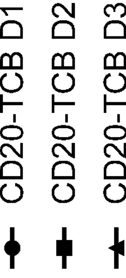


FIG. 34



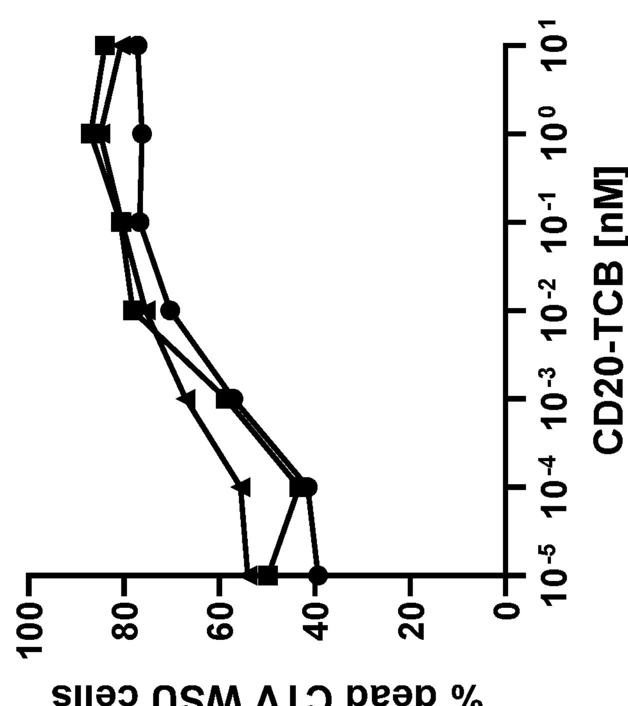
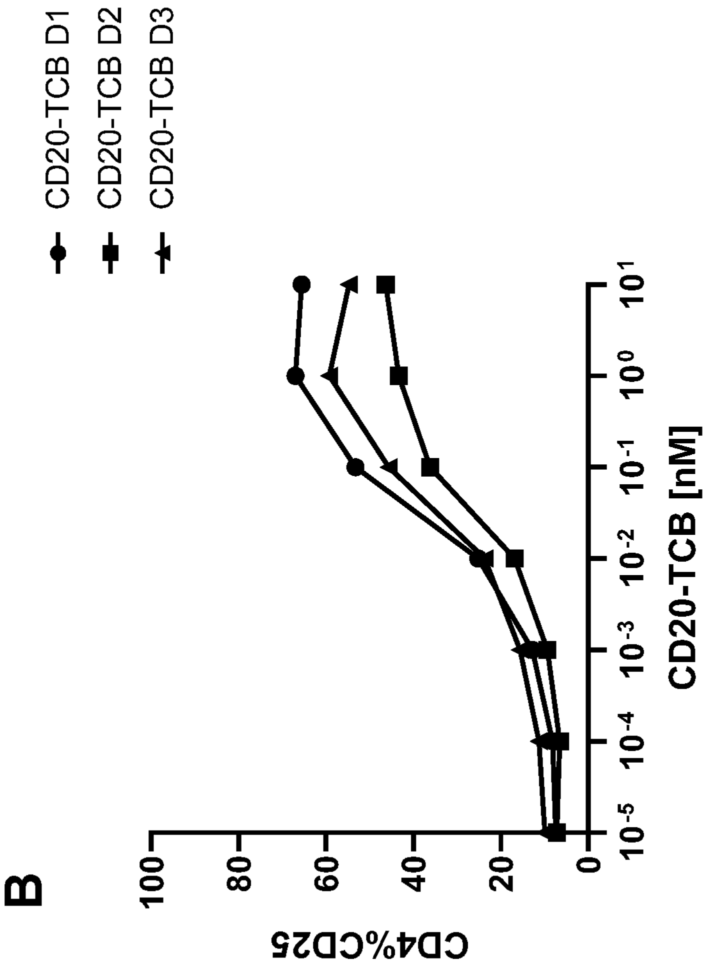
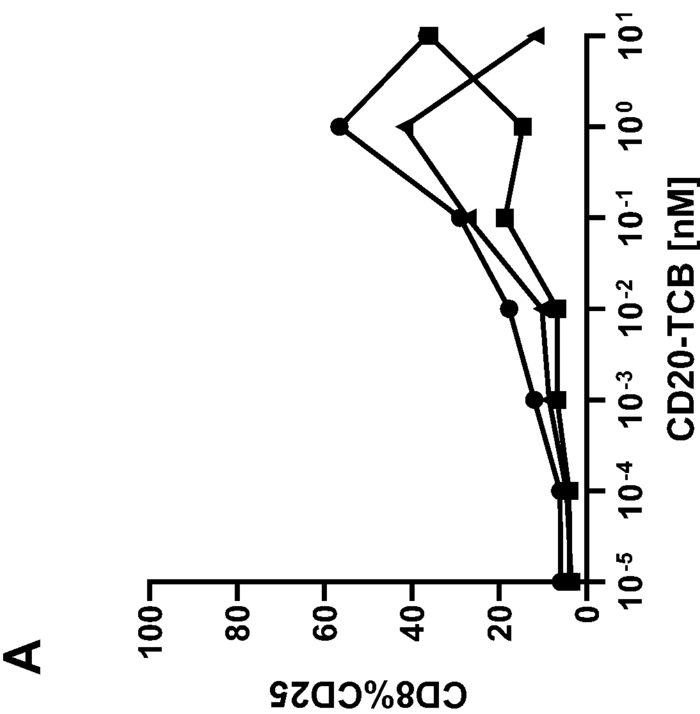


FIG. 35

FIG. 36


B
CD4%CD25
100
80
60
40
20
0
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
CD20-TCB [nM]
CD20-TCB D1
CD20-TCB D2
CD20-TCB D3


A
CD8%CD25
100
80
60
40
20
0
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
CD20-TCB [nM]

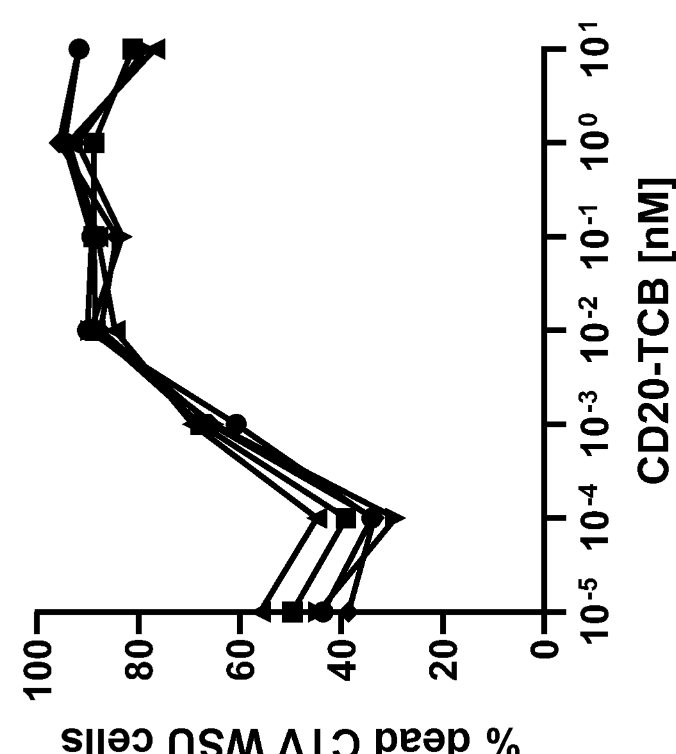


FIG. 37

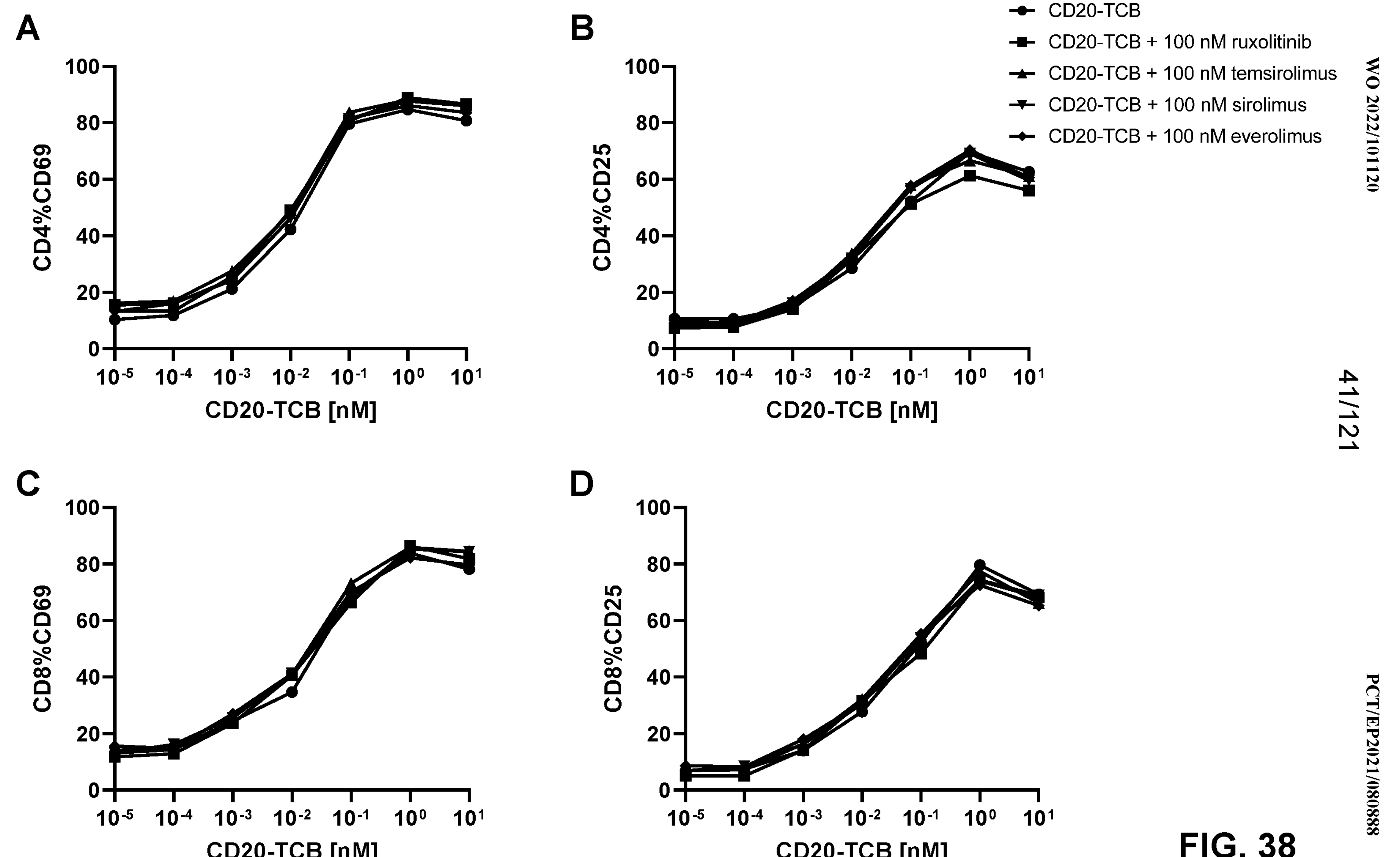


FIG. 38

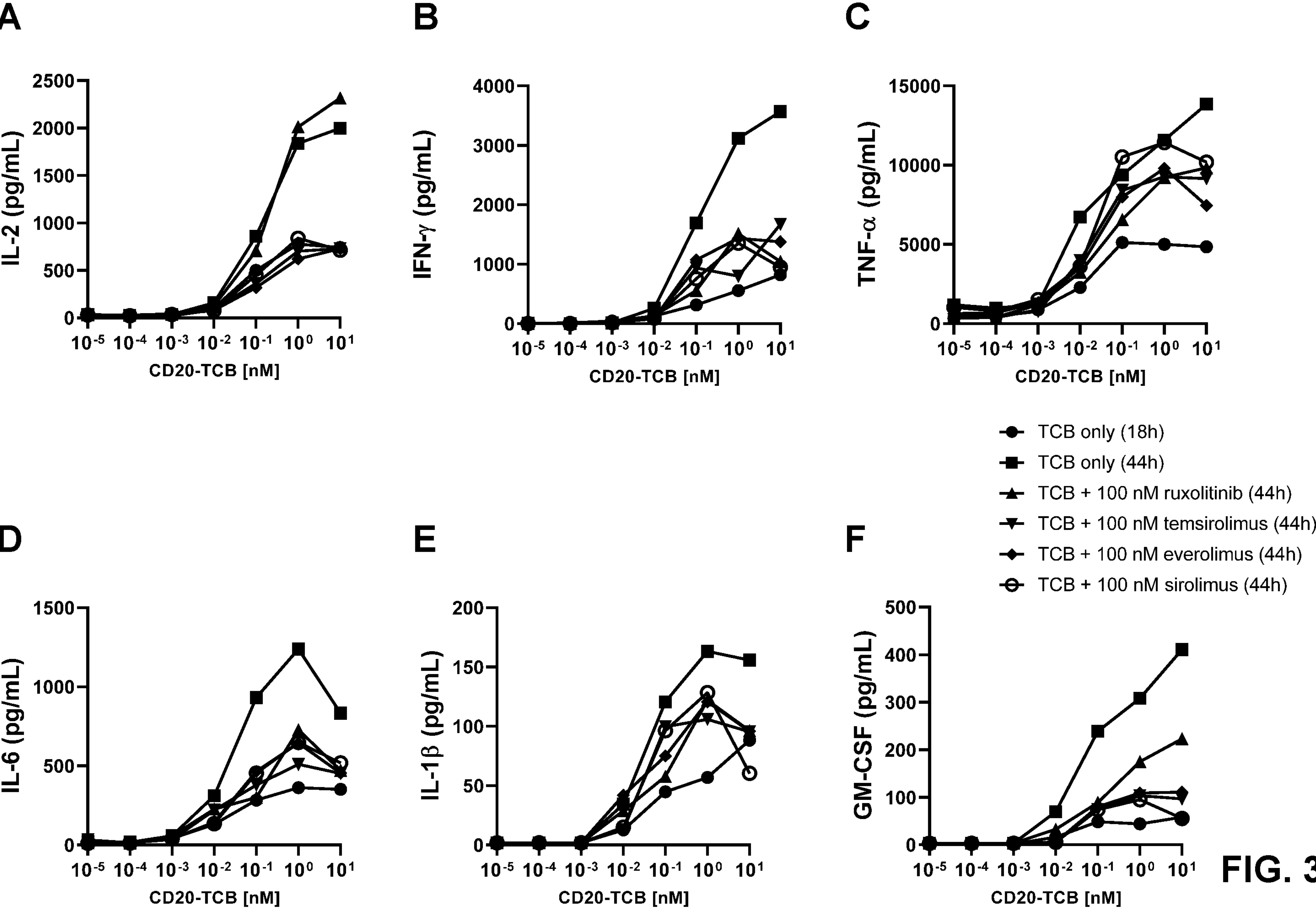


FIG. 39

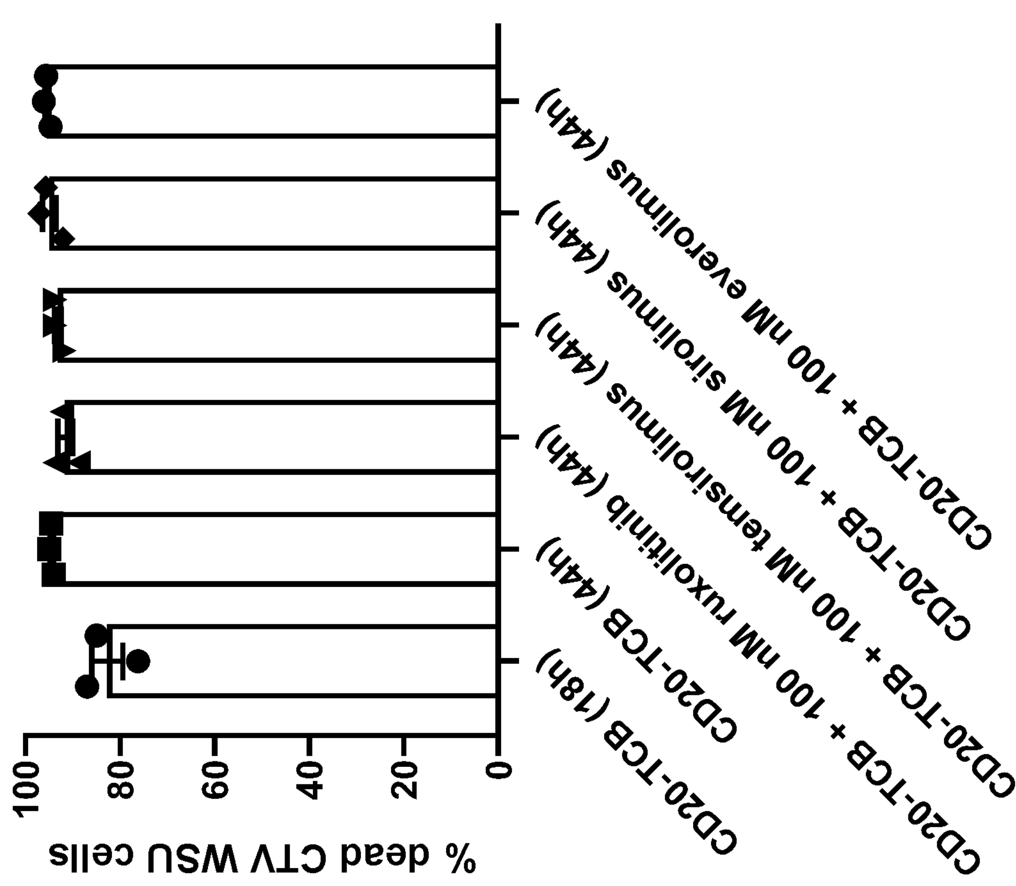
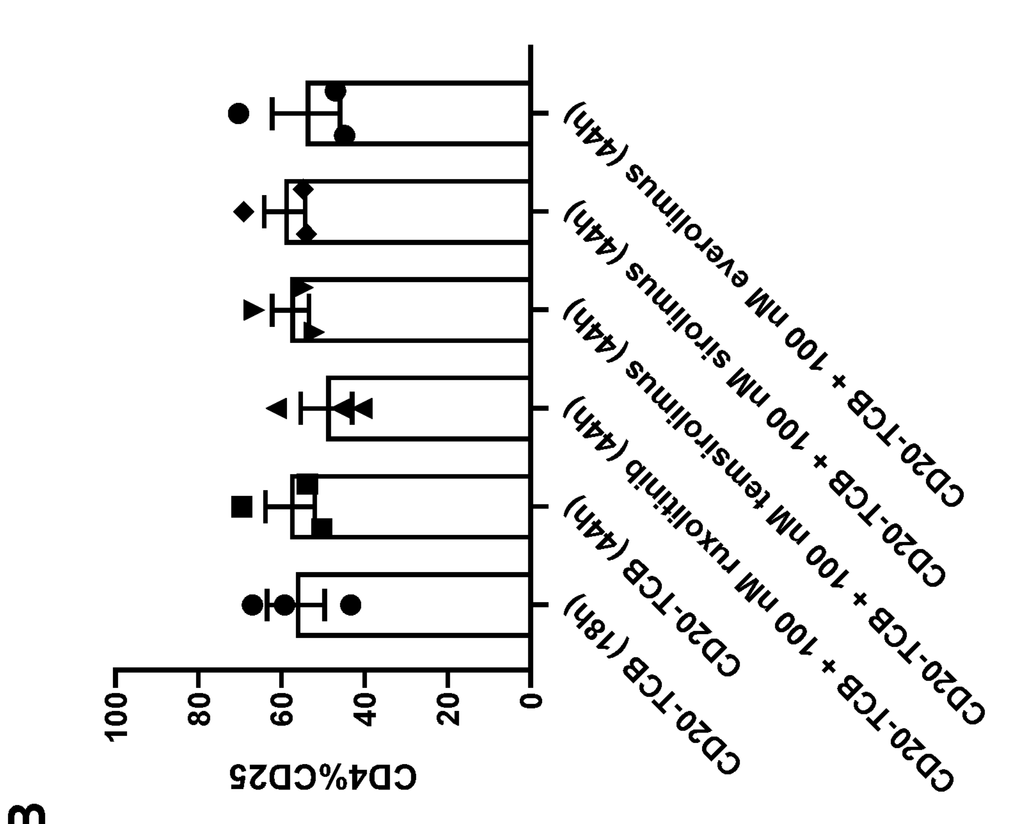


FIG. 40



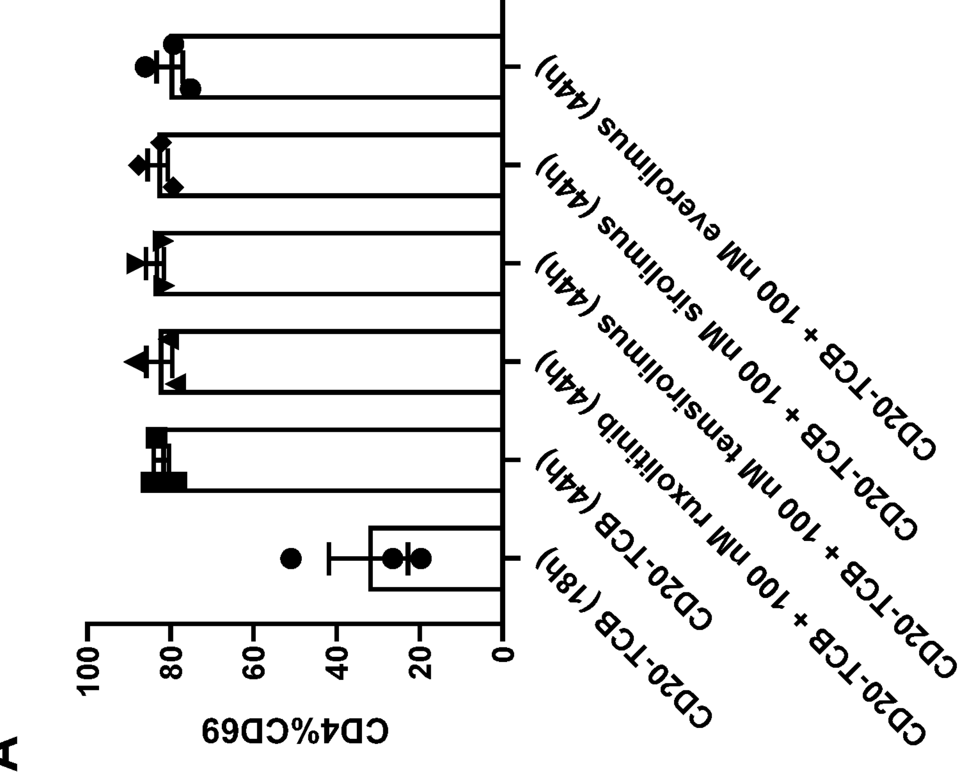
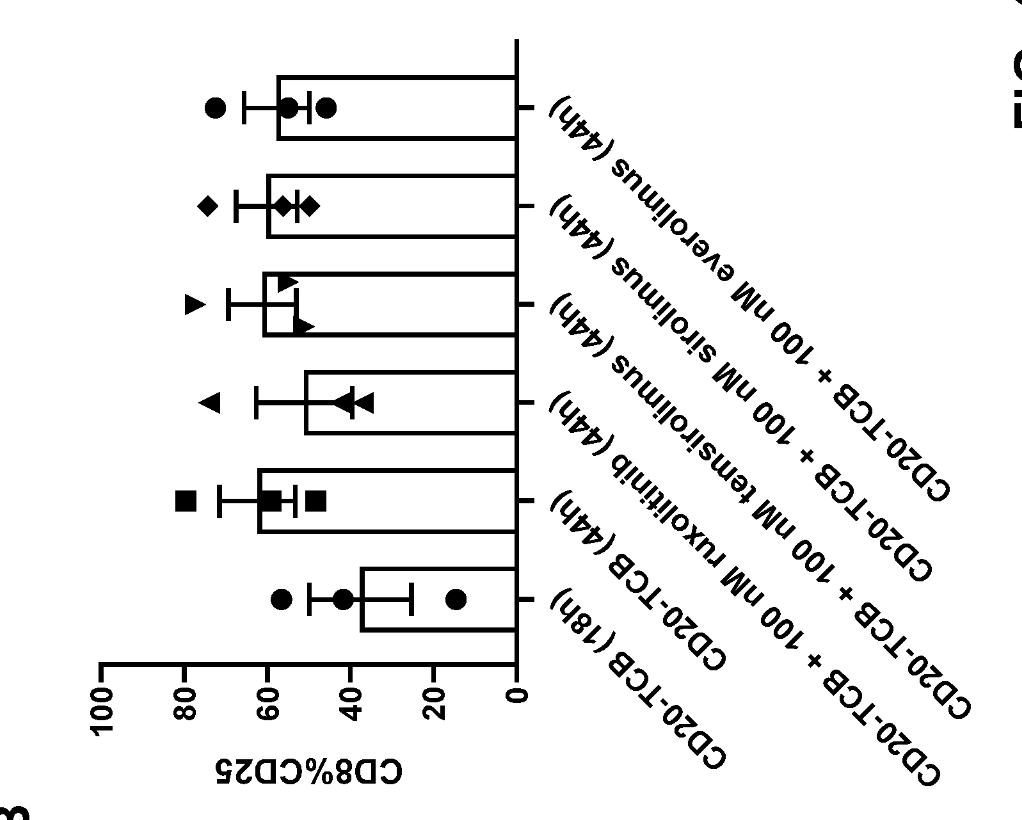


FIG. 41



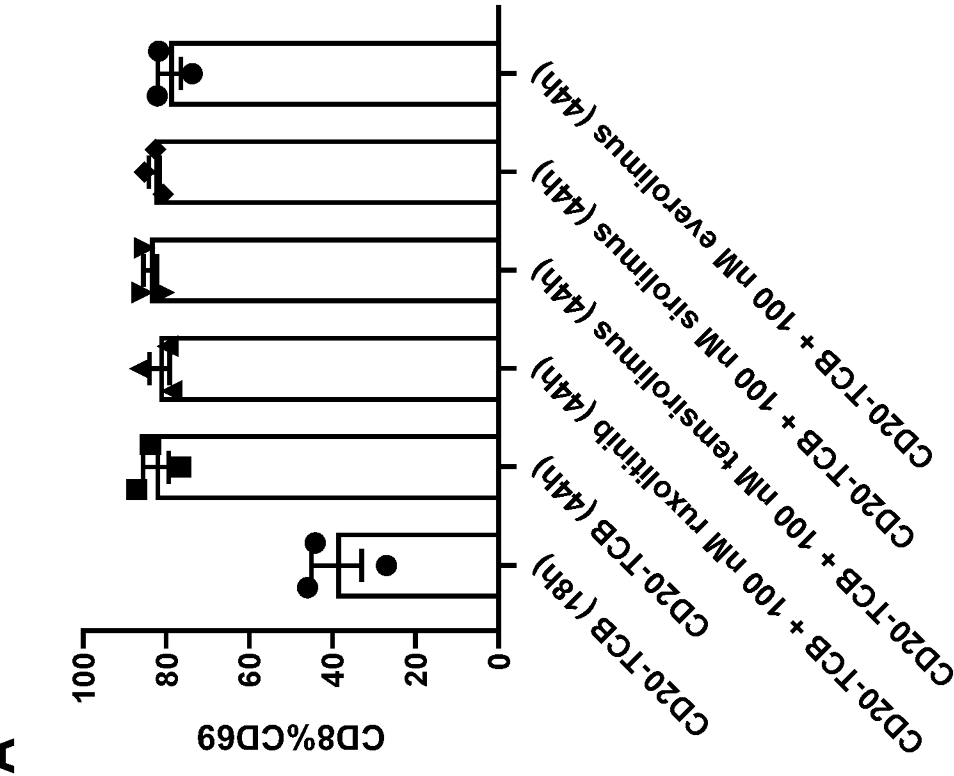
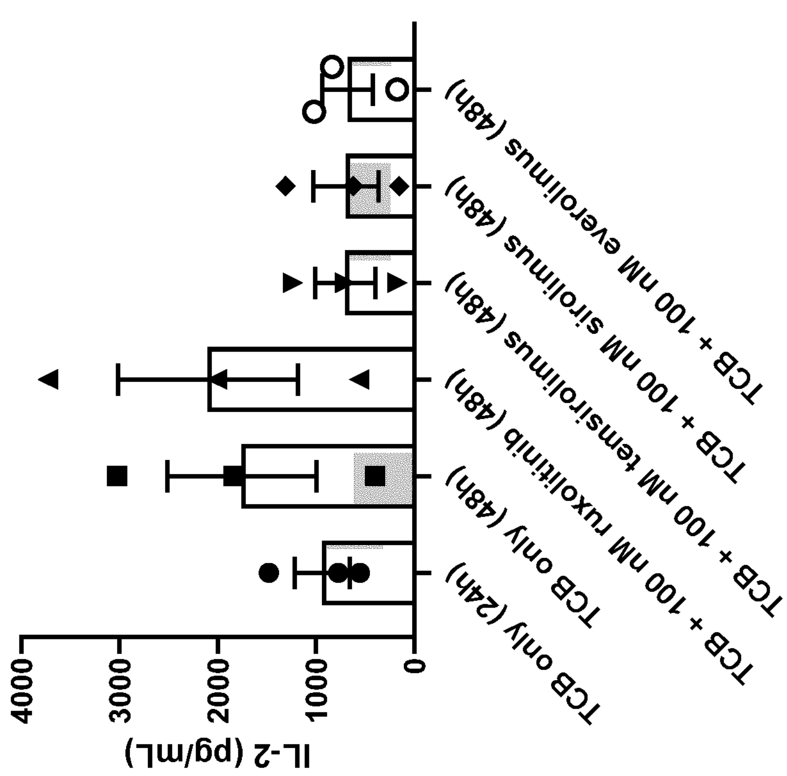


FIG. 42

FIG. 43


B
IL-2 (pg/mL)
4000
3000
2000
1000
0
TCB only (24h)
TCB only (48h)
TCB + 100 nM ruxolitinib (48h)
TCB + 100 nM temsirolimus (48h)
TCB + 100 nM sirolimus (48h)
TCB + 100 nM everolimus (48h)

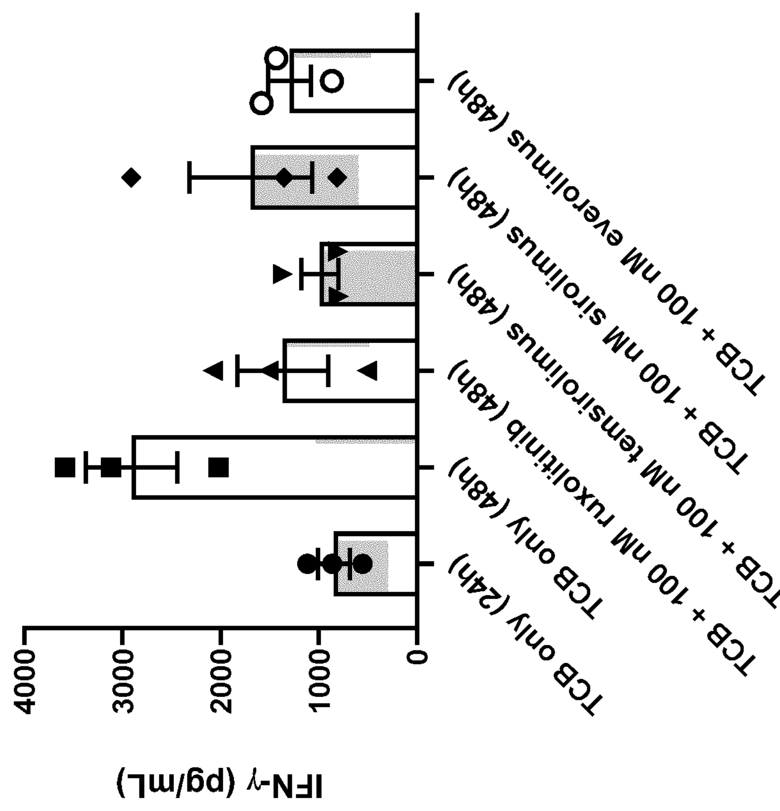

A
IFN-γ (pg/mL)
4000
3000
2000
1000
0
TCB only (24h)
TCB only (48h)
TCB + 100 nM ruxolitinib (48h)
TCB + 100 nM temsirolimus (48h)
TCB + 100 nM sirolimus (48h)
TCB + 100 nM everolimus (48h)

FIG. 43

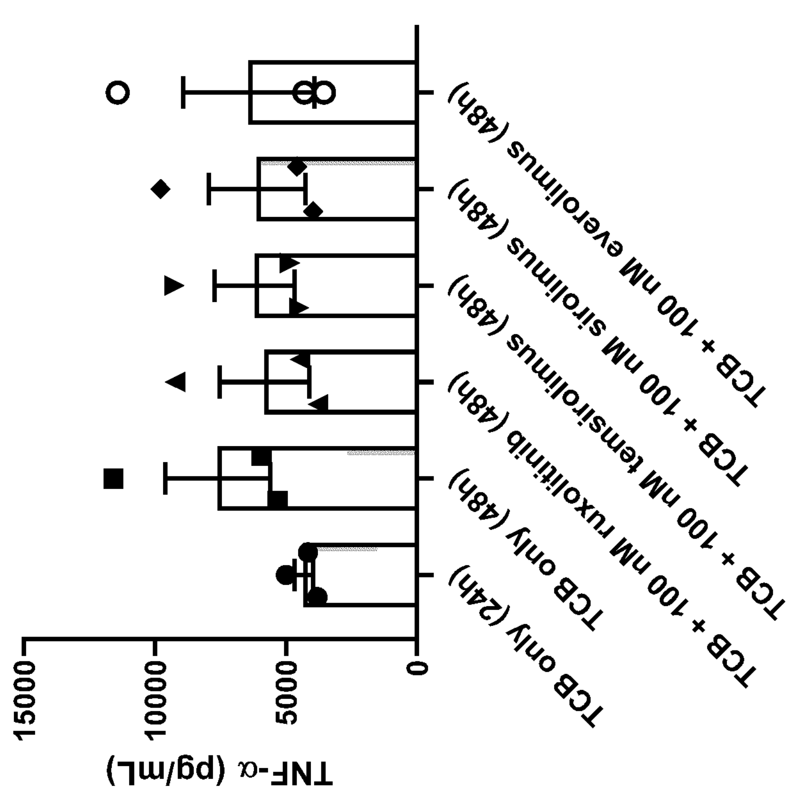
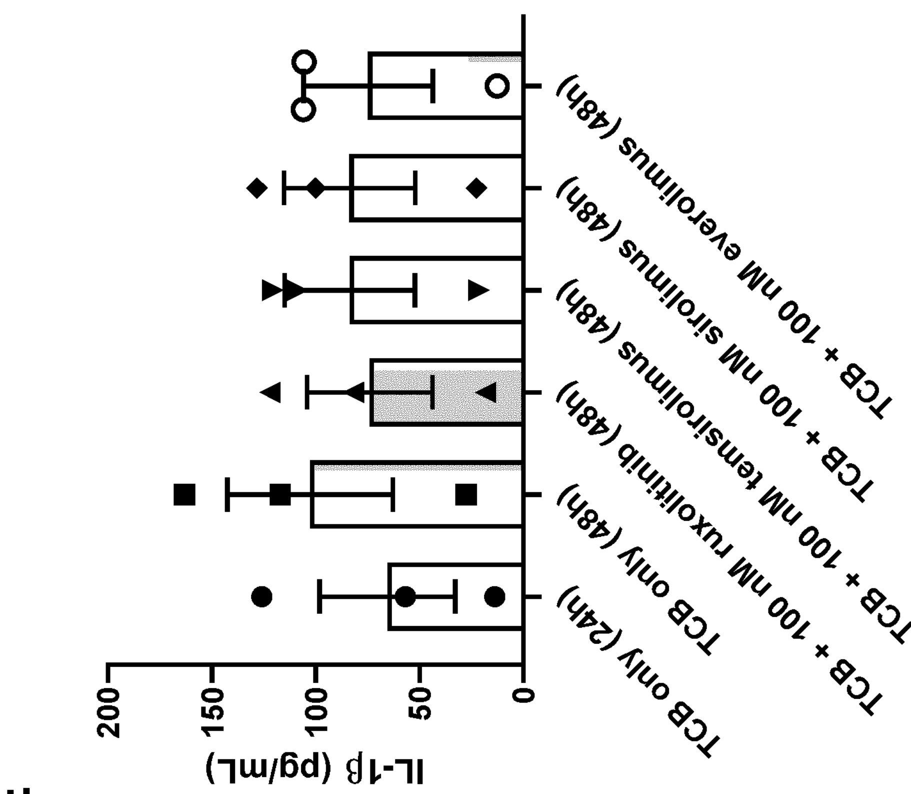


FIG. 43


E
IL-1β (pg/mL)
200
150
100
50
0
TCB only (24h)
TCB only (48h)
TCB + 100 nM ruxolitinib (48h)
TCB + 100 nM temsirolimus (48h)
TCB + 100 nM sirolimus (48h)
TCB + 100 nM everolimus (48h)

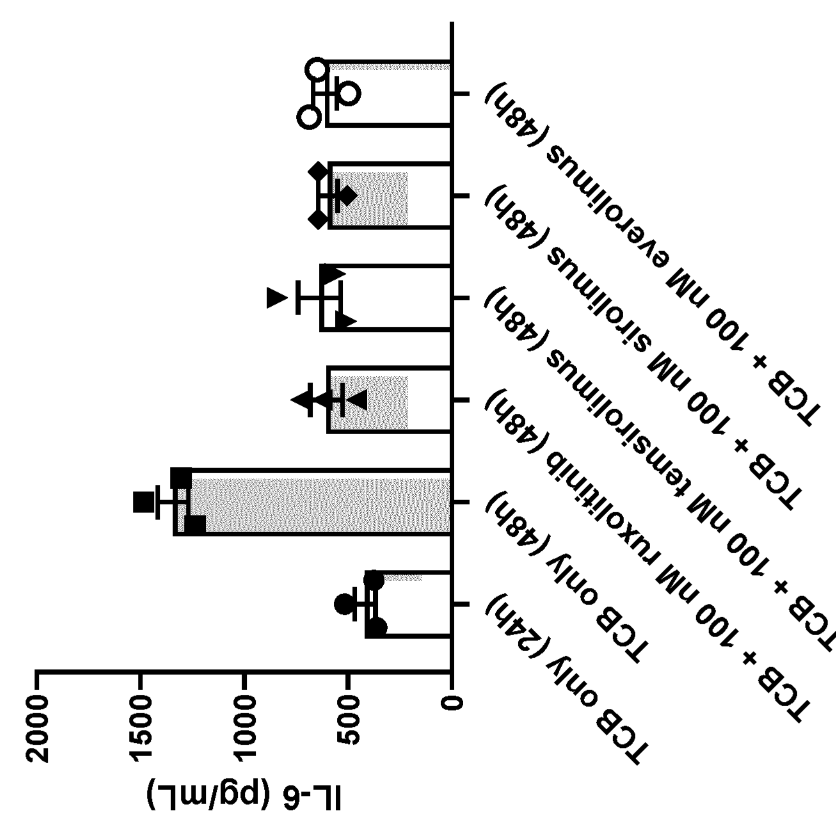
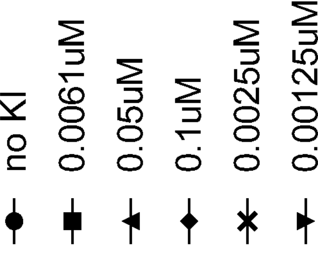
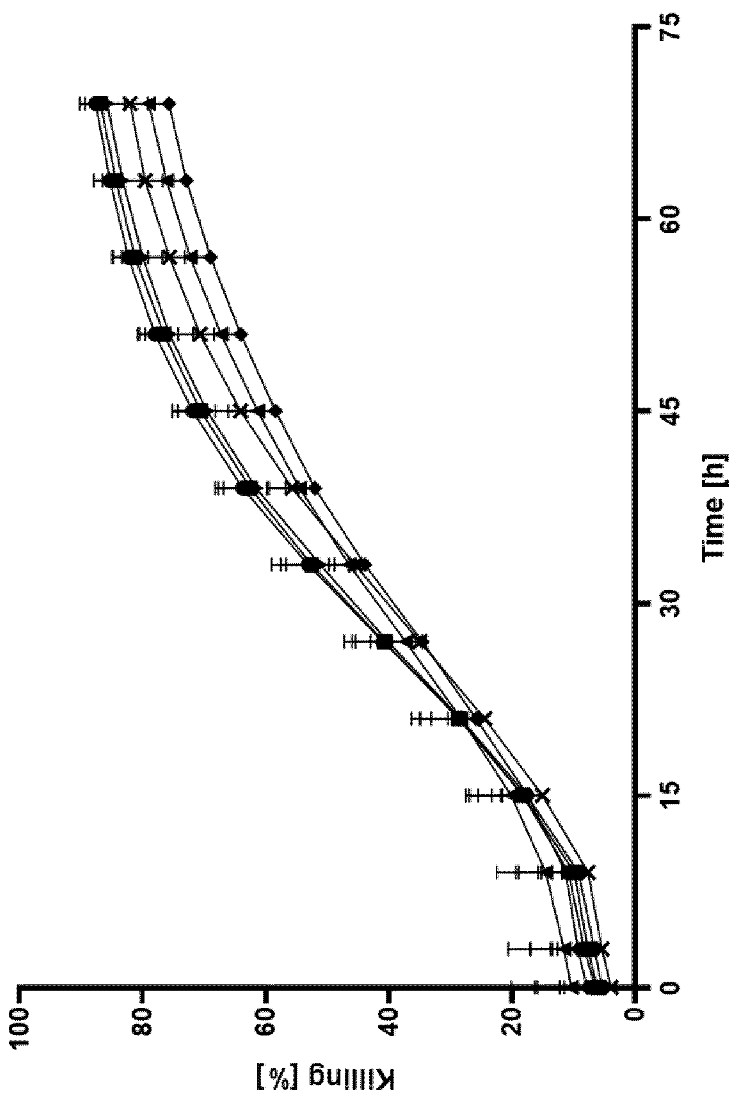
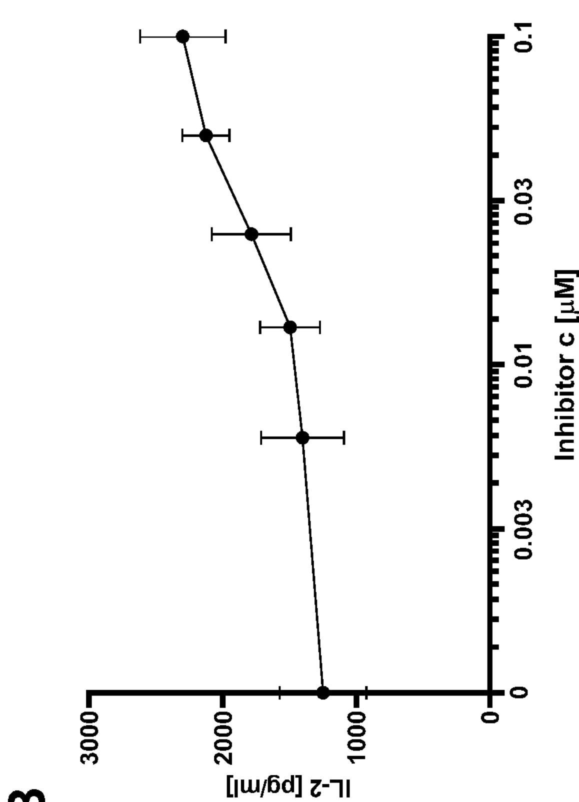

D
IL-6 (pg/mL)
2000
1500
1000
500
0
TCB only (24h)
TCB only (48h)
TCB + 100 nM ruxolitinib (48h)
TCB + 100 nM temsirolimus (48h)
TCB + 100 nM sirolimus (48h)
TCB + 100 nM everolimus (48h)

FIG. 44







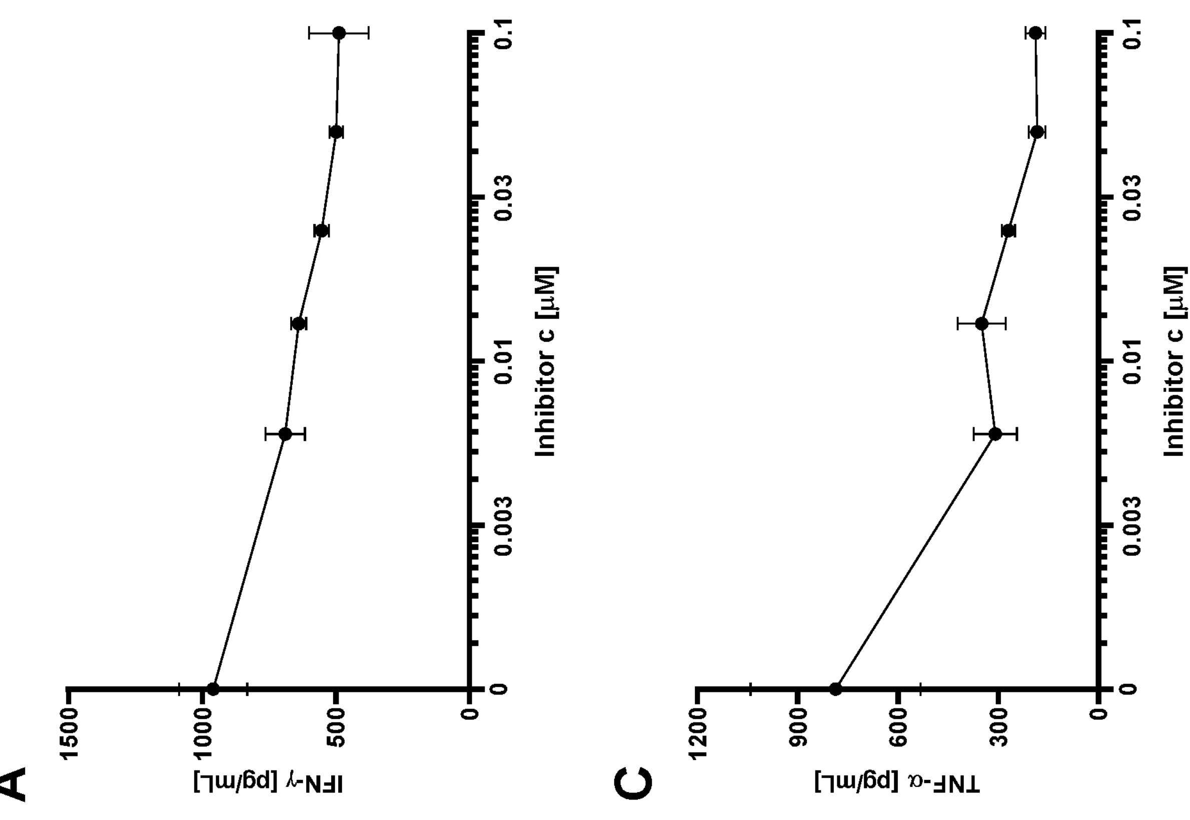
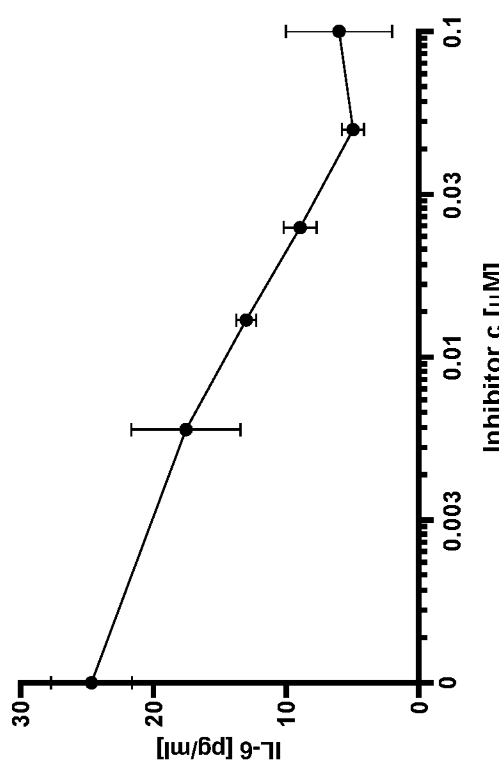
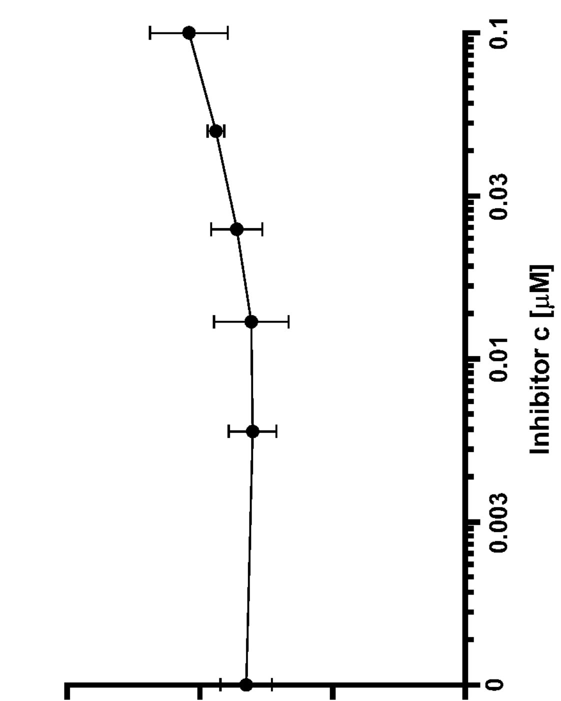


FIG. 45





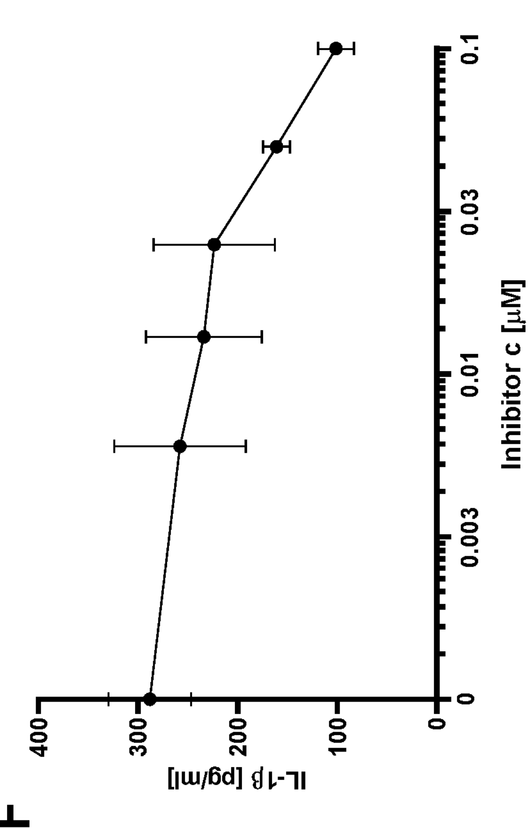


FIG. 45

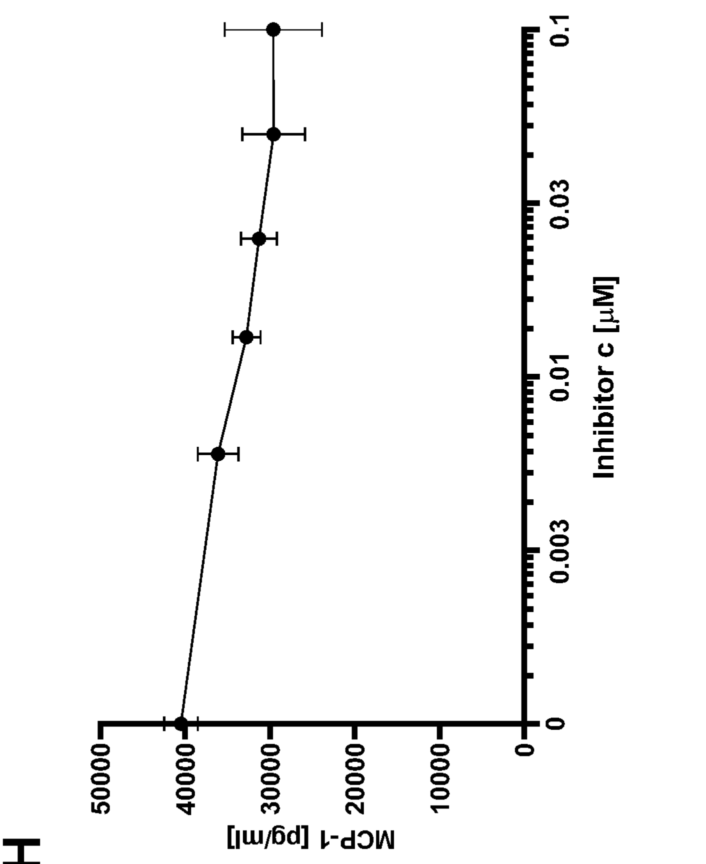
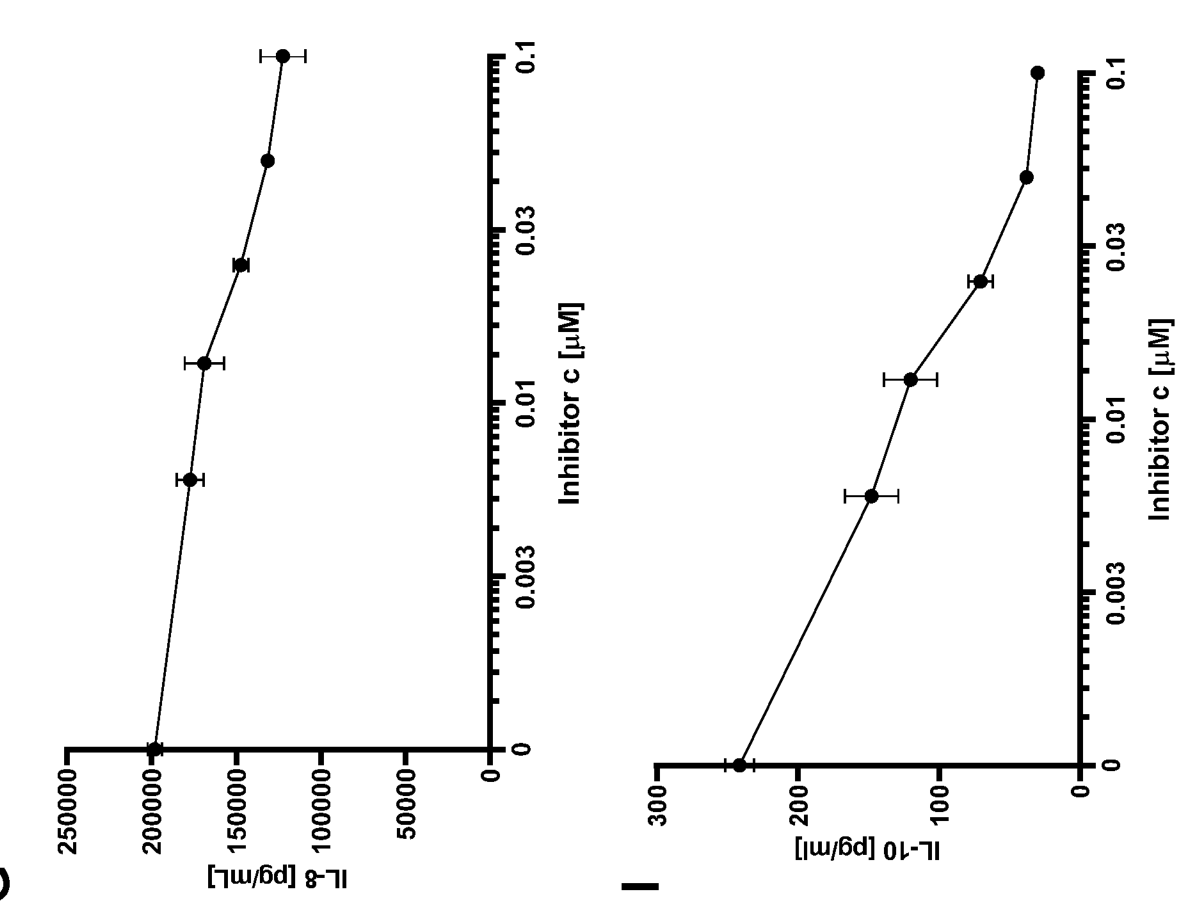

H
MCP-1 [pg/ml]
50000
40000
30000
20000
10000
0
0
0.003
0.01
0.03
0.1
Inhibitor c [μM]

FIG. 45


G
IL-8 [pg/mL]
250000
200000
150000
100000
50000
0
0
0.003
0.01
0.03
0.1
Inhibitor c [μM]
I
IL-10 [pg/ml]
300
200
100
0
0
0.003
0.01
0.03
0.1
Inhibitor c [μM]

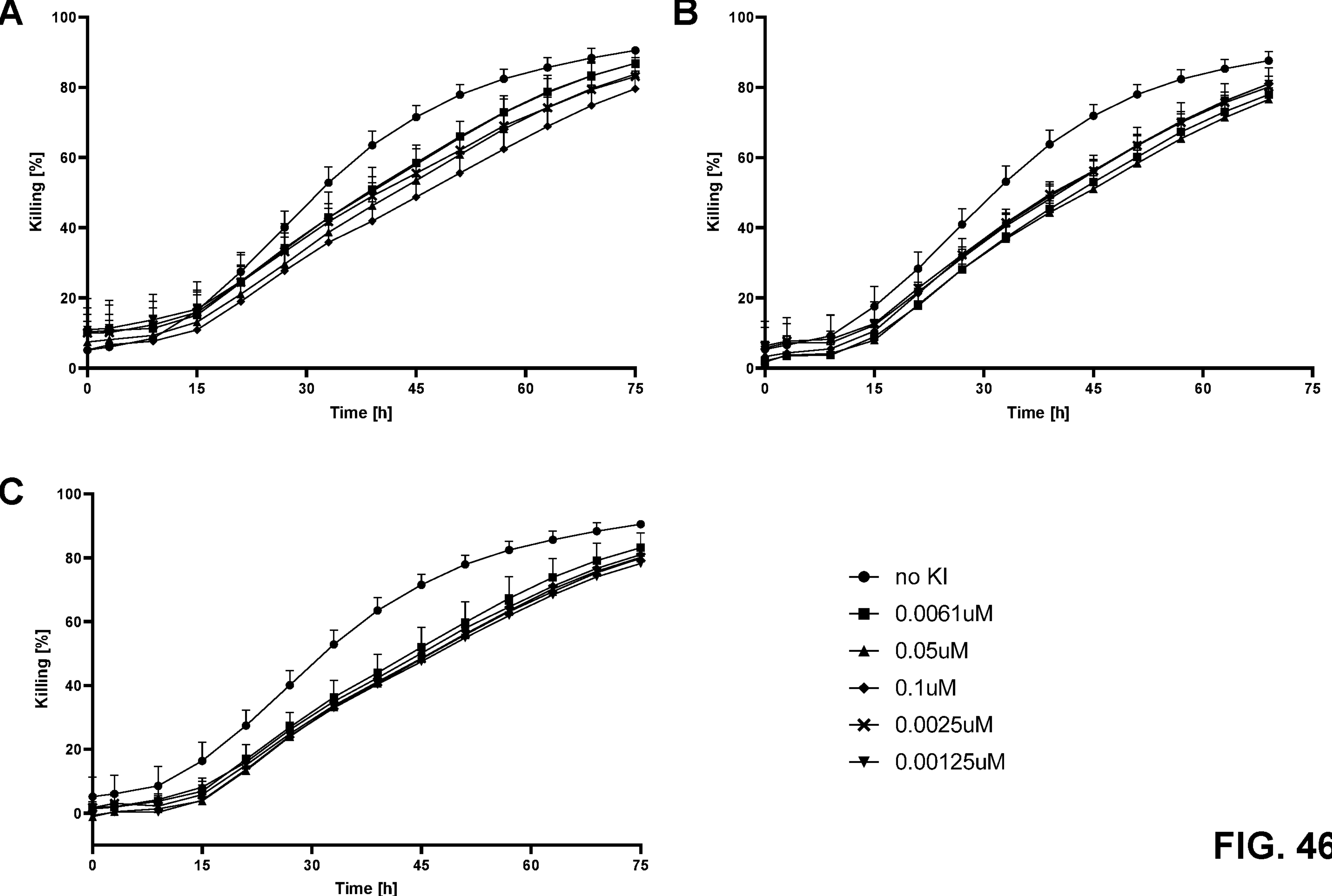
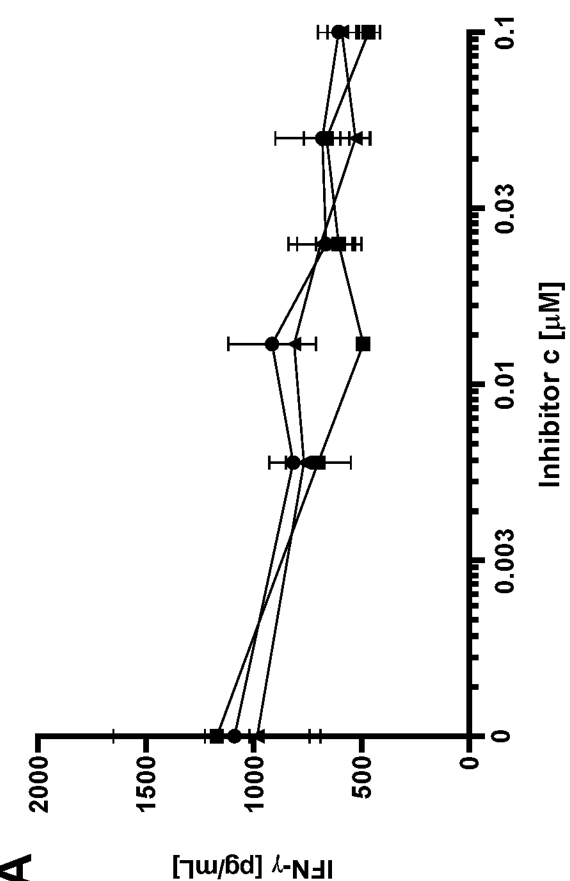
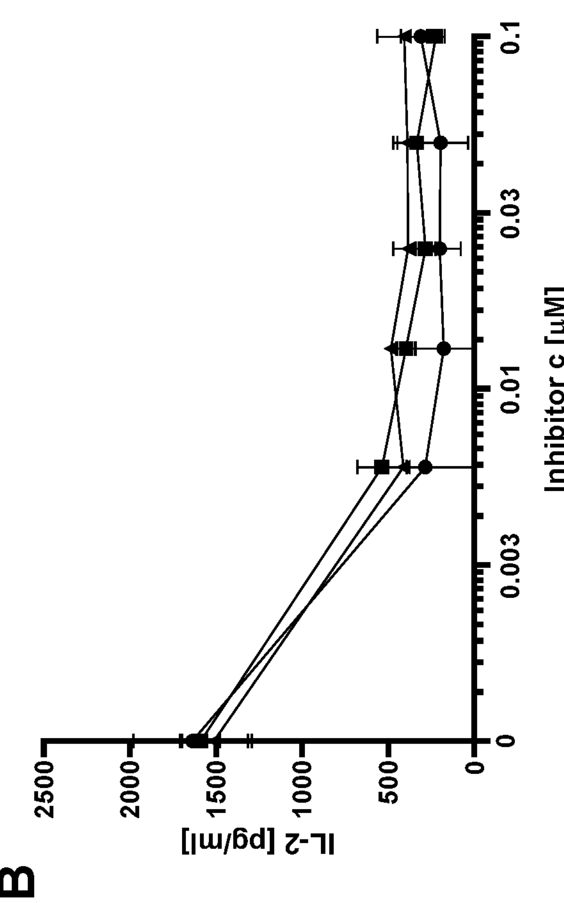
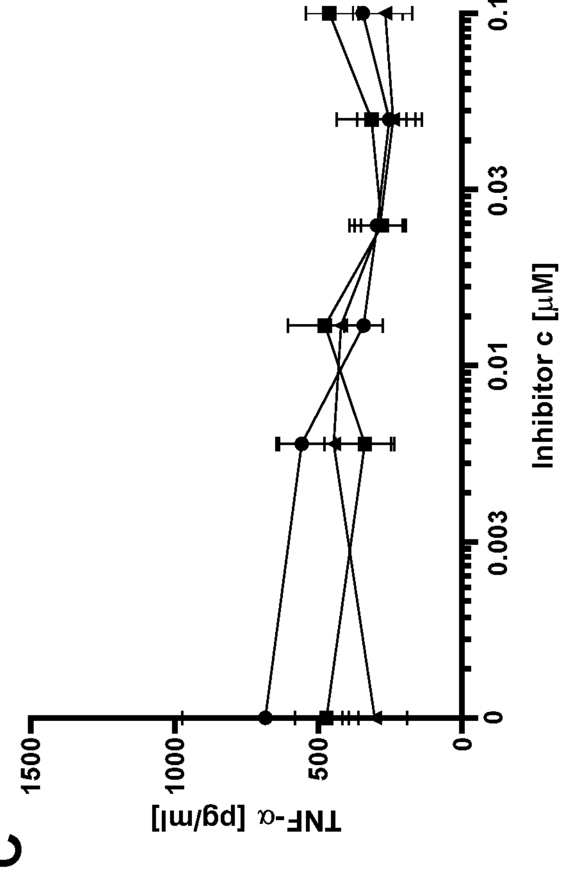


FIG. 46


A
IFN-γ [pg/mL]
2000
1500
1000
500
0
0
0.003
0.01
0.03
0.1
Inhibitor c [μM]


B
IL-2 [pg/ml]
2500
2000
1500
1000
500
0
0
0.003
0.01
0.03
0.1
Inhibitor c [μM]


C
TNF-α [pg/ml]
1500
1000
500
0
0
0.003
0.01
0.03
0.1
Inhibitor c [μM]

Everolimus
Sirolimus
Temsirolimus

FIG. 47

E

IL-6 [pg/ml]

50 40 30 20 10 0

0 0.003 0.01 0.03 0.1

Inhibitor c [μM]

Everolimus

Sirolimus

Temsirolimus

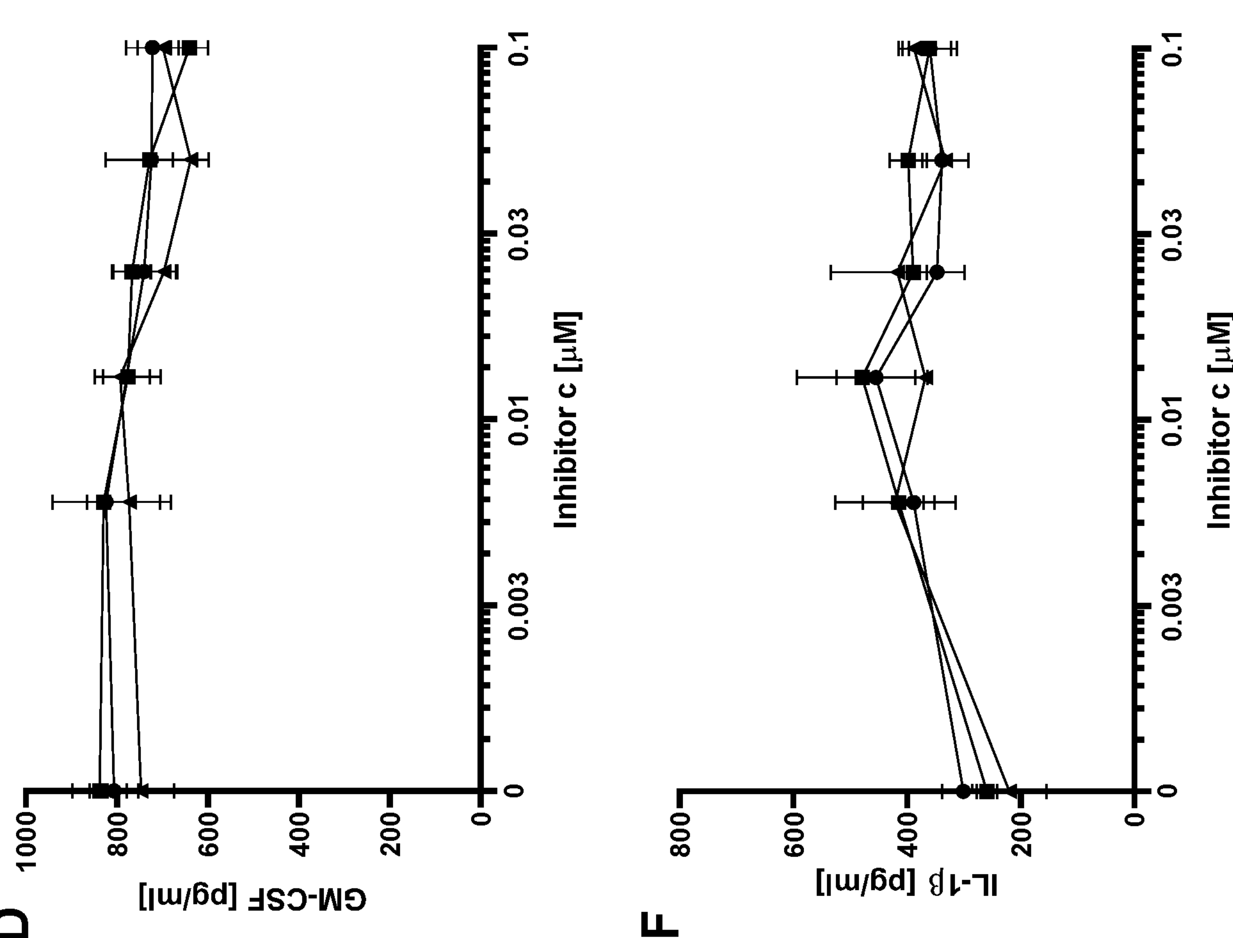


FIG. 47

G

IL-8 [pg/ml]

250000 200000 150000 100000 50000 0

0 0.003 0.01 0.03 0.1

Inhibitor c [μM]

H

MCP-1 [pg/ml]

60000 40000 20000 0

0 0.003 0.01 0.03 0.1

Inhibitor c [μM]

I

IL-10 [pg/ml]

500 400 300 200 100 0

0 0.003 0.01 0.03 0.1

Inhibitor c [μM]

Everolimus

Sirolimus

Temsirolimus

FIG. 47

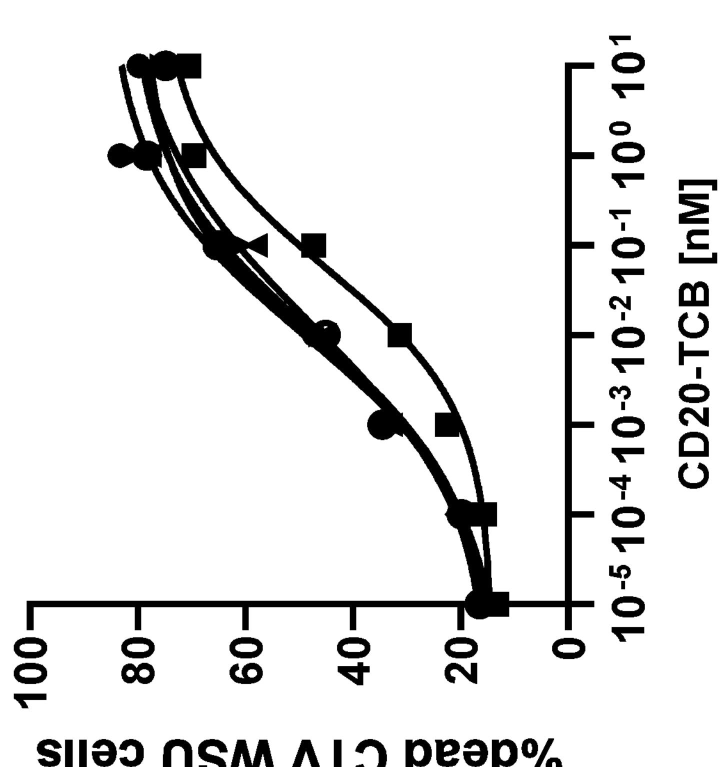


FIG. 48

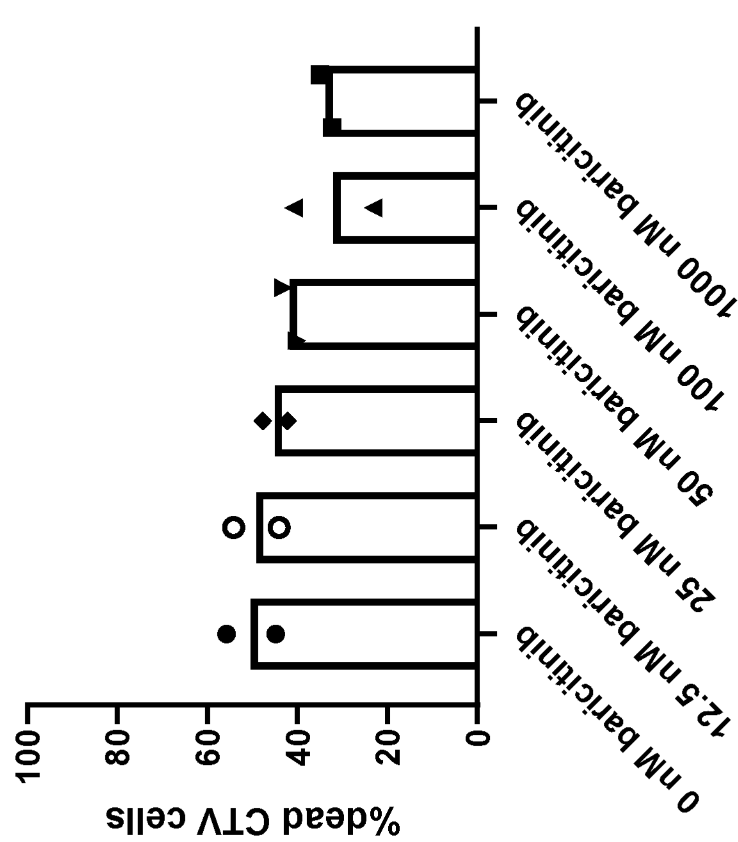
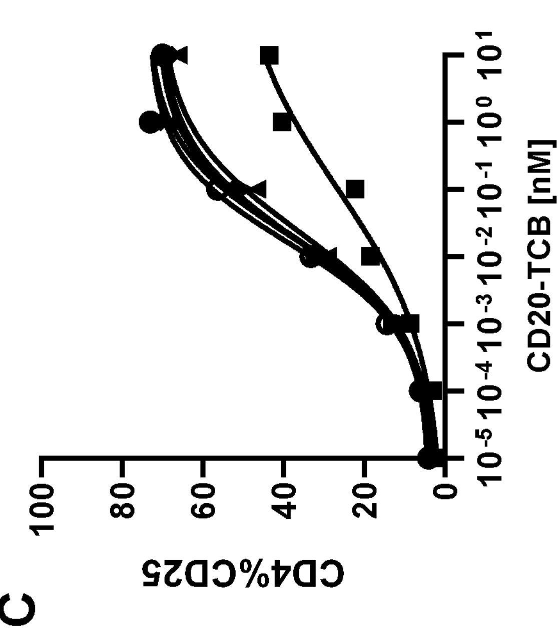
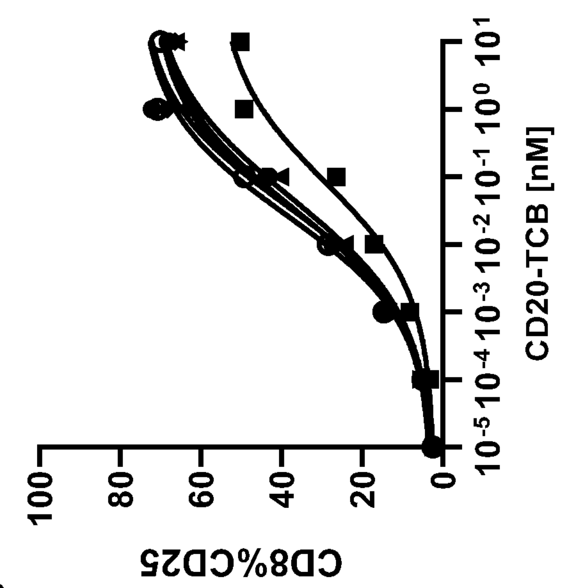


FIG. 49

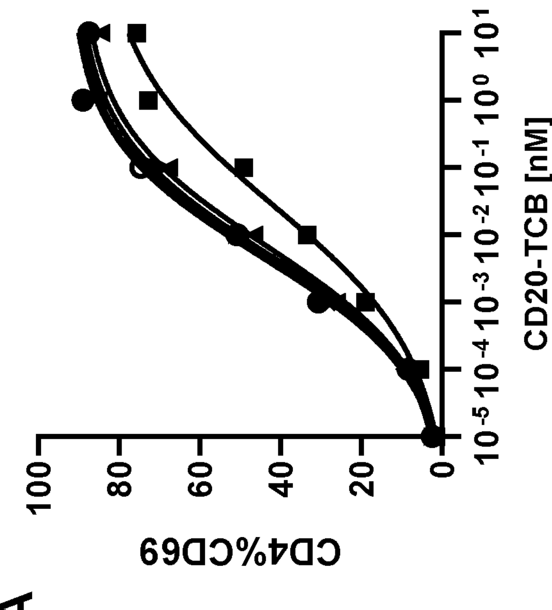
A



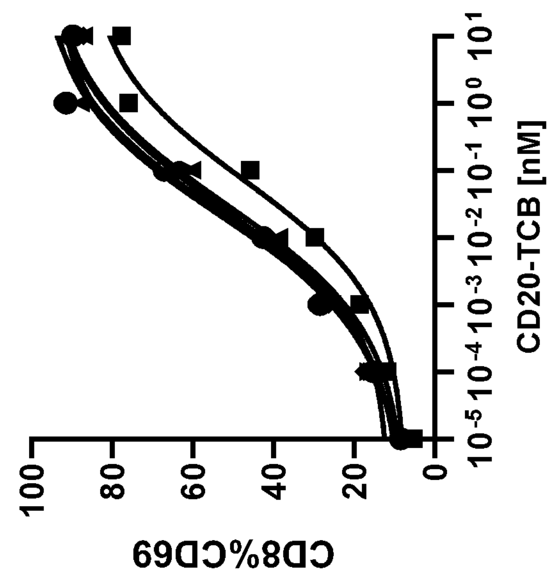
C



B

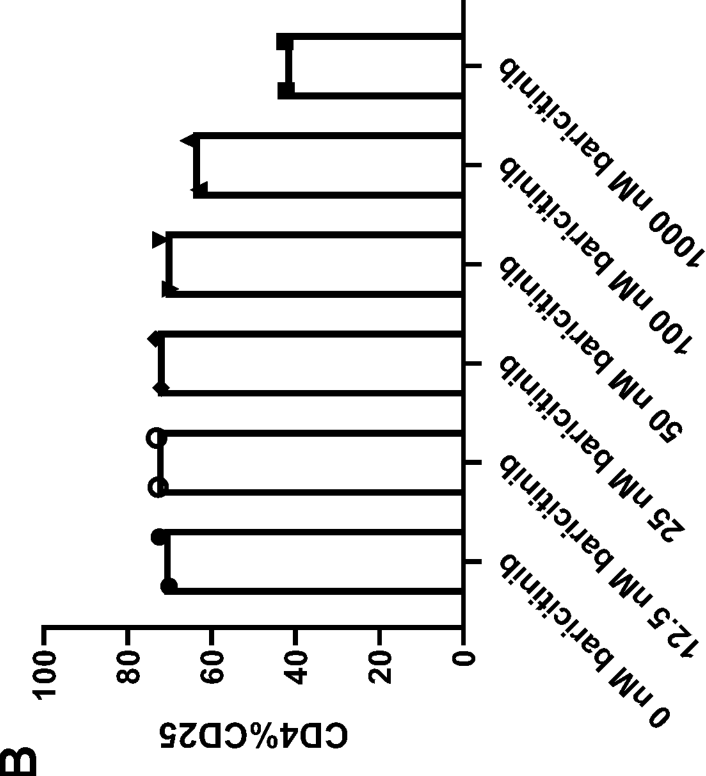


D



1000 nM baricitinib
100 nM baricitinib
50 nM baricitinib
25 nM baricitinib
12.5 nM baricitinib
0 nM baricitinib

FIG. 50



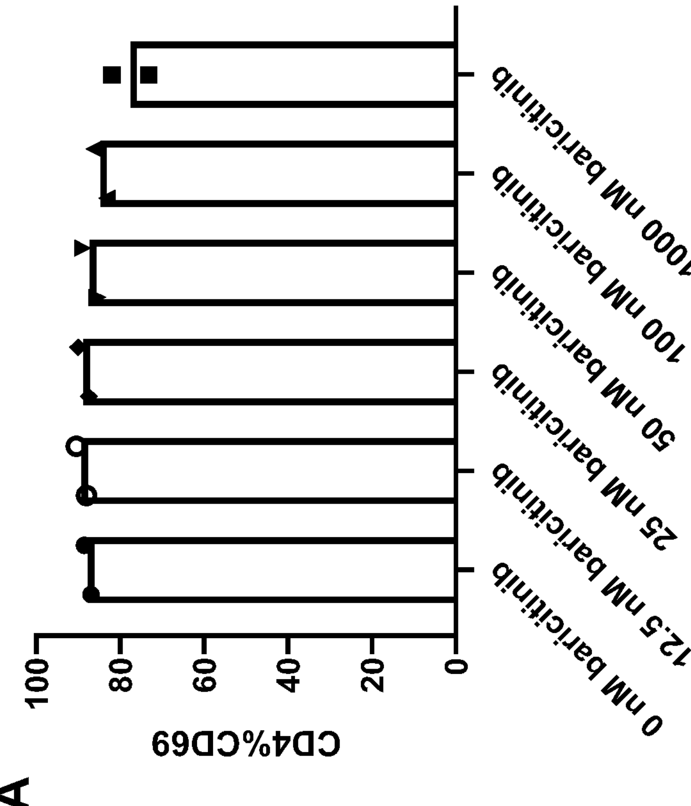
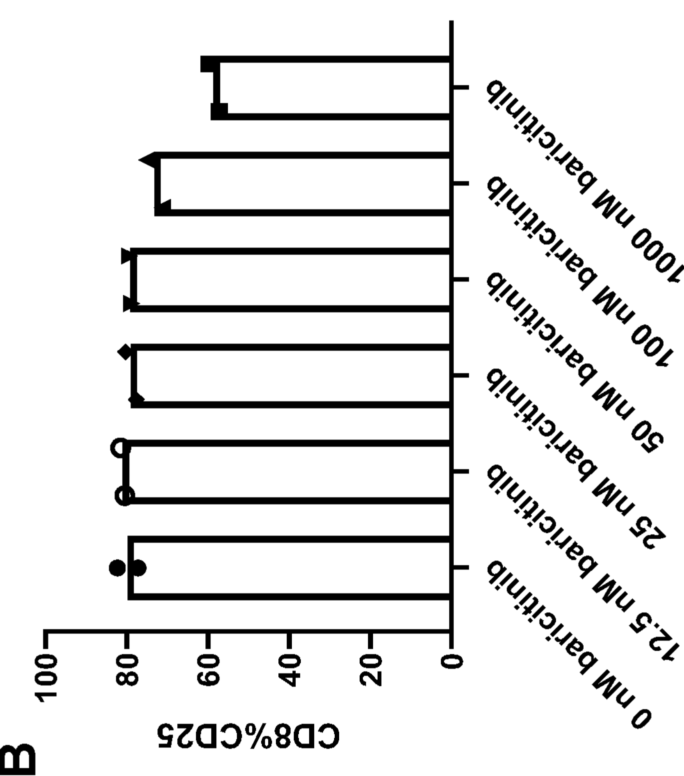


FIG. 51



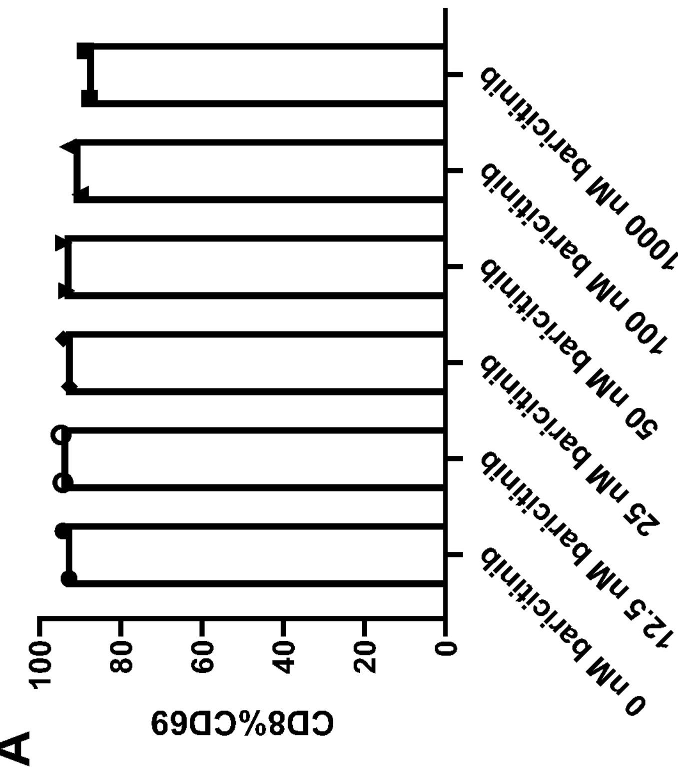
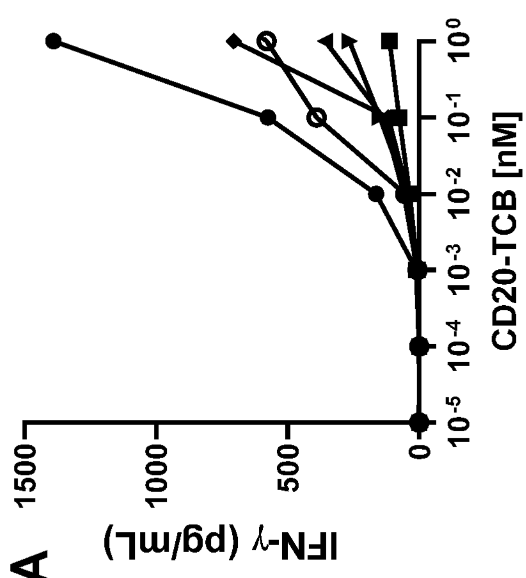
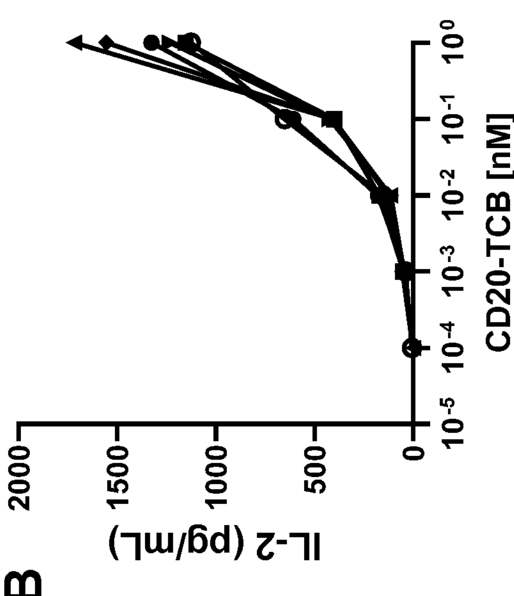
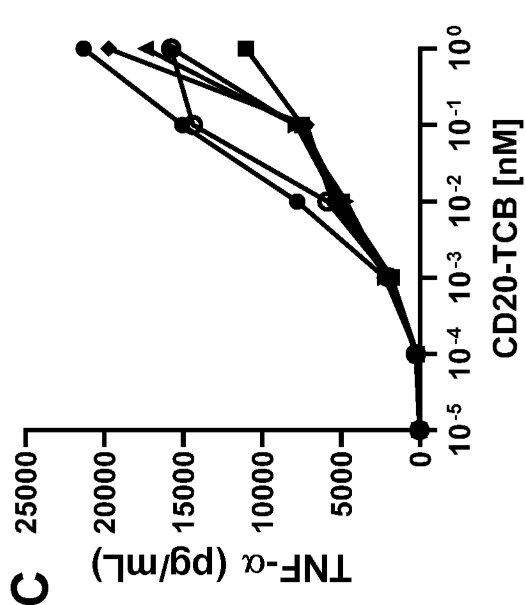


FIG. 52

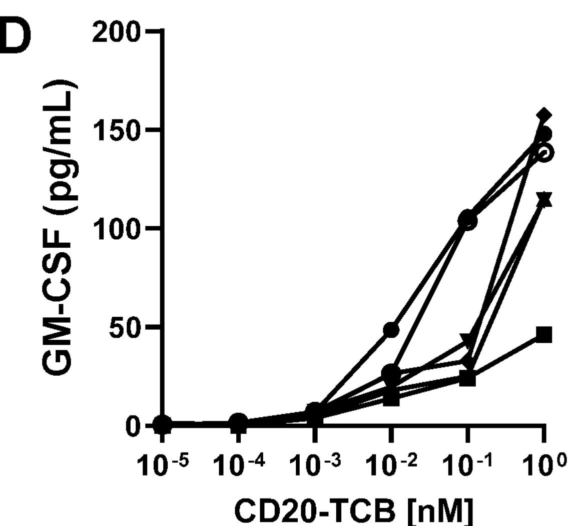


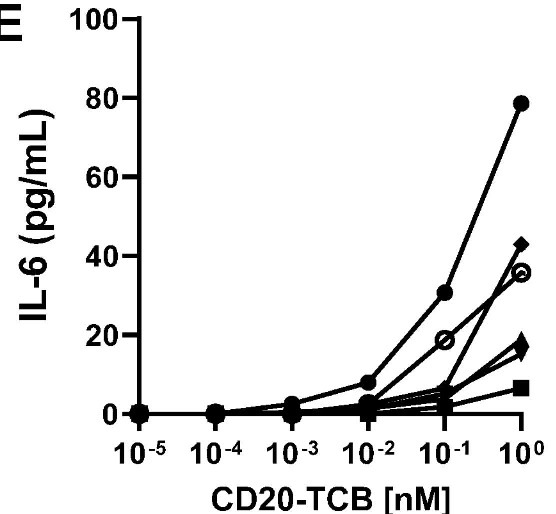


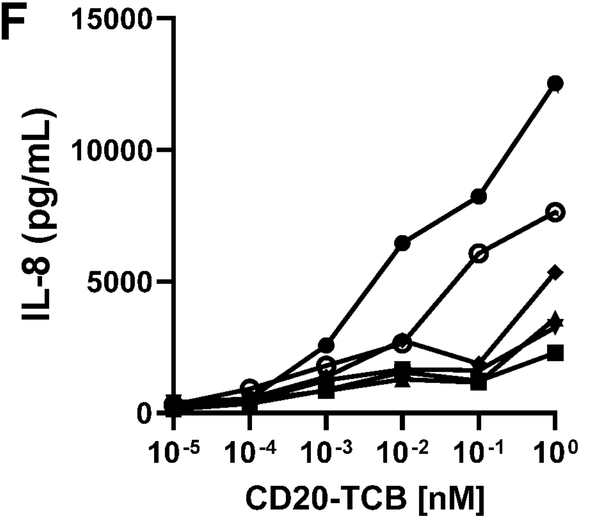


1000 nM baricitinib
100 nM baricitinib
50 nM baricitinib
25 nM baricitinib
12.5 nM baricitinib
0 nM baricitinib

FIG. 53

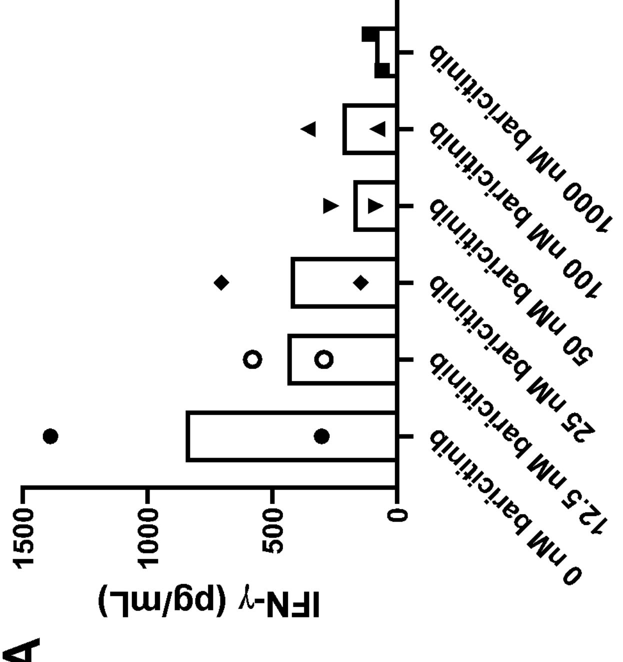


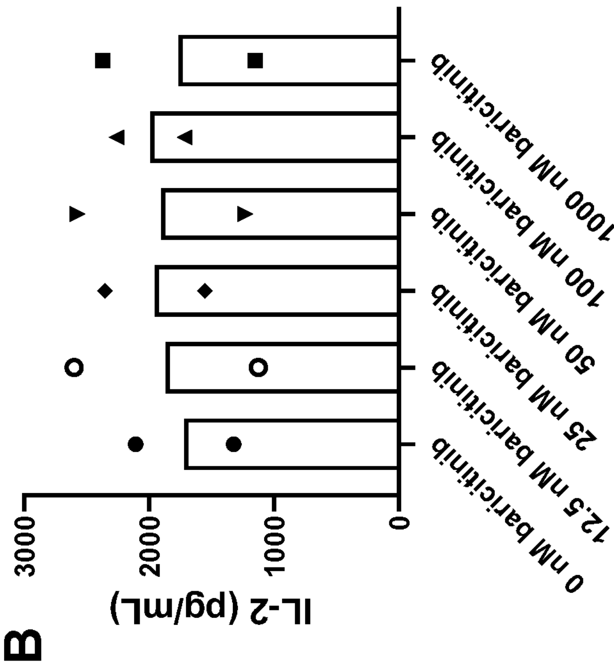




1000 nM baricitinib
100 nM baricitinib
50 nM baricitinib
25 nM baricitinib
12.5 nM baricitinib
0 nM baricitinib

FIG. 53





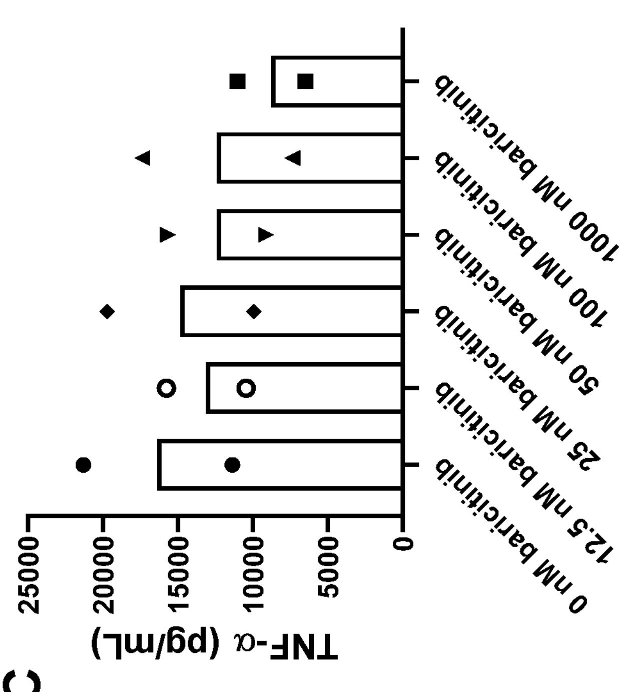
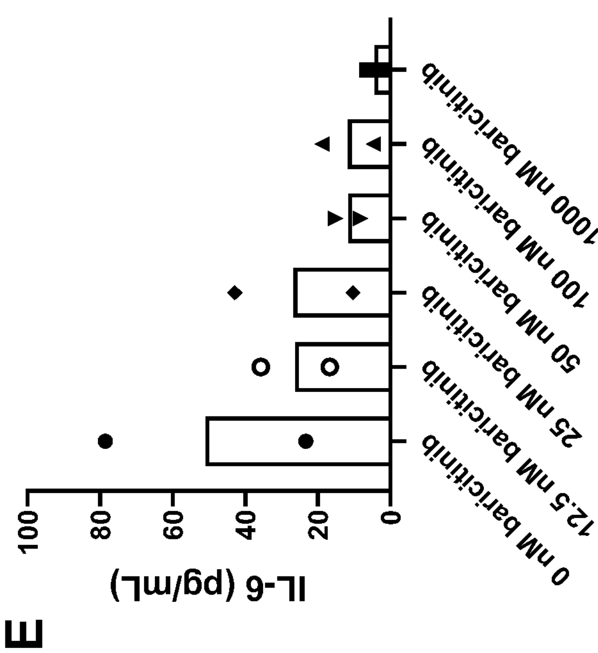
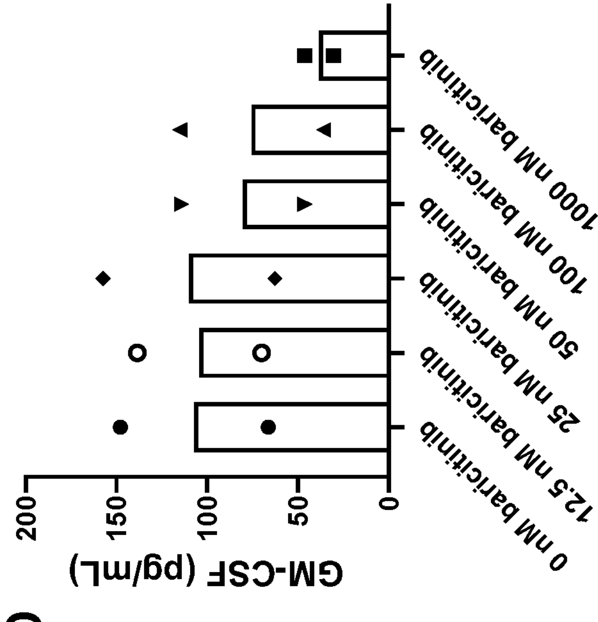


FIG. 54





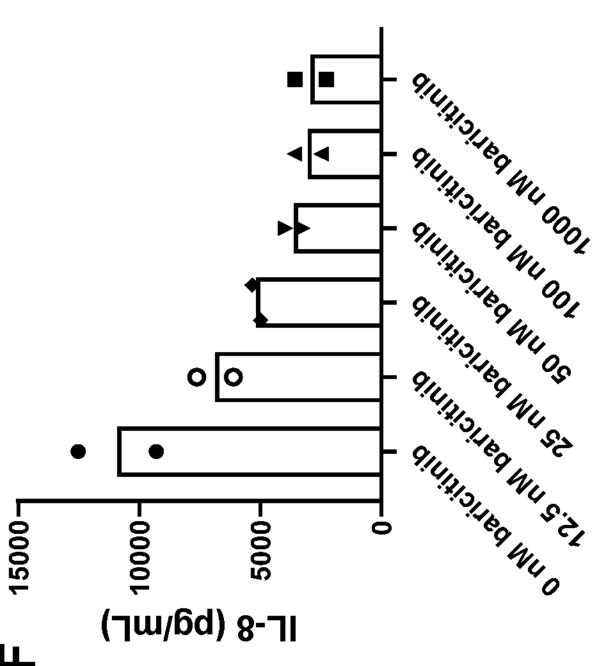


FIG. 54

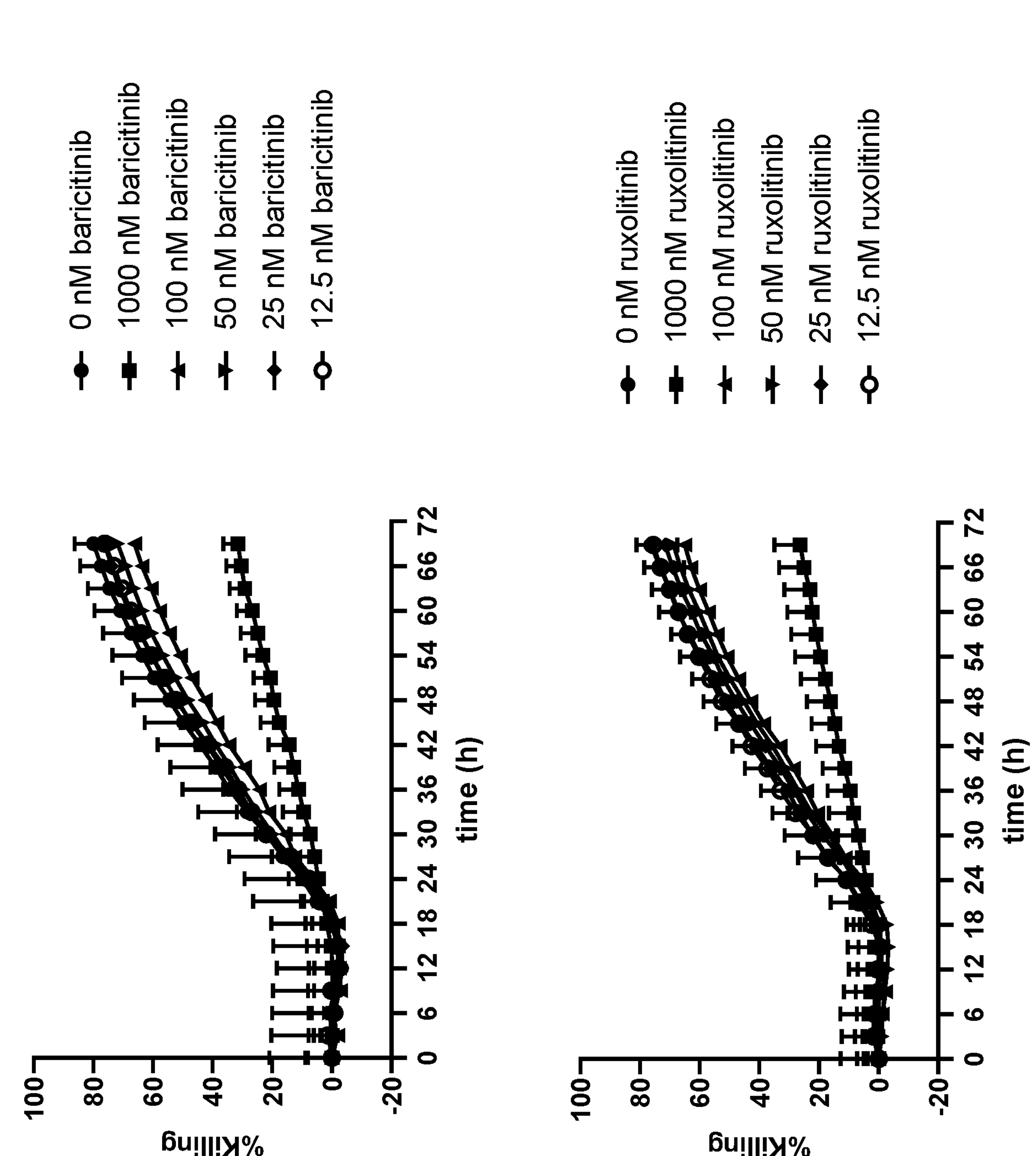


FIG. 55

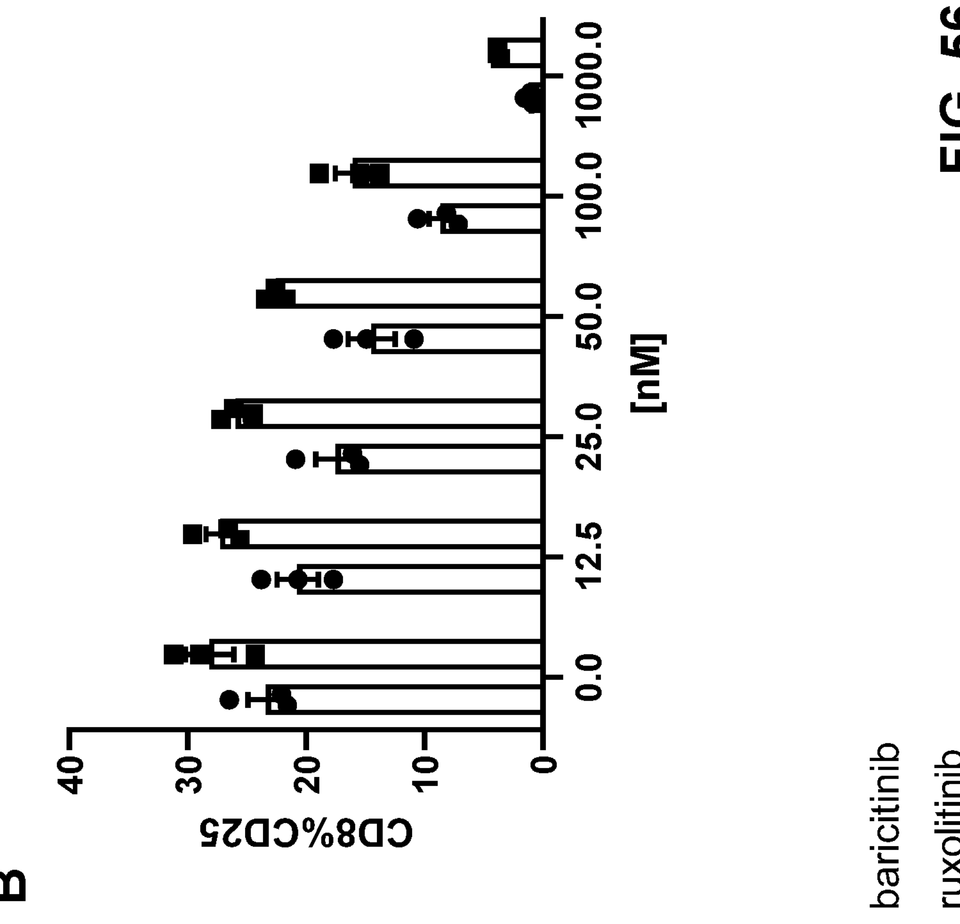
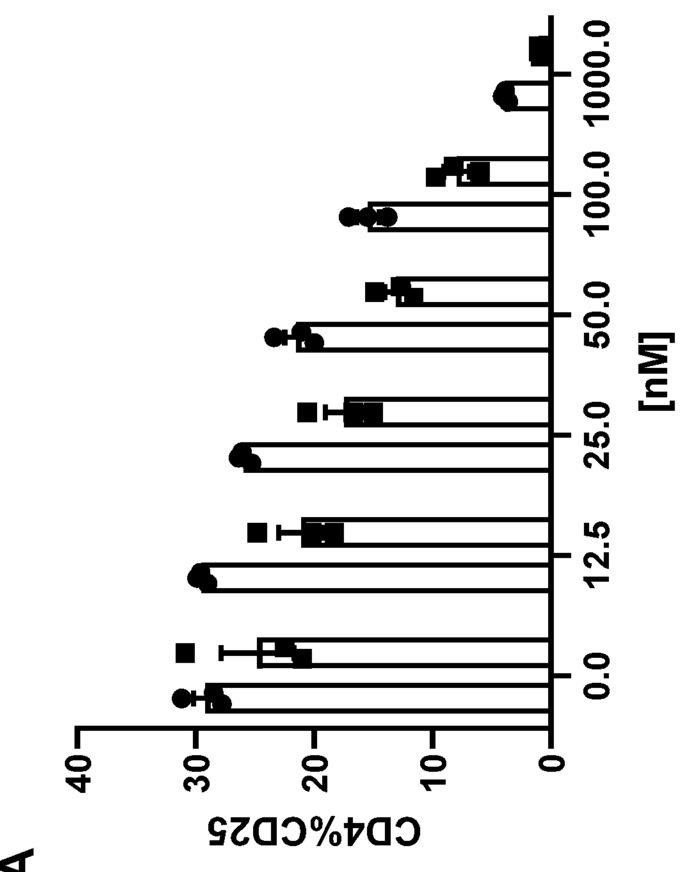


FIG. 56



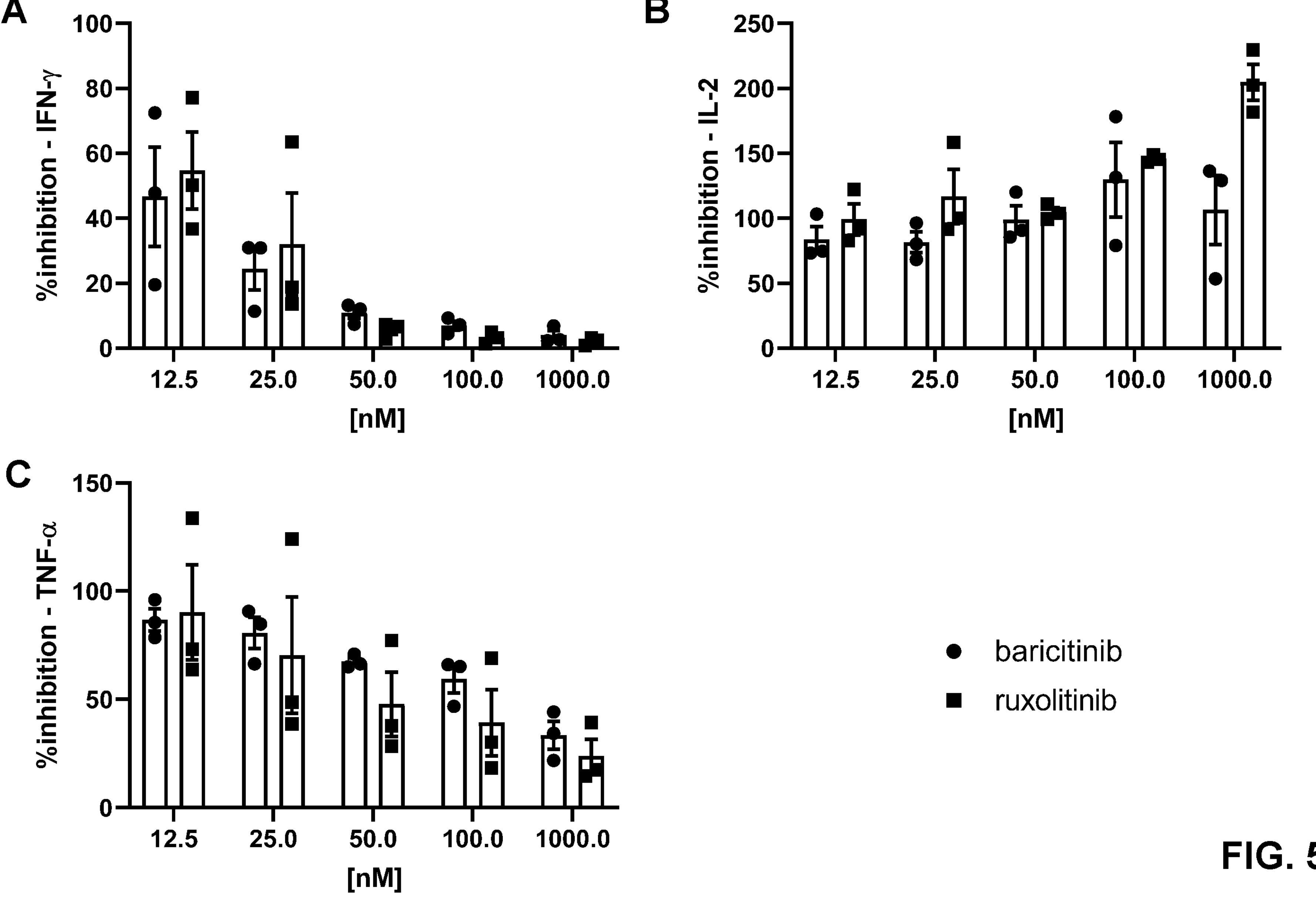
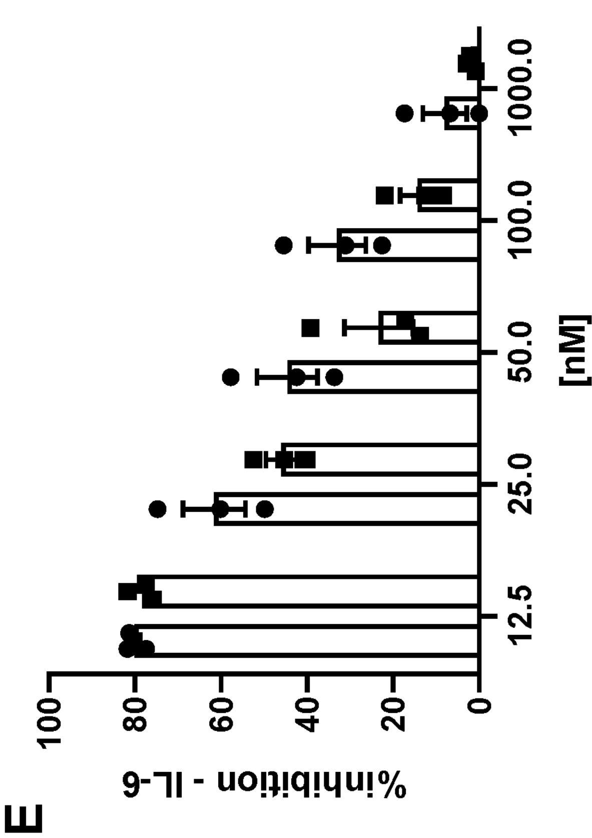
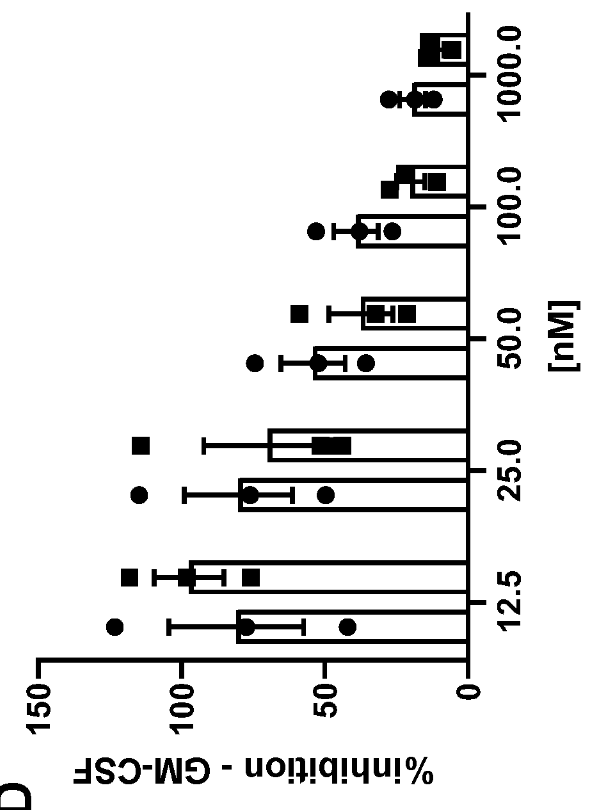


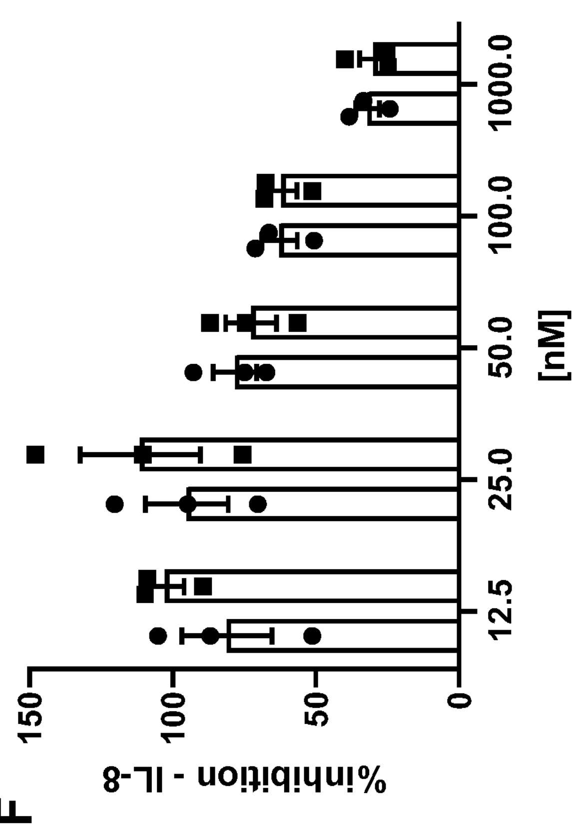
FIG. 57

FIG. 57


E
%inhibition - IL-6
100
80
60
40
20
0
12.5
25.0
50.0
100.0
1000.0
[nM]

baricitinib
ruxolitinib

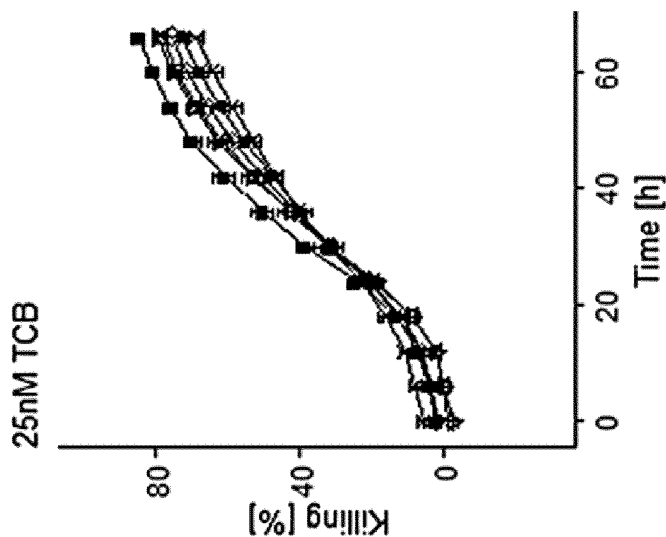

D
%inhibition - GM-CSF
150
100
50
0
12.5
25.0
50.0
100.0
1000.0
[nM]


F
%inhibition - IL-8
150
100
50
0
12.5
25.0
50.0
100.0
1000.0
[nM]

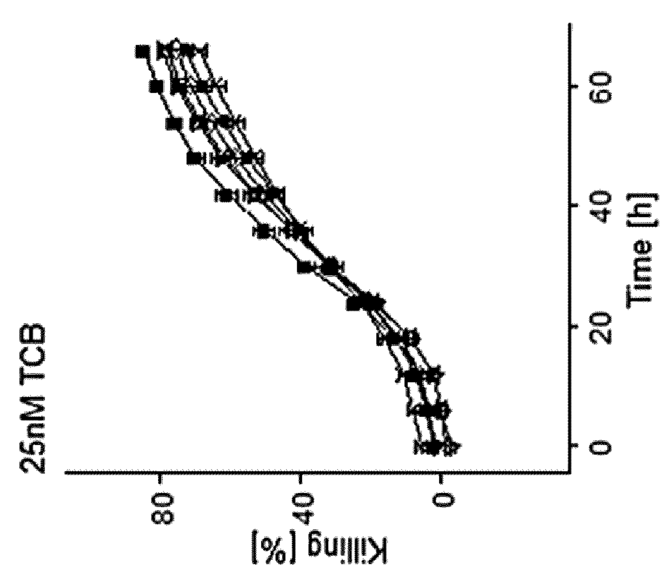
× 100nM
● 50nM
◇ 25nM
△ 12.5nM
▽ 6.25nM
■ no KI

FIG. 58

B



A



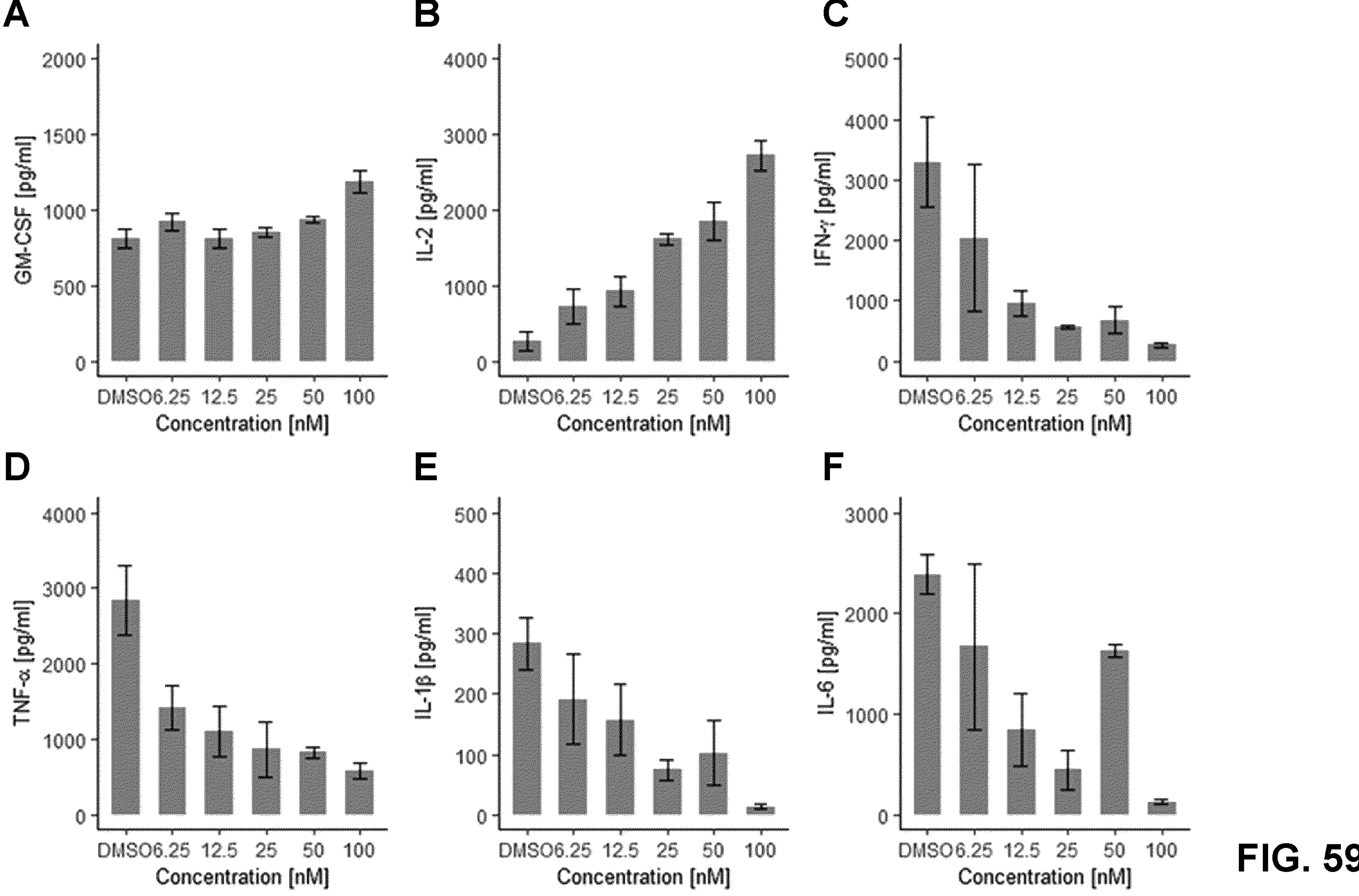


FIG. 59

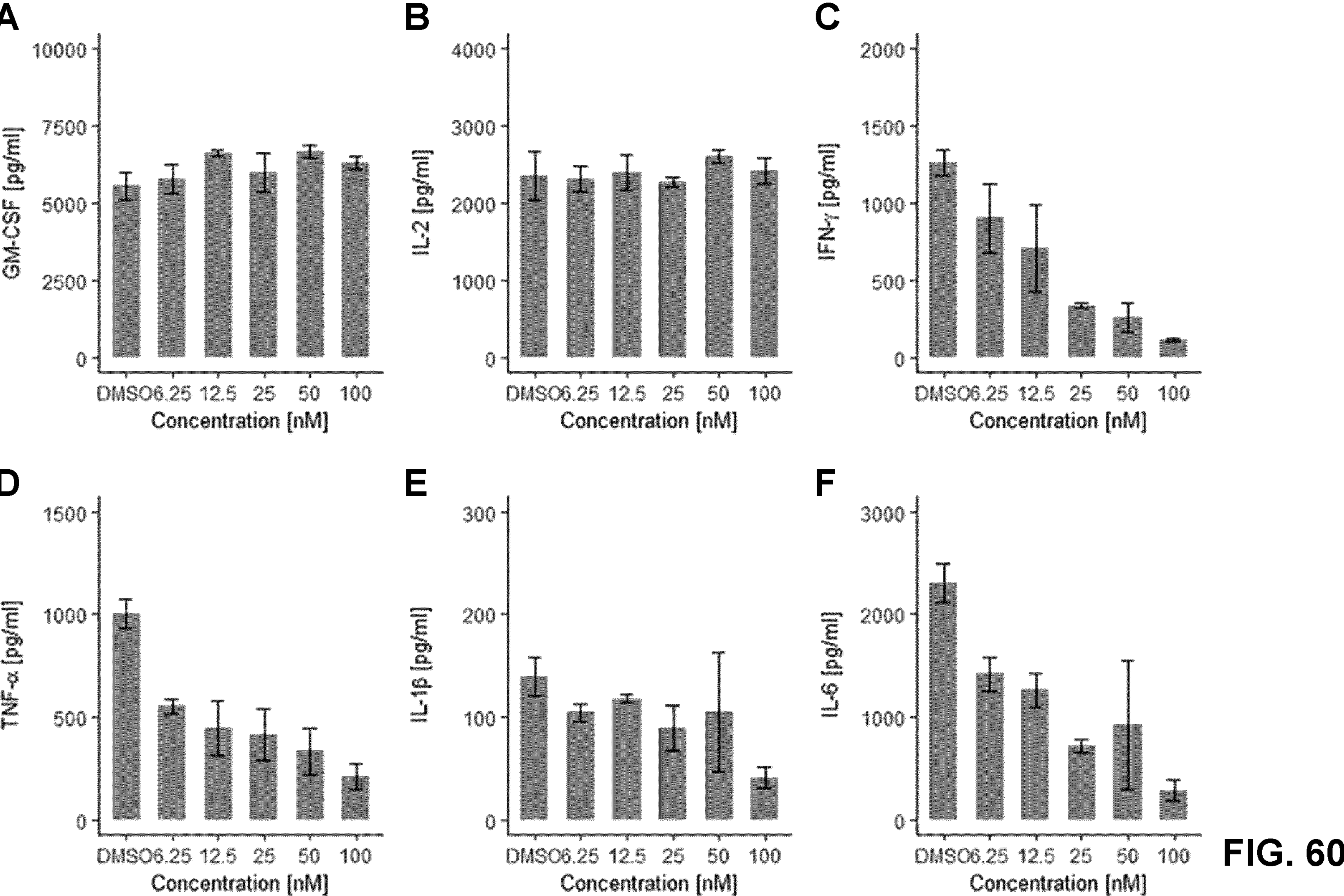
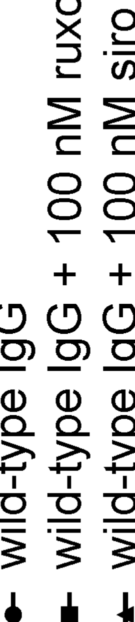


FIG. 60

PGLALA IgG + 100 nM ruxo
PGLALA IgG + 100 nM siro
PGLALA IgG



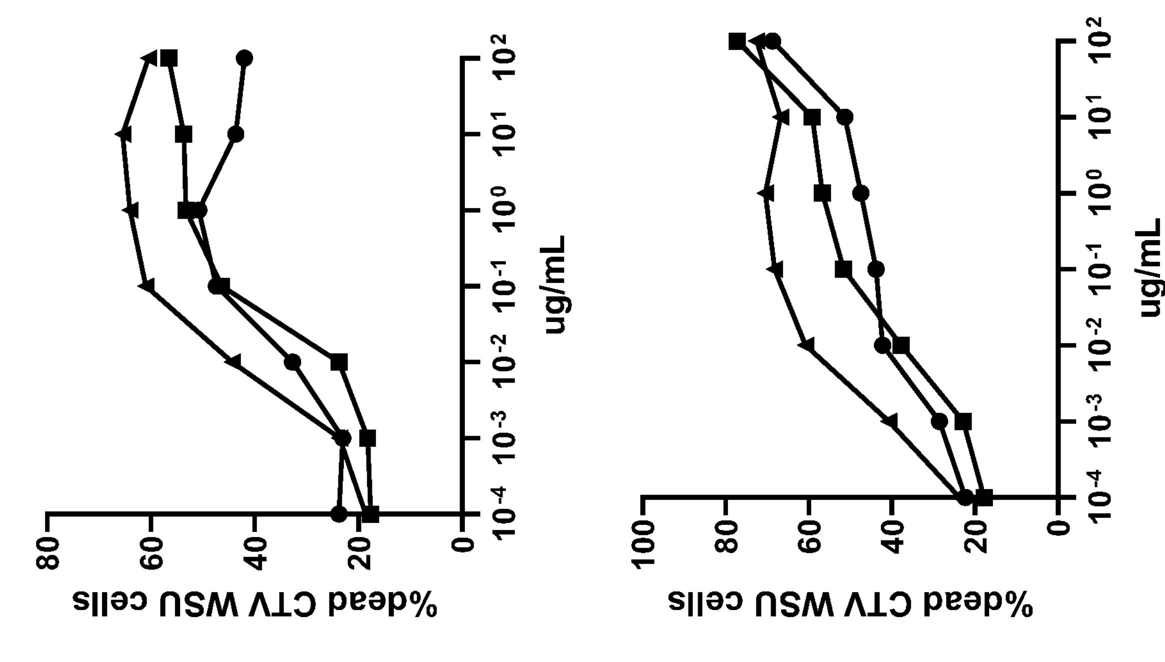


FIG. 61

● PGLALA IgG
■ PGLALA IgG + 100 nM ruxo
▲ PGLALA IgG + 100 nM siro

A

GM-CSF (pg/mL)

250
200
150
100
50
0

10 1 0.1 0.01

μg/mL

B

IFN-γ (pg/mL)

100
80
60
40
20
0

10 1 0.1 0.01

μg/mL

C

IL-2 (pg/mL)

25
20
15
10
5
0

10 1 0.1 0.01

μg/mL

D

TNF-α (pg/mL)

2500
2000
1500
1000
500
0

10 1 0.1 0.01

μg/mL

FIG. 62

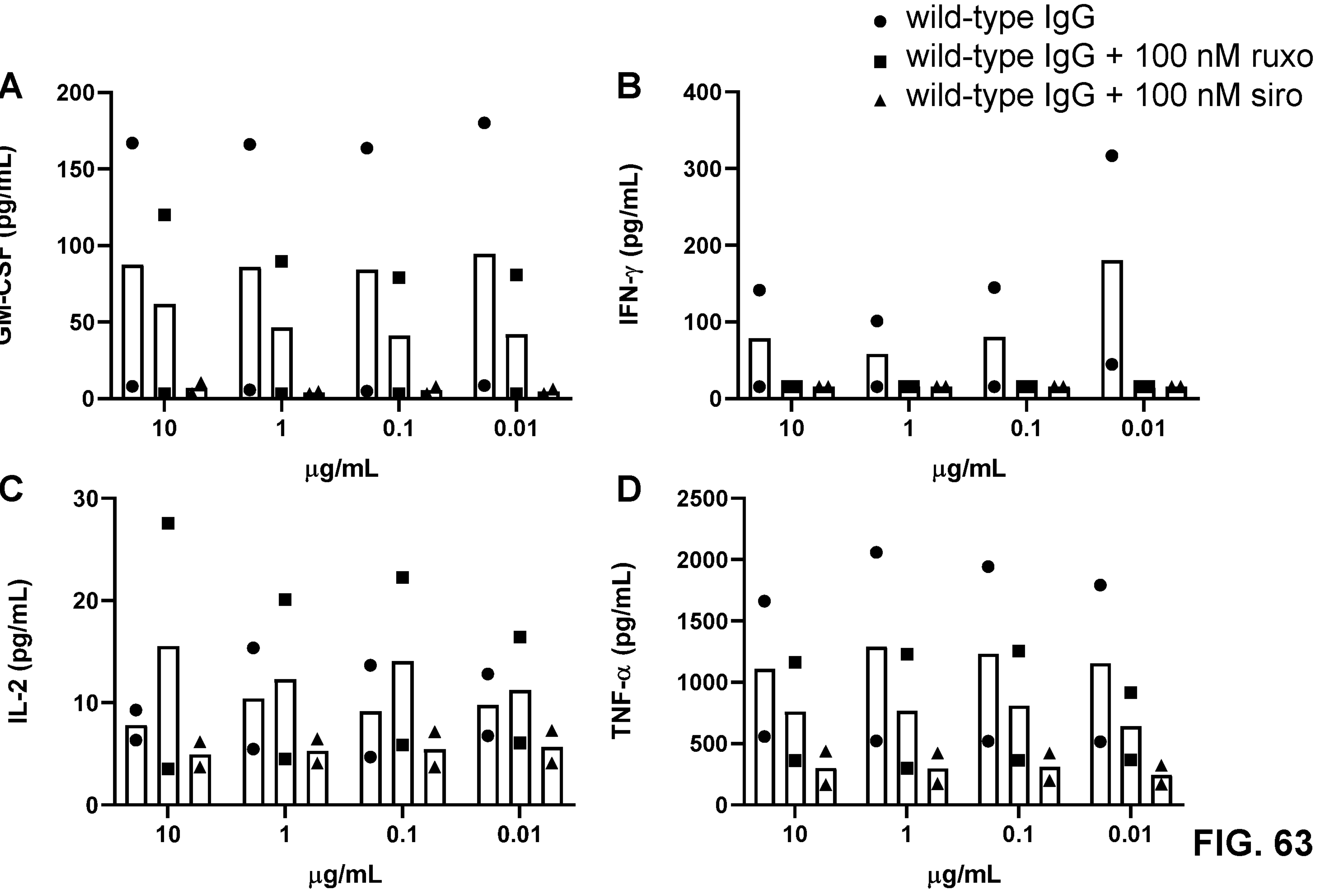


FIG. 63

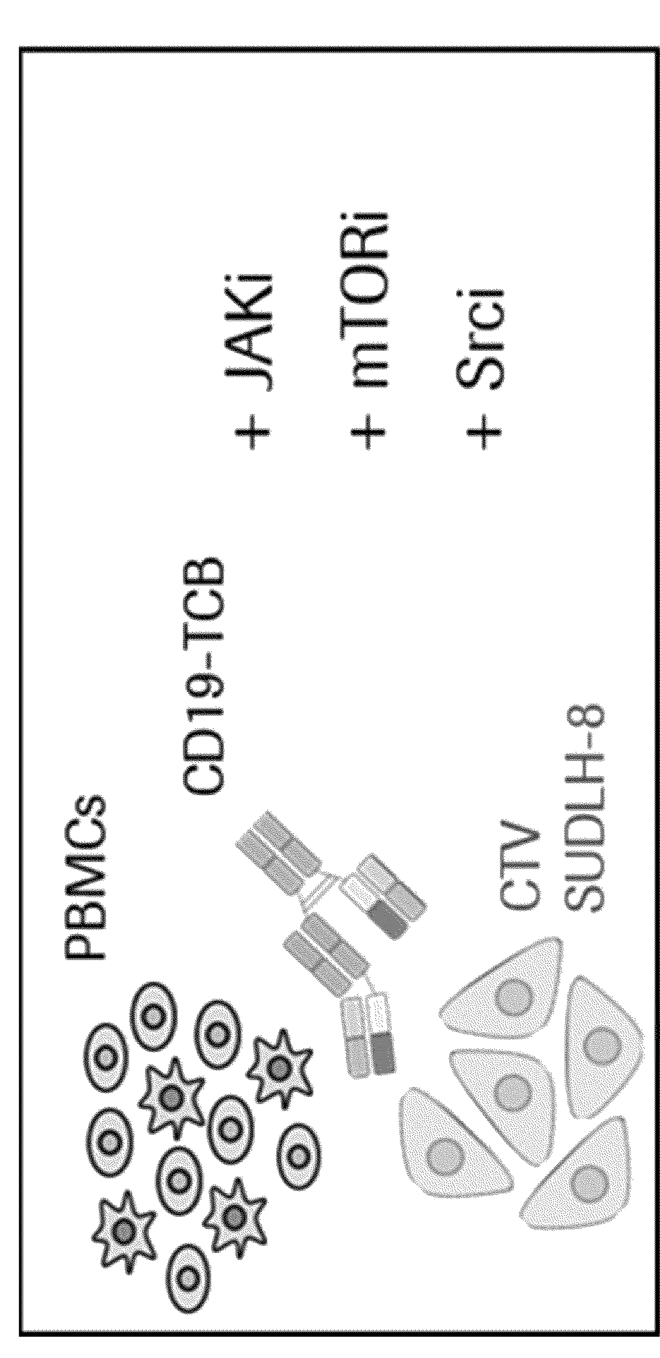


FIG. 64

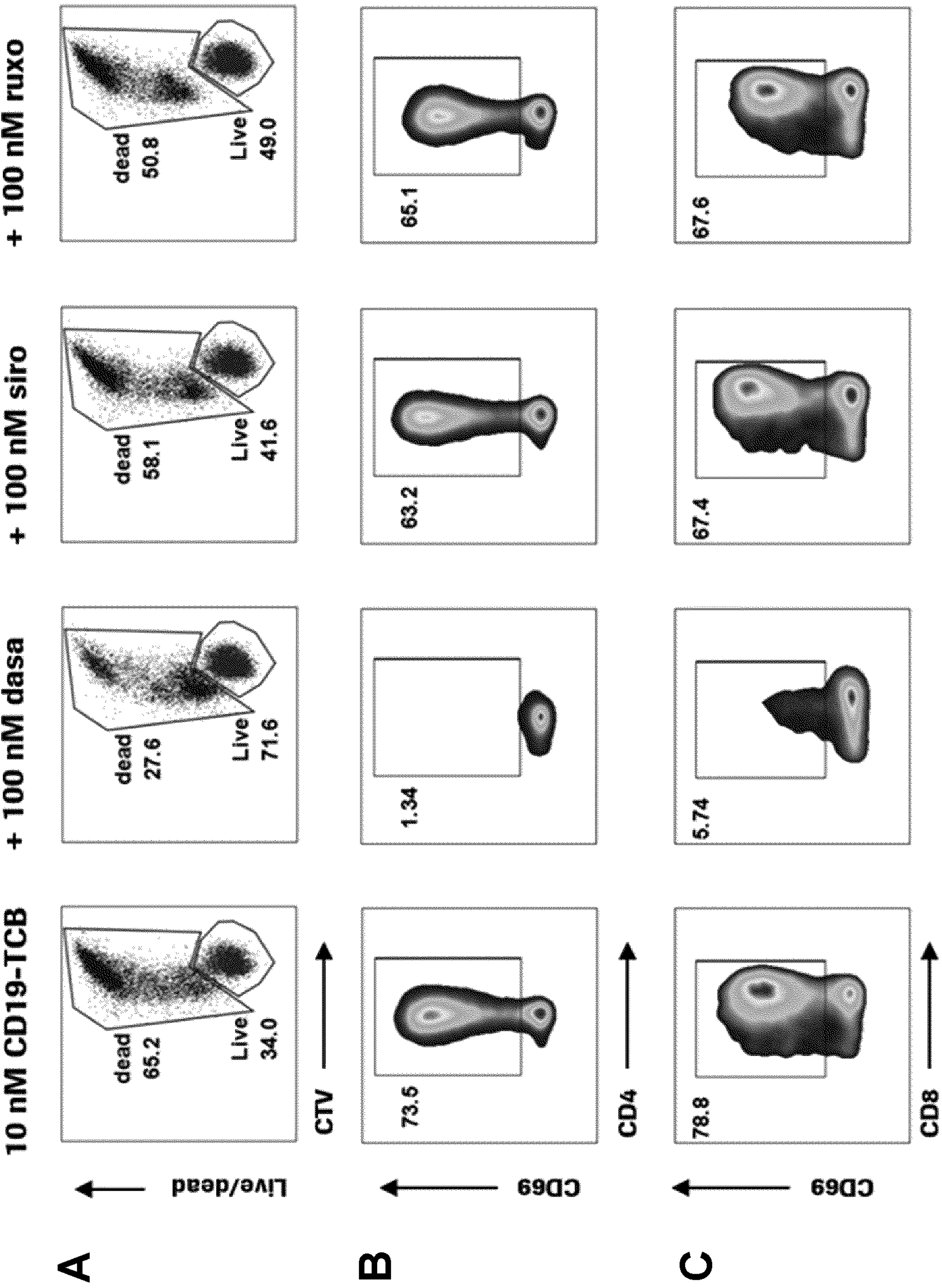


FIG. 65

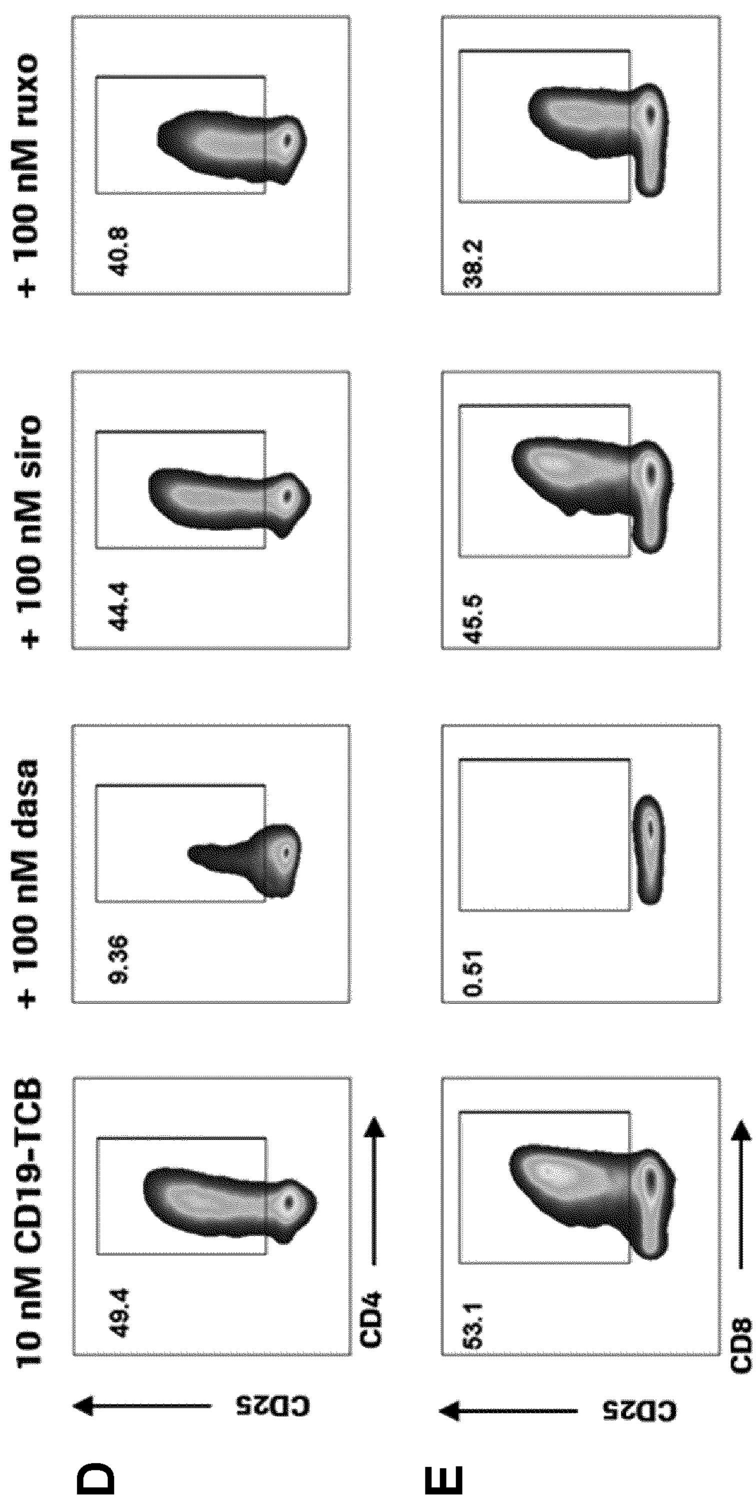
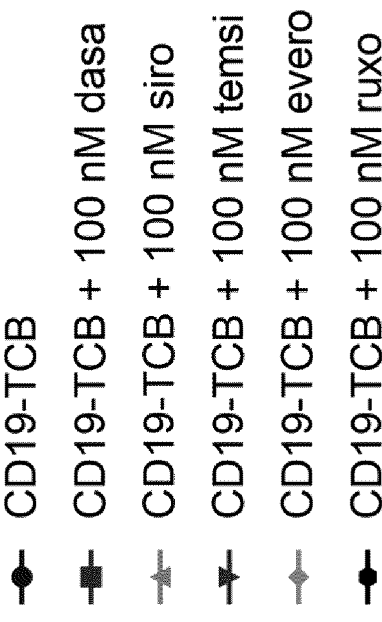
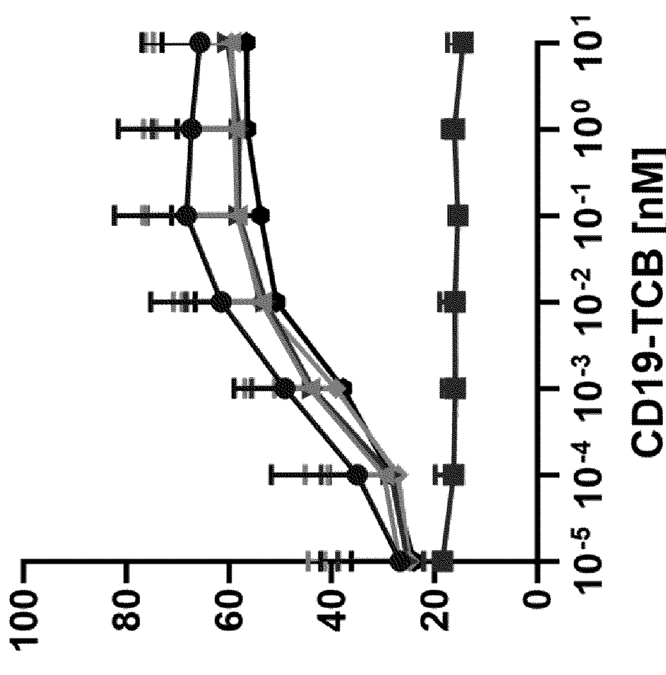


FIG. 65

FIG. 66





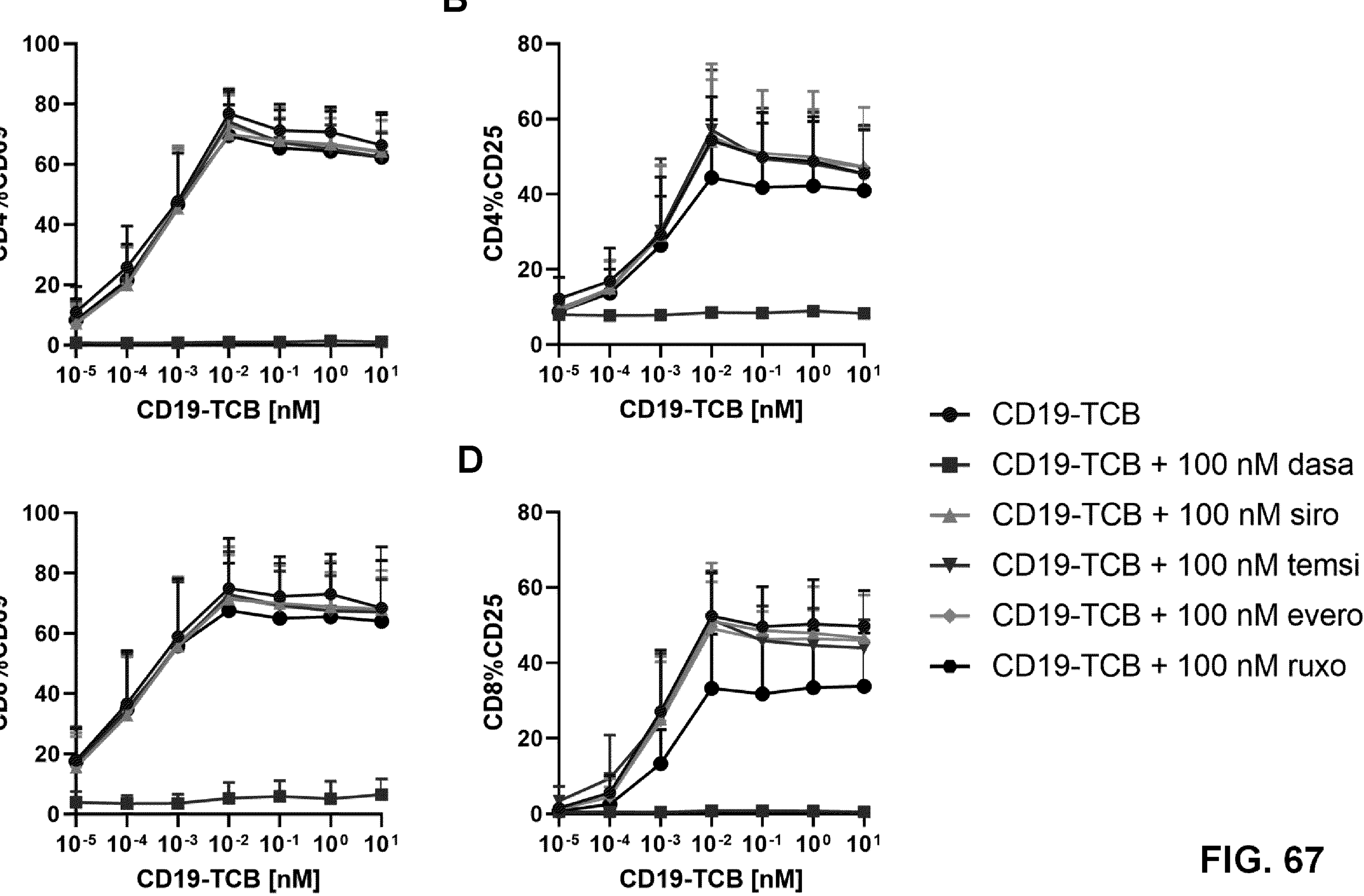


FIG. 67

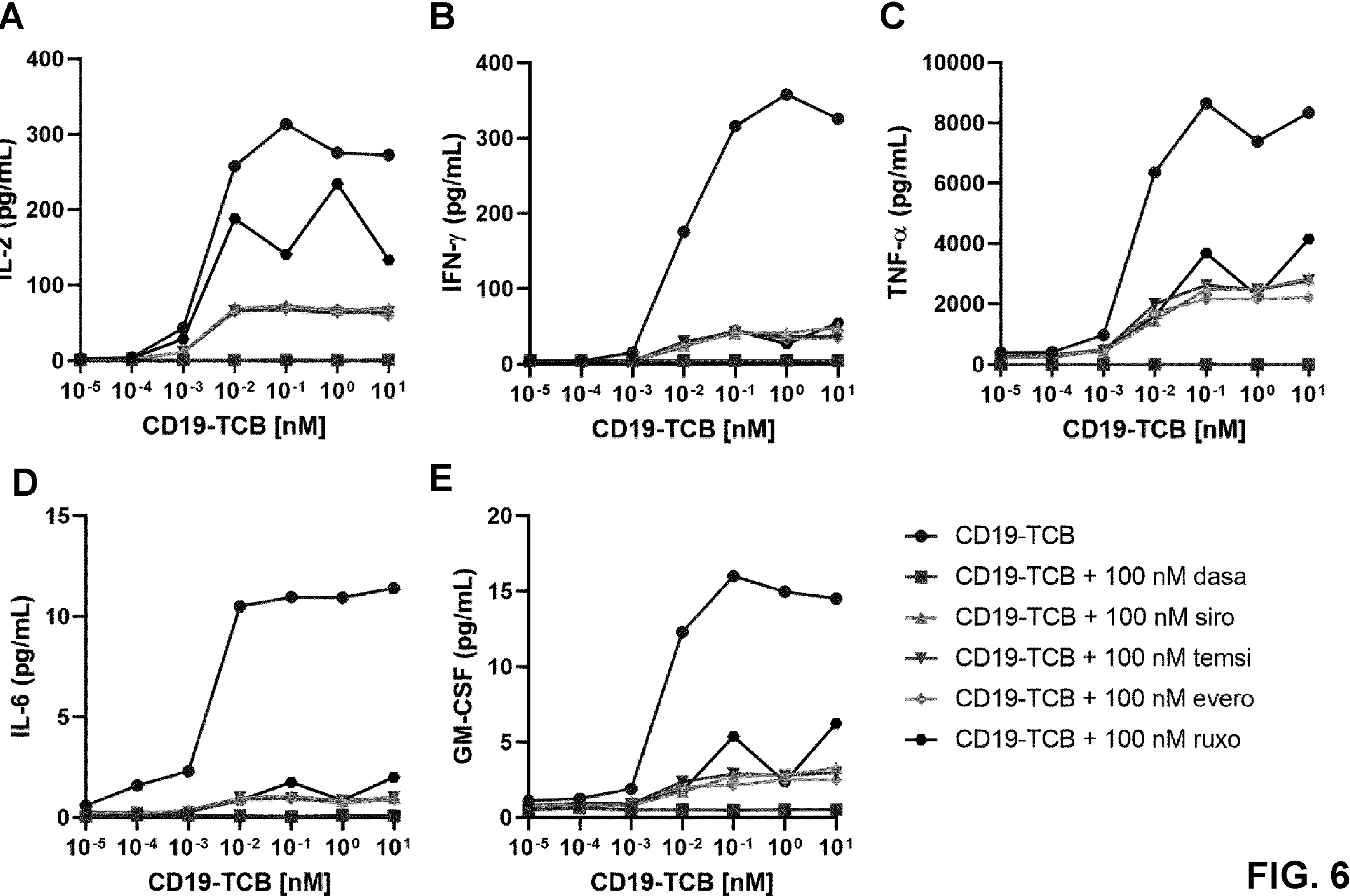
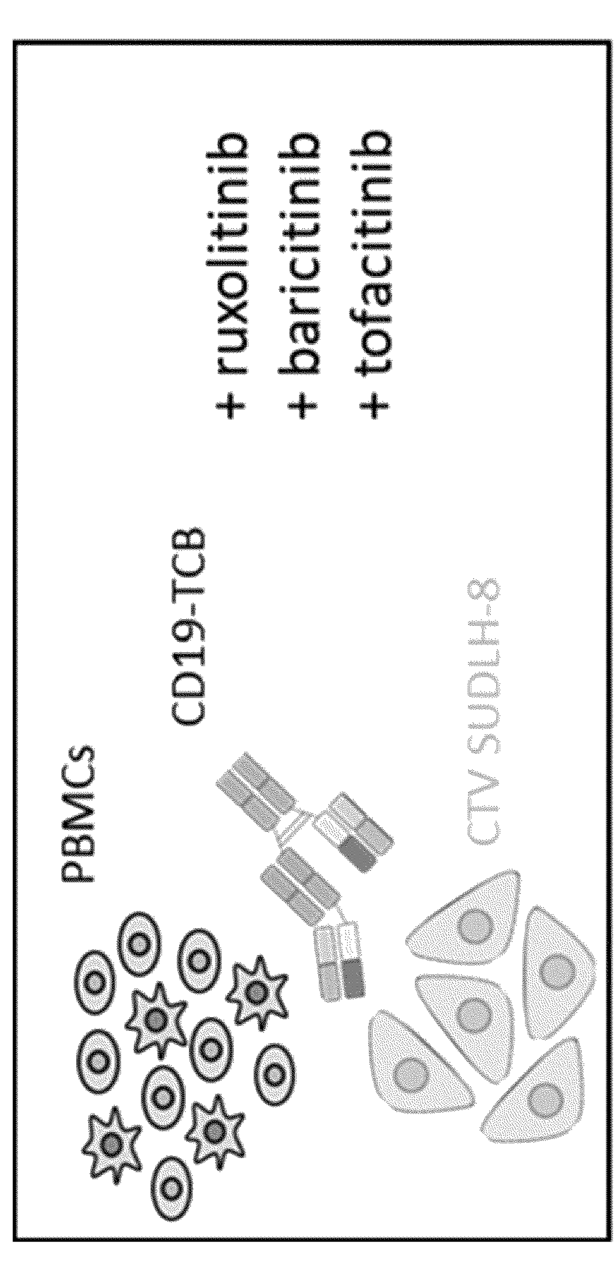
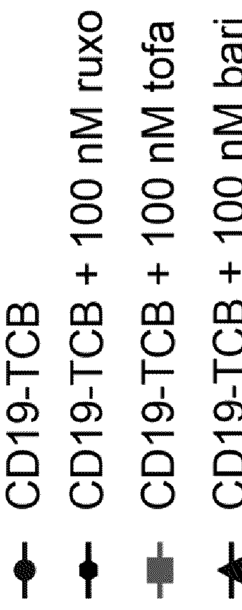


FIG. 68

FIG. 69





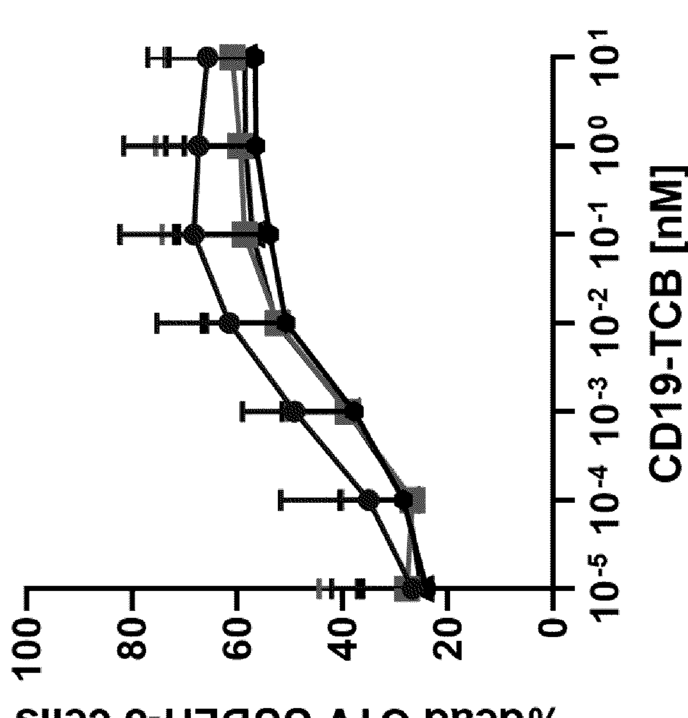


FIG. 70

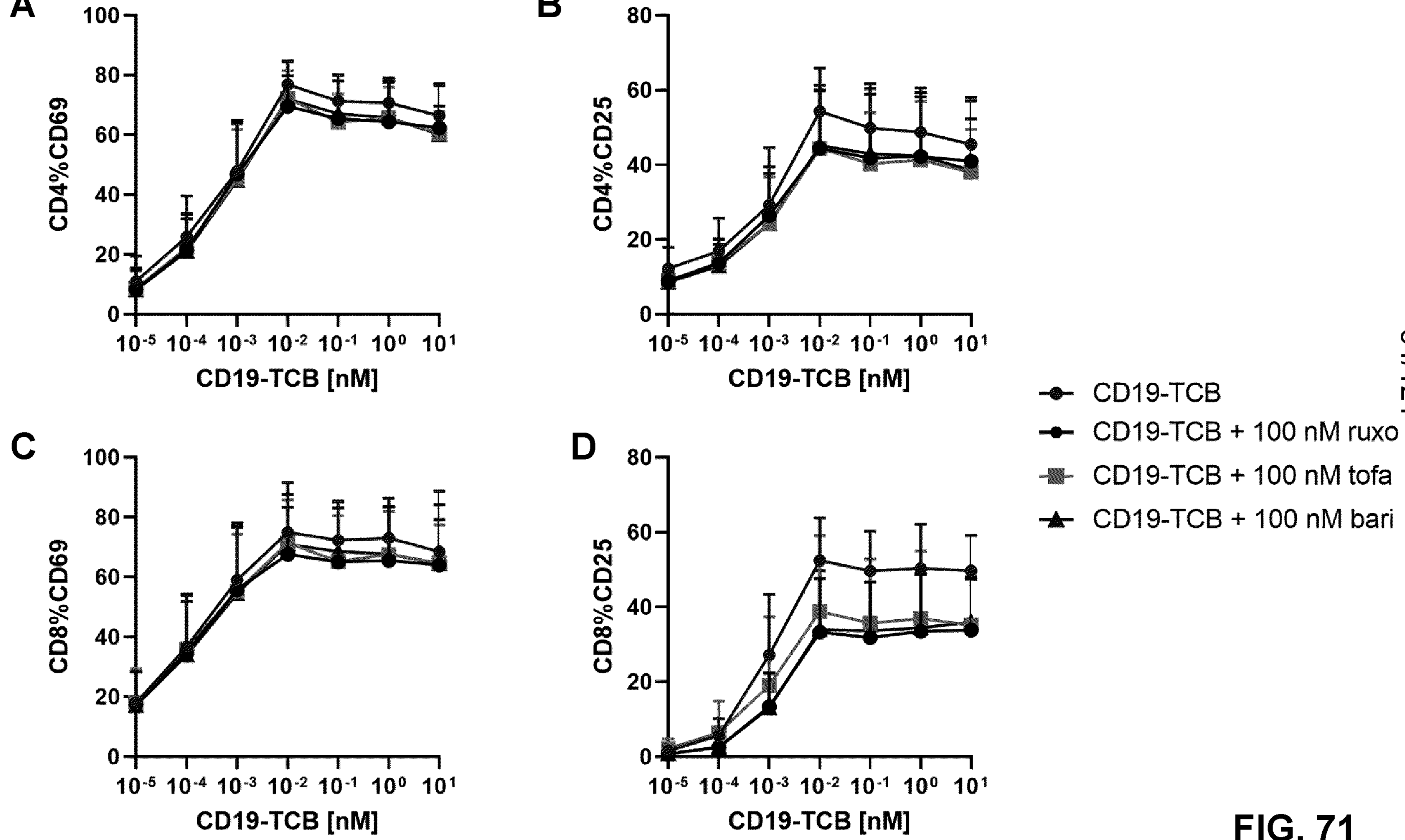
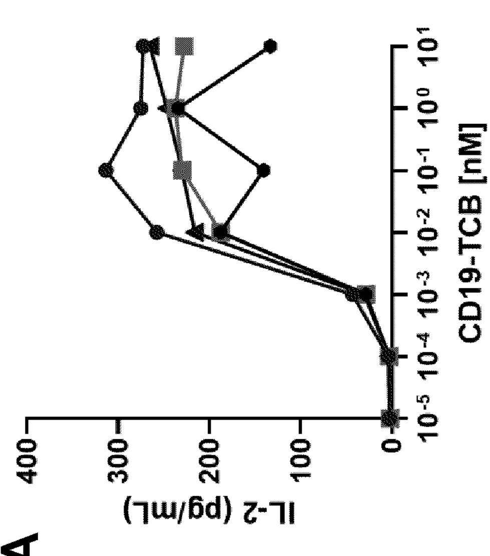
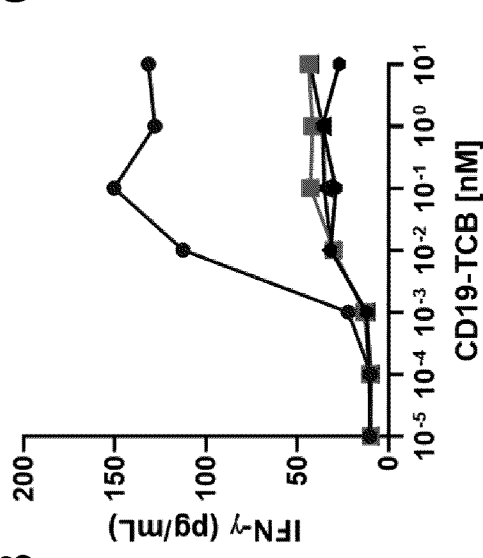
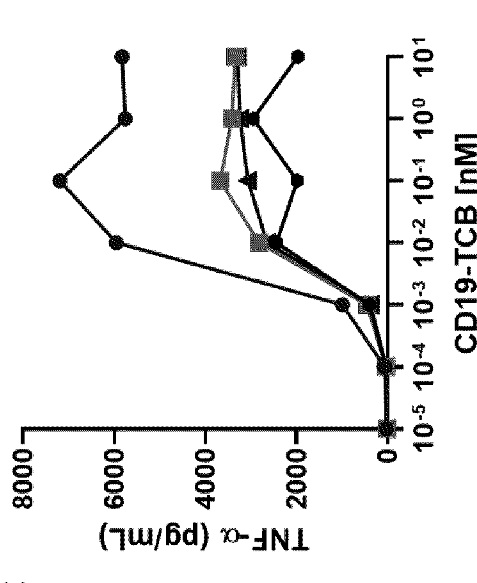
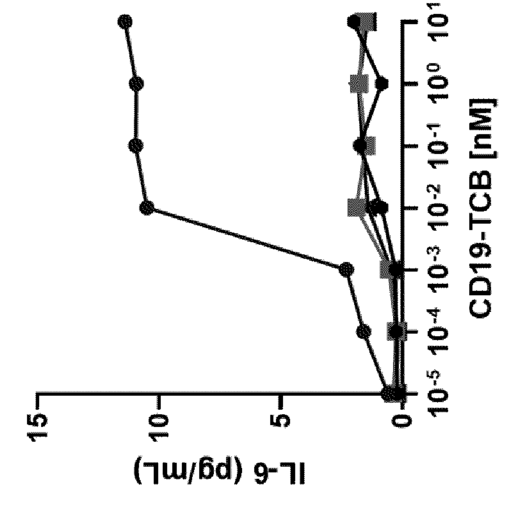


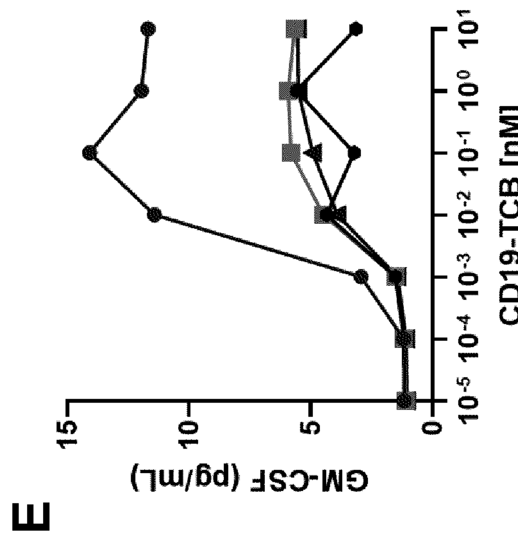
FIG. 71











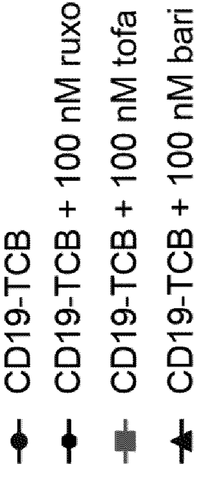


FIG. 72

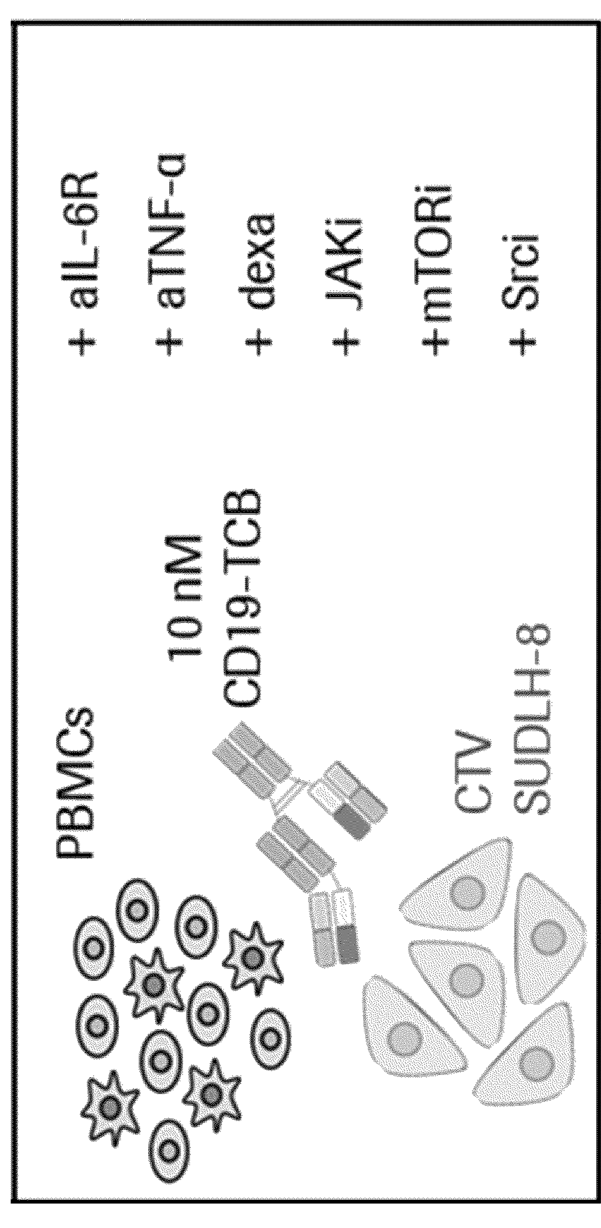
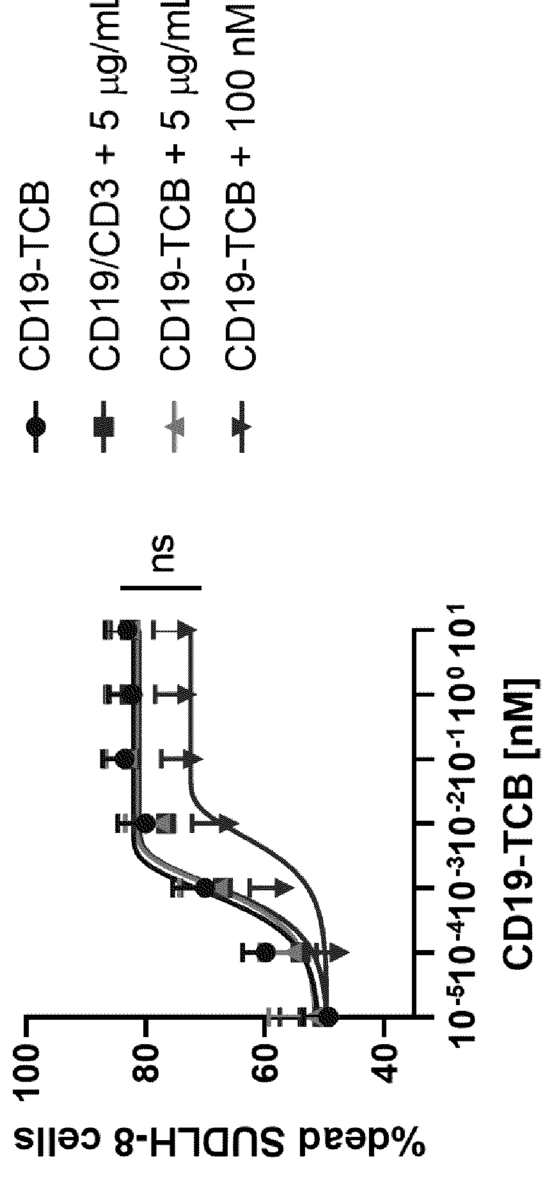


FIG. 73

A



B

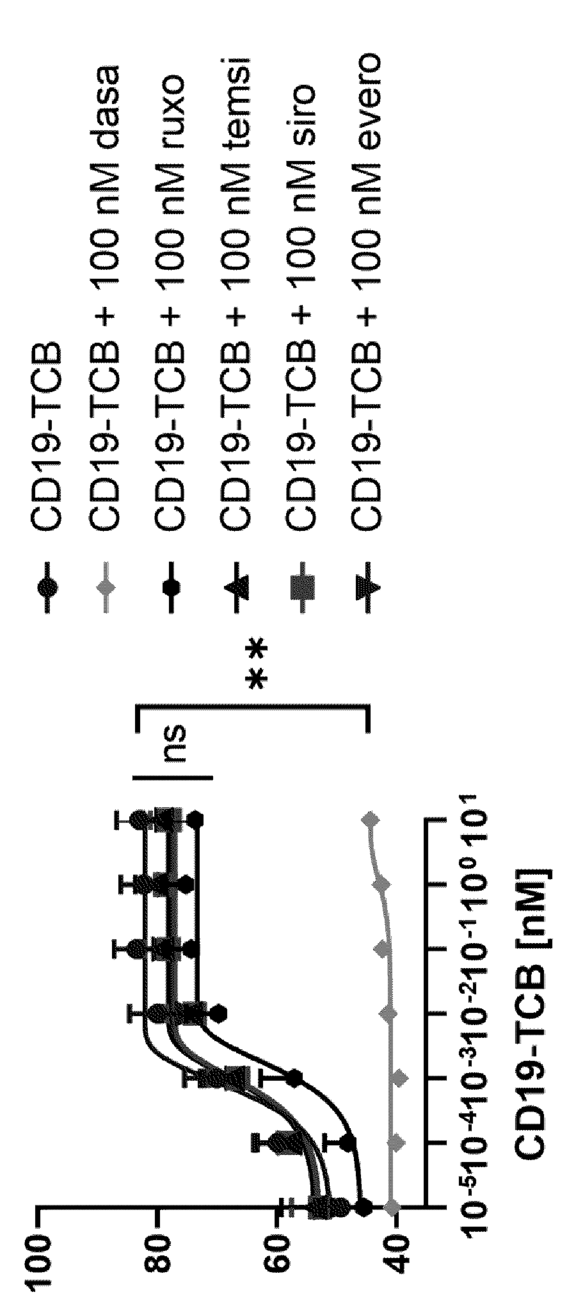
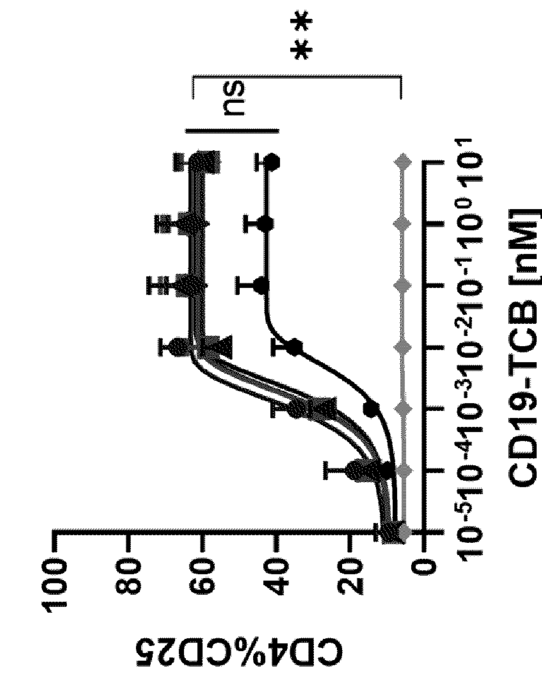
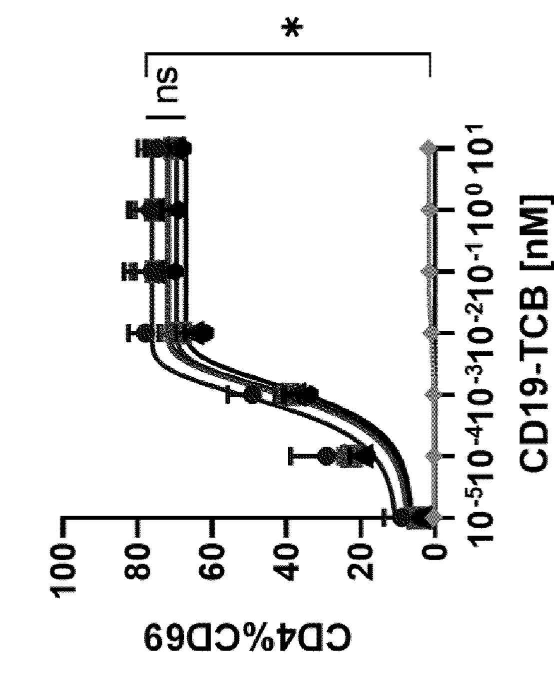


FIG. 74

A

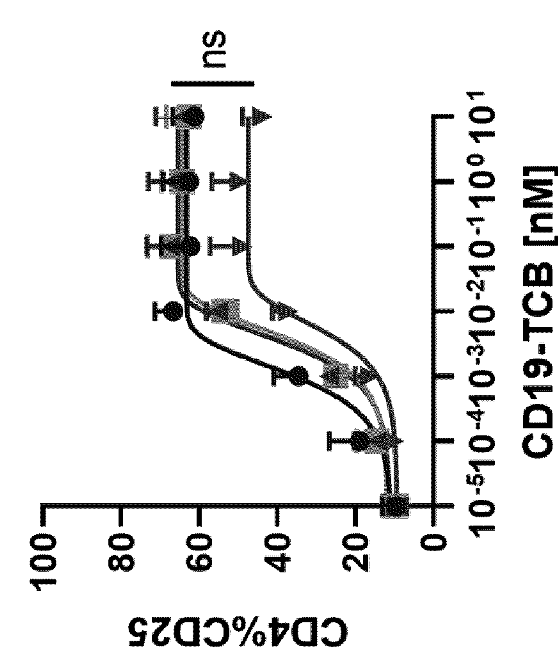


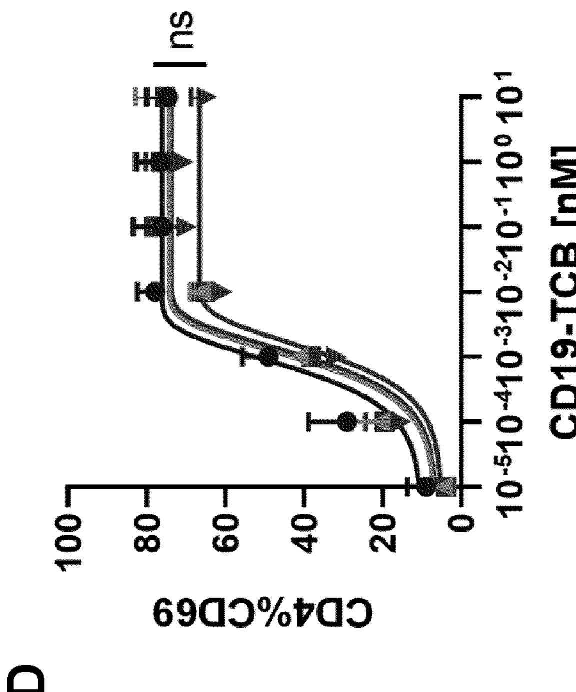
B



CD19-TCB
CD19/CD3 + 5 μg/mL aTNF-α
CD19-TCB + 5 μg/mL aIL-6R
CD19-TCB + 100 nM dexa

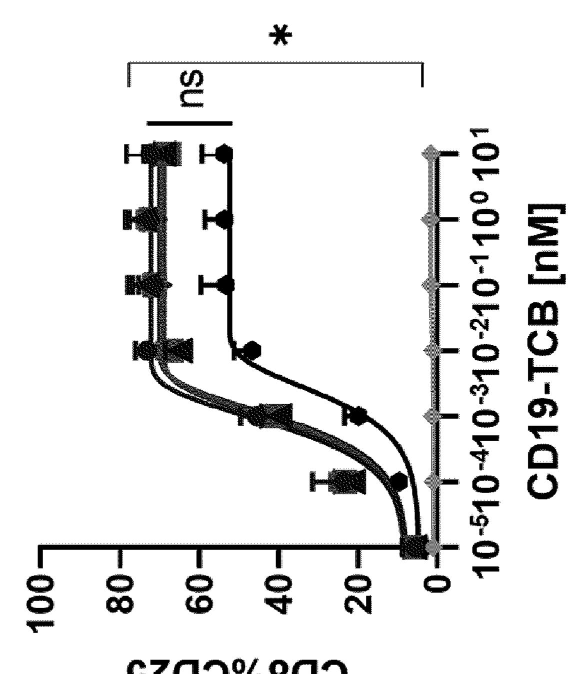
FIG. 75

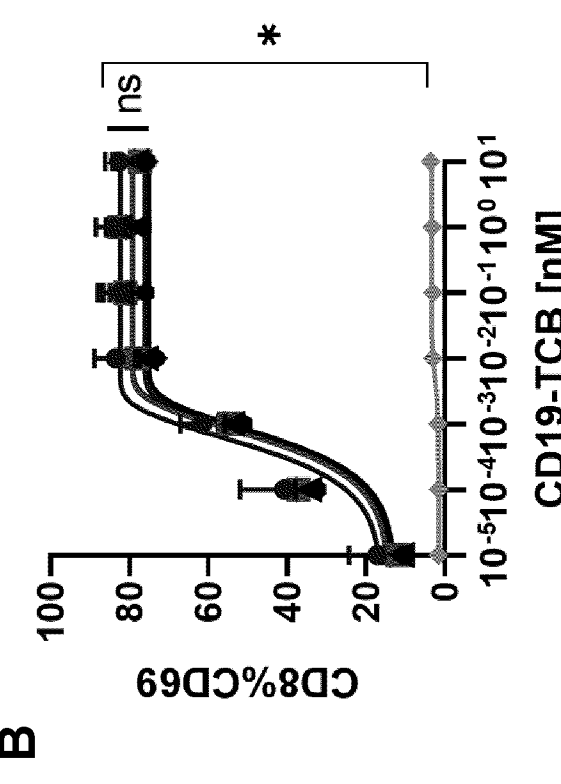




CD19-TCB
CD19-TCB + 100 nM dasa
CD19-TCB + 100 nM ruxo
CD19-TCB + 100 nM temsi
CD19-TCB + 100 nM siro
CD19-TCB + 100 nM evero

FIG. 75





- CD19-TCB
- CD19/CD3 + 5 µg/mL aTNF-α
- CD19-TCB + 5 µg/mL aIL-6R
- CD19-TCB + 100 nM dexa

FIG. 76

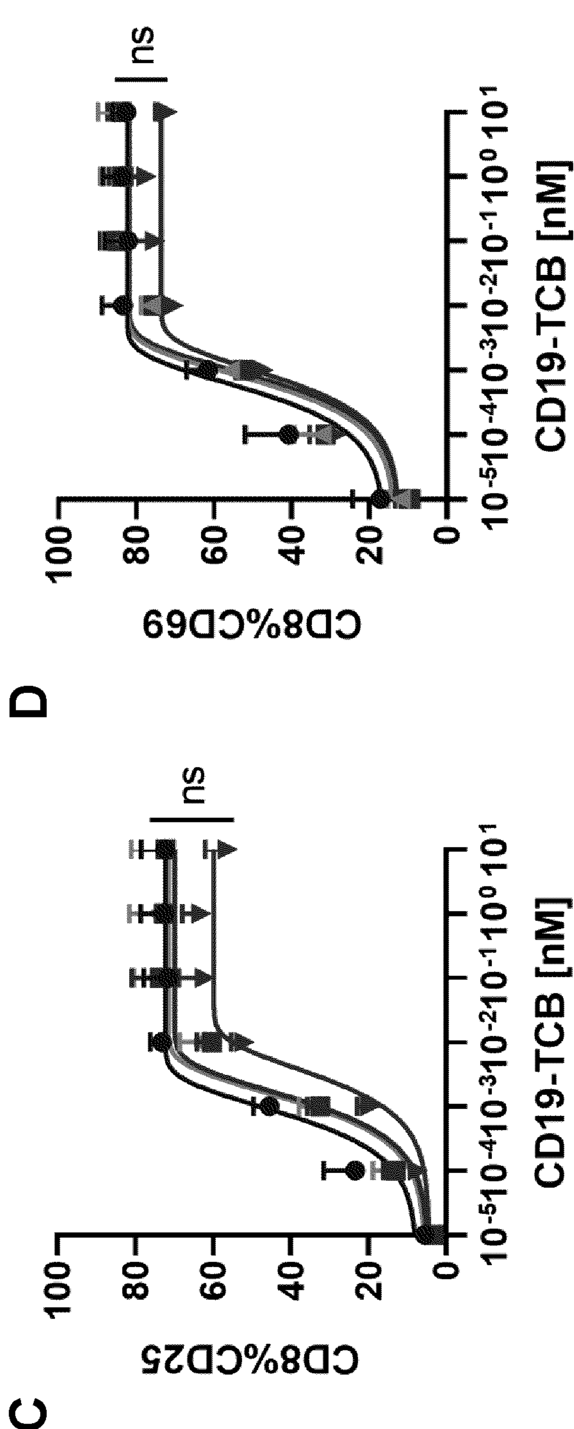
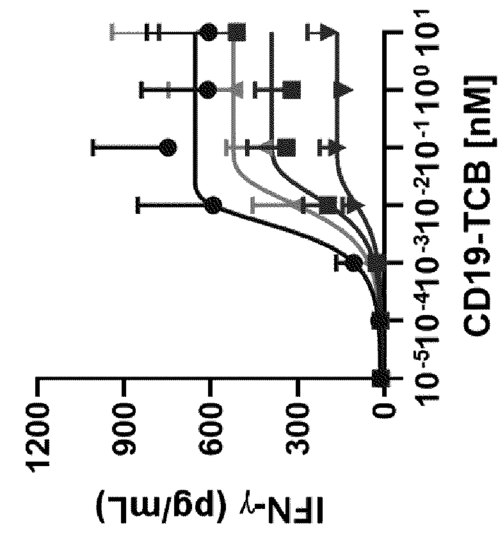
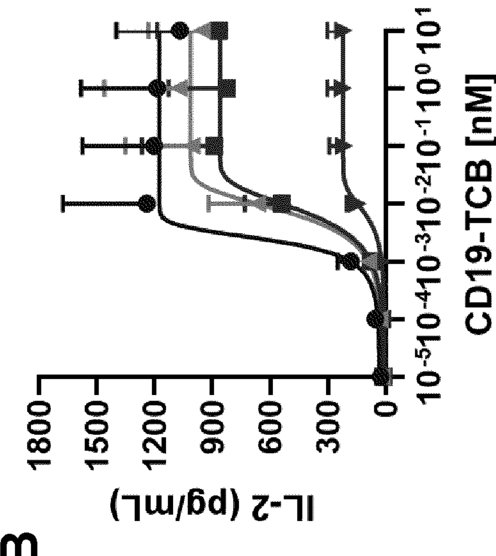
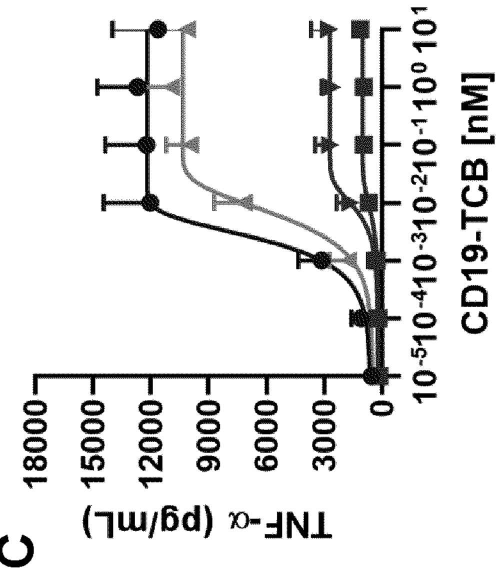
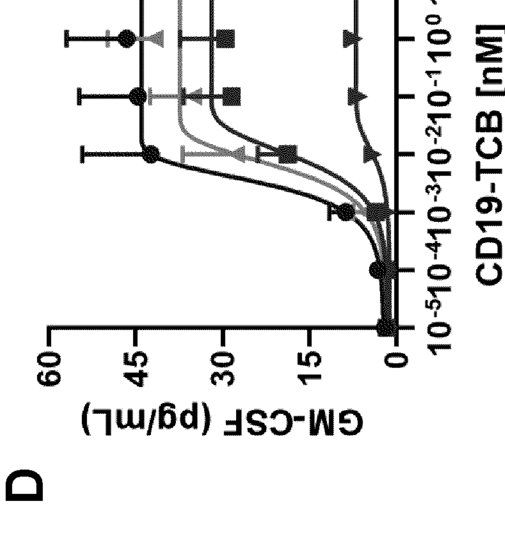


FIG. 76









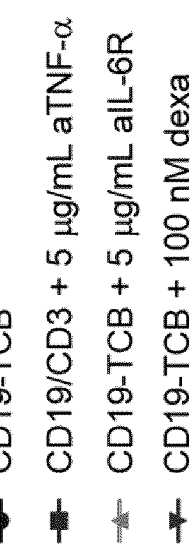


FIG. 77

E
IFN-γ (pg/mL)
CD19-TCB [nM]

F
IL-2 (pg/mL)
CD19-TCB [nM]

G
TNF-α (pg/mL)
CD19-TCB [nM]

H
GM-CSF (pg/mL)
CD19-TCB [nM]

FIG. 77

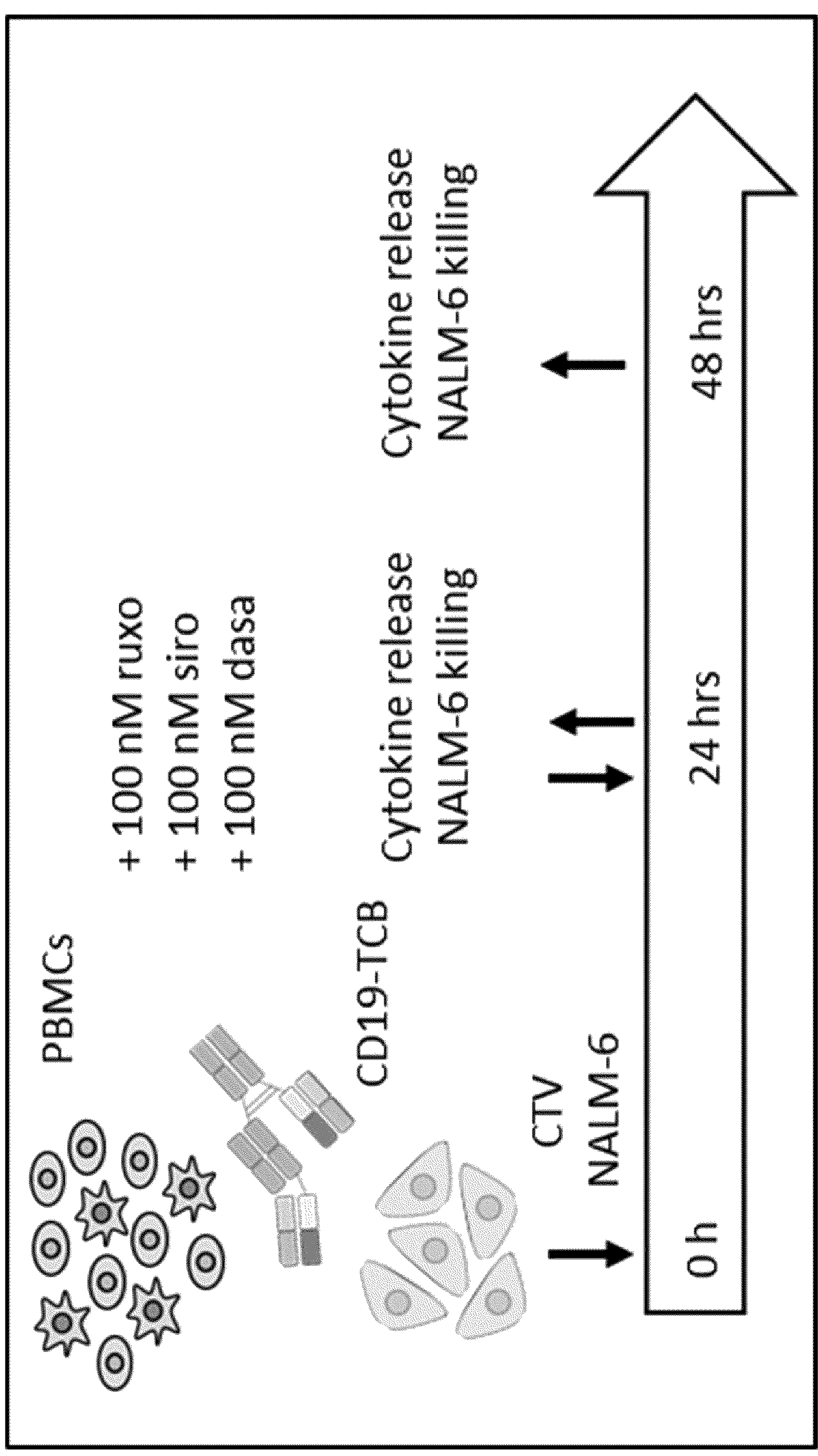
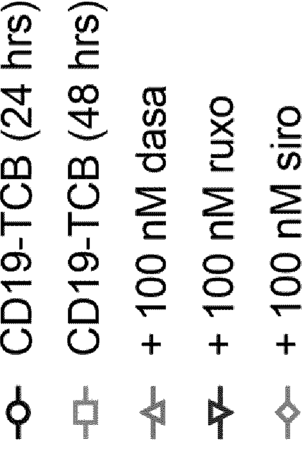
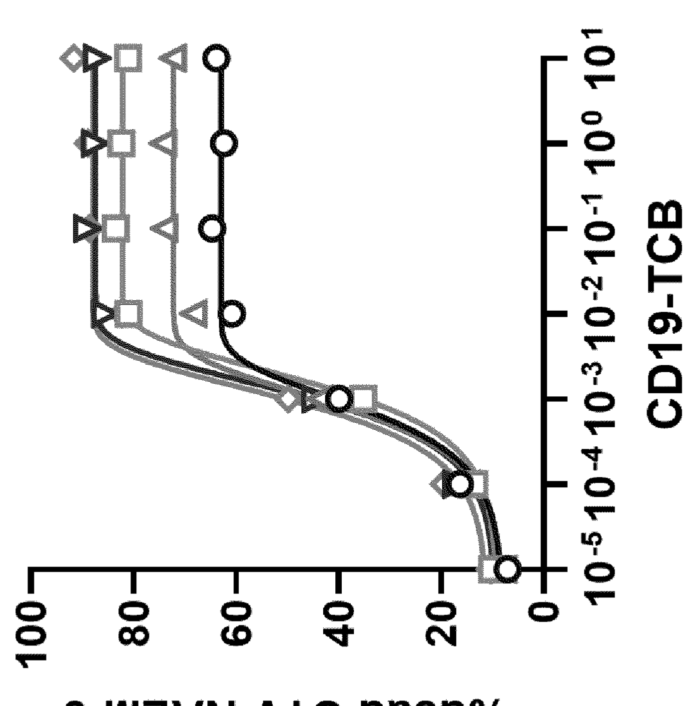


FIG. 78

FIG. 79


CD19-TCB (24 hrs)
CD19-TCB (48 hrs)
+ 100 nM dasa
+ 100 nM ruxo
+ 100 nM siro


%dead CTV NALM-6
100
80
60
40
20
0
10⁻⁵
10⁻⁴
10⁻³
10⁻²
10⁻¹
10⁰
10¹
CD19-TCB

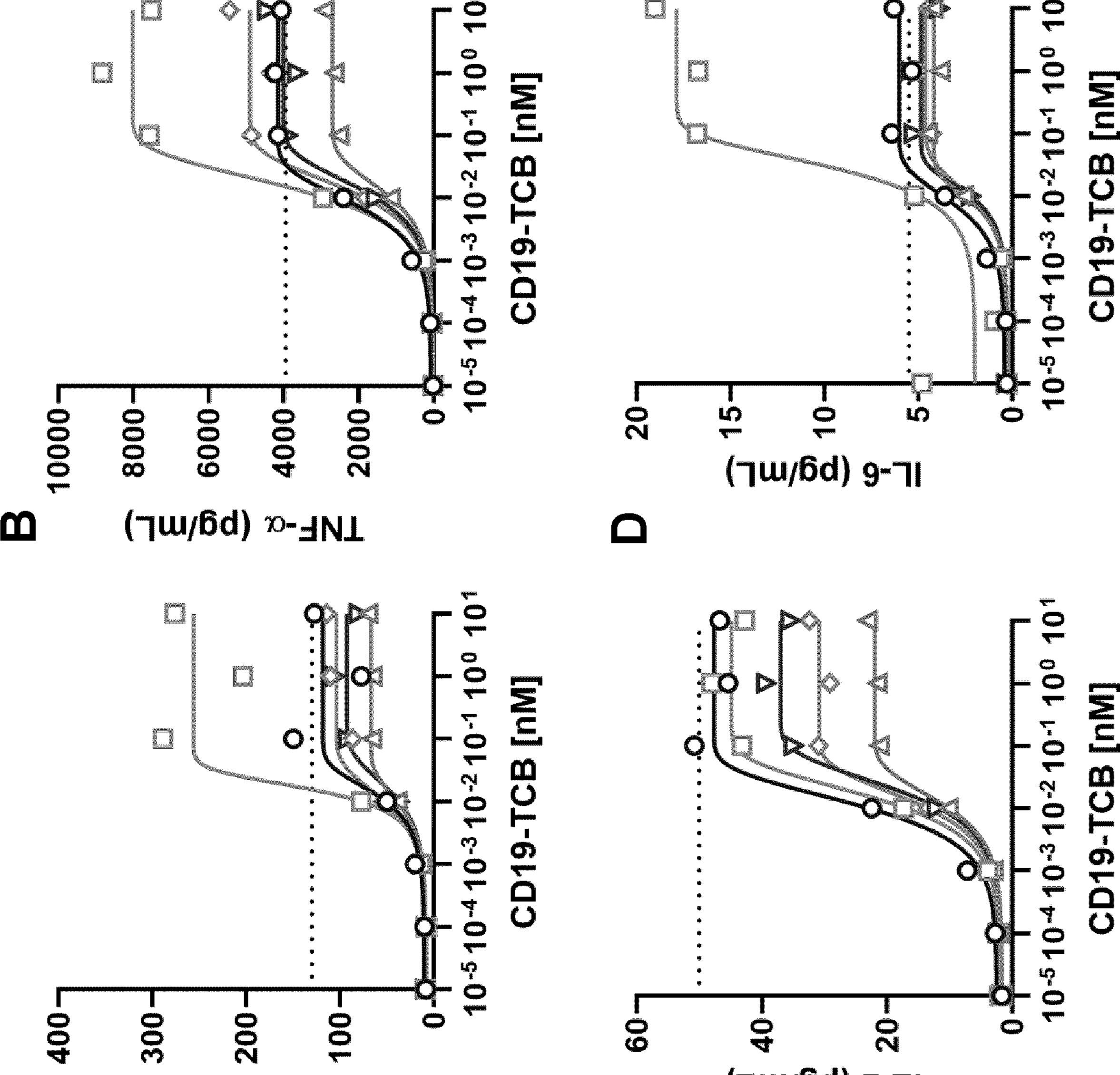


FIG. 80

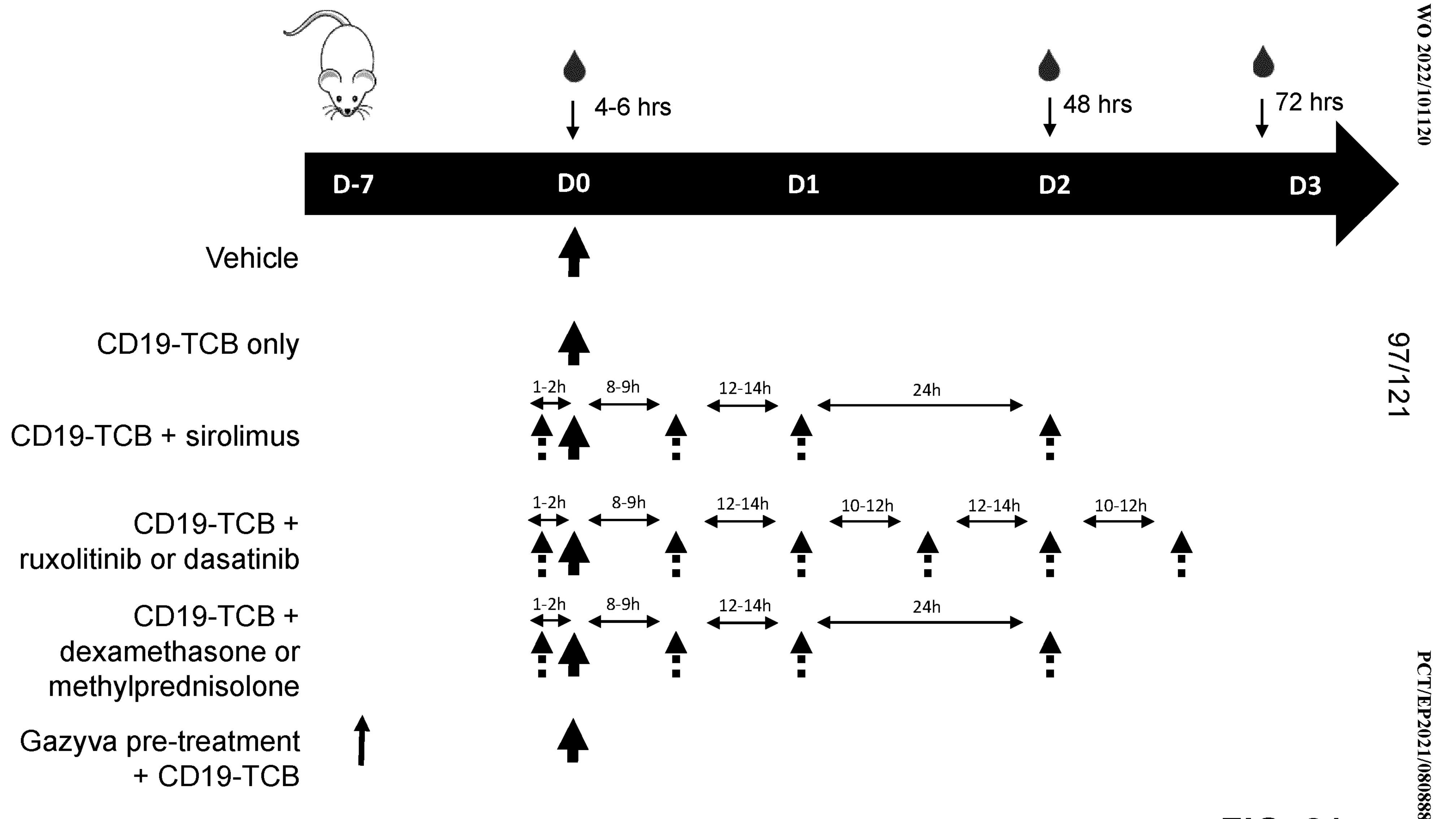
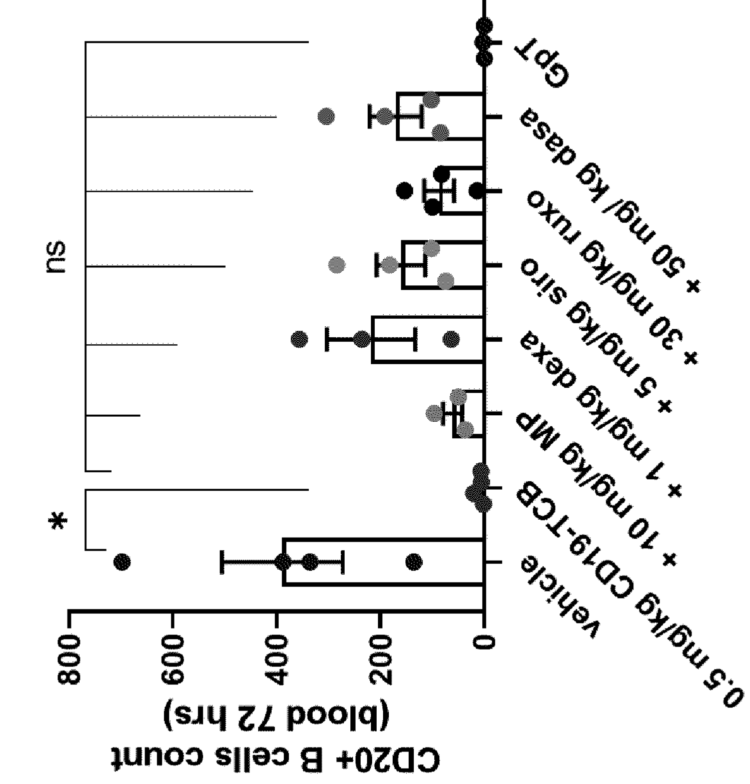
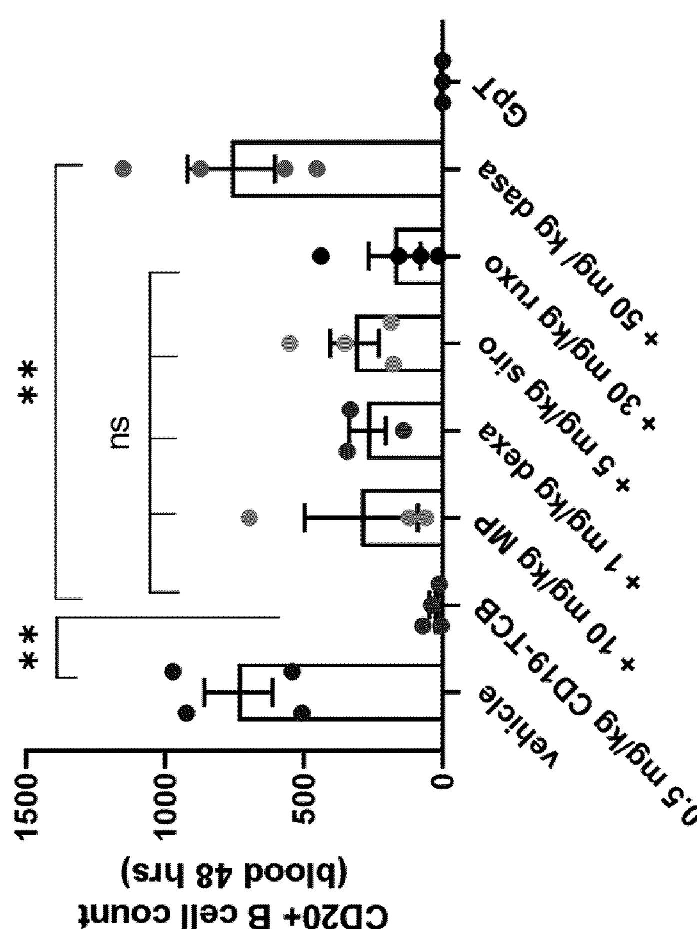


FIG. 81

FIG. 82


B
CD20+ B cells count
(blood 72 hrs)
800
600
400
200
0
*
ns
vehicle
0.5 mg/kg CD19-TCB
+ 10 mg/kg MP
+ 1 mg/kg dexa
+ 5 mg/kg siro
+ 30 mg/kg ruxo
+ 50 mg/ kg dasa
Gpt


A
CD20+ B cell count
(blood 48 hrs)
1500
1000
500
0
**
ns
**
vehicle
0.5 mg/kg CD19-TCB
+ 10 mg/kg MP
+ 1 mg/kg dexa
+ 5 mg/kg siro
+ 30 mg/kg ruxo
+ 50 mg/ kg dasa
Gpt

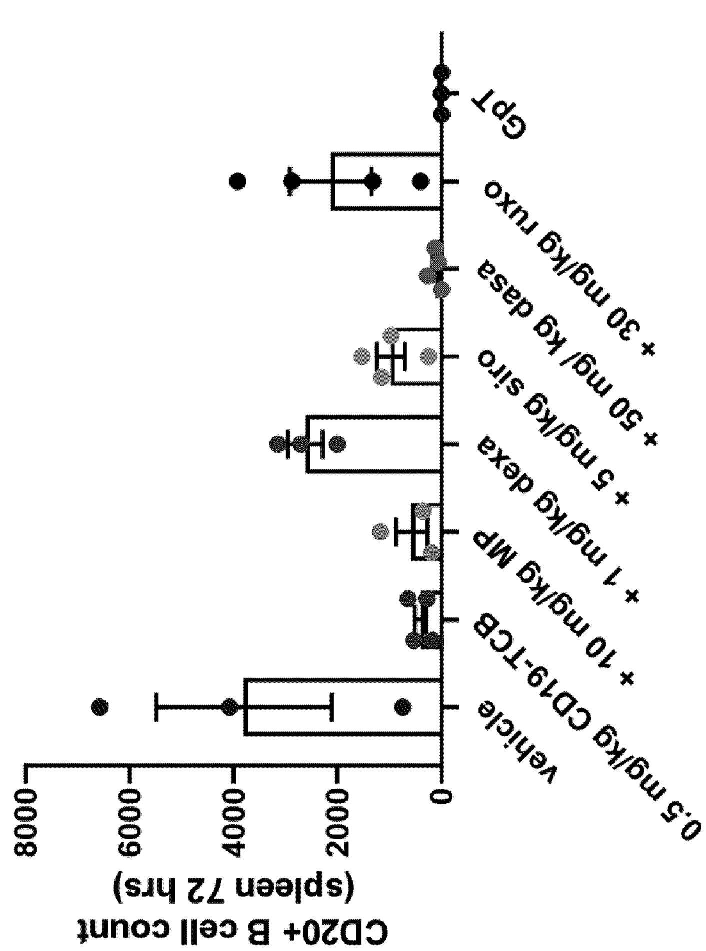


FIG. 83

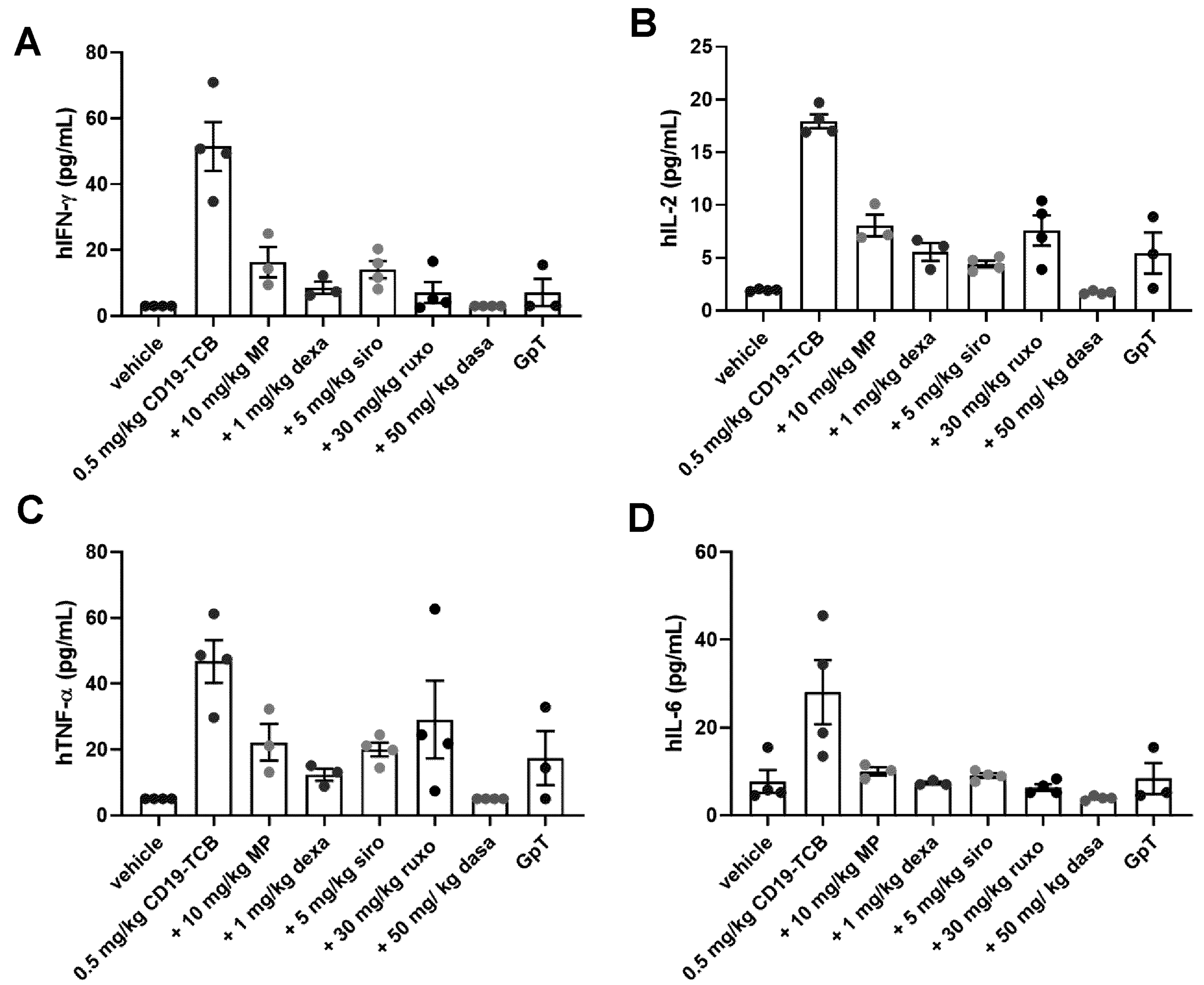
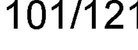
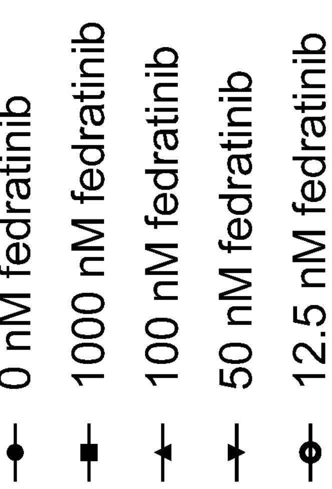


FIG. 84





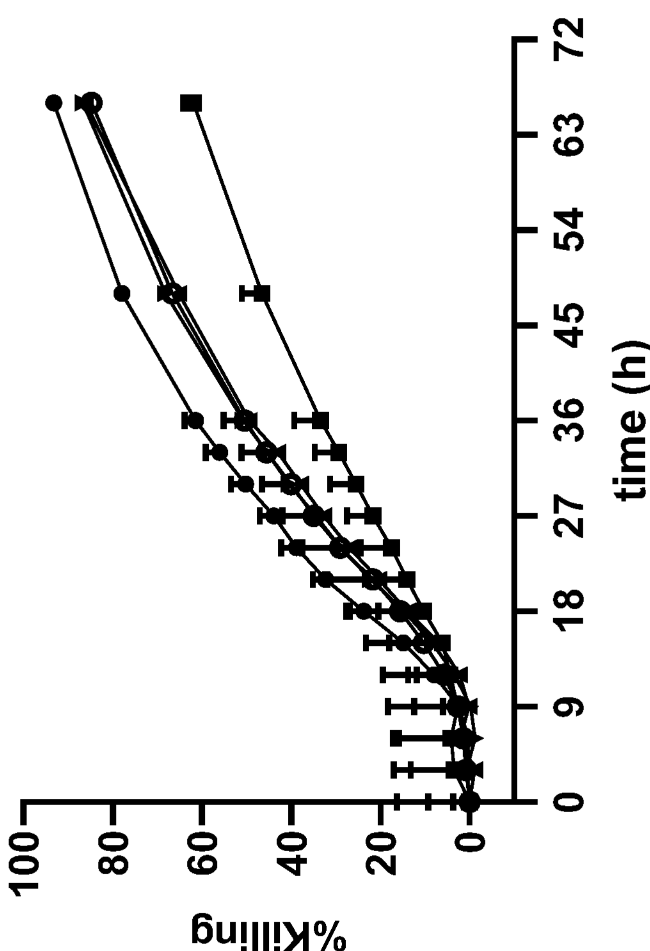


FIG. 85

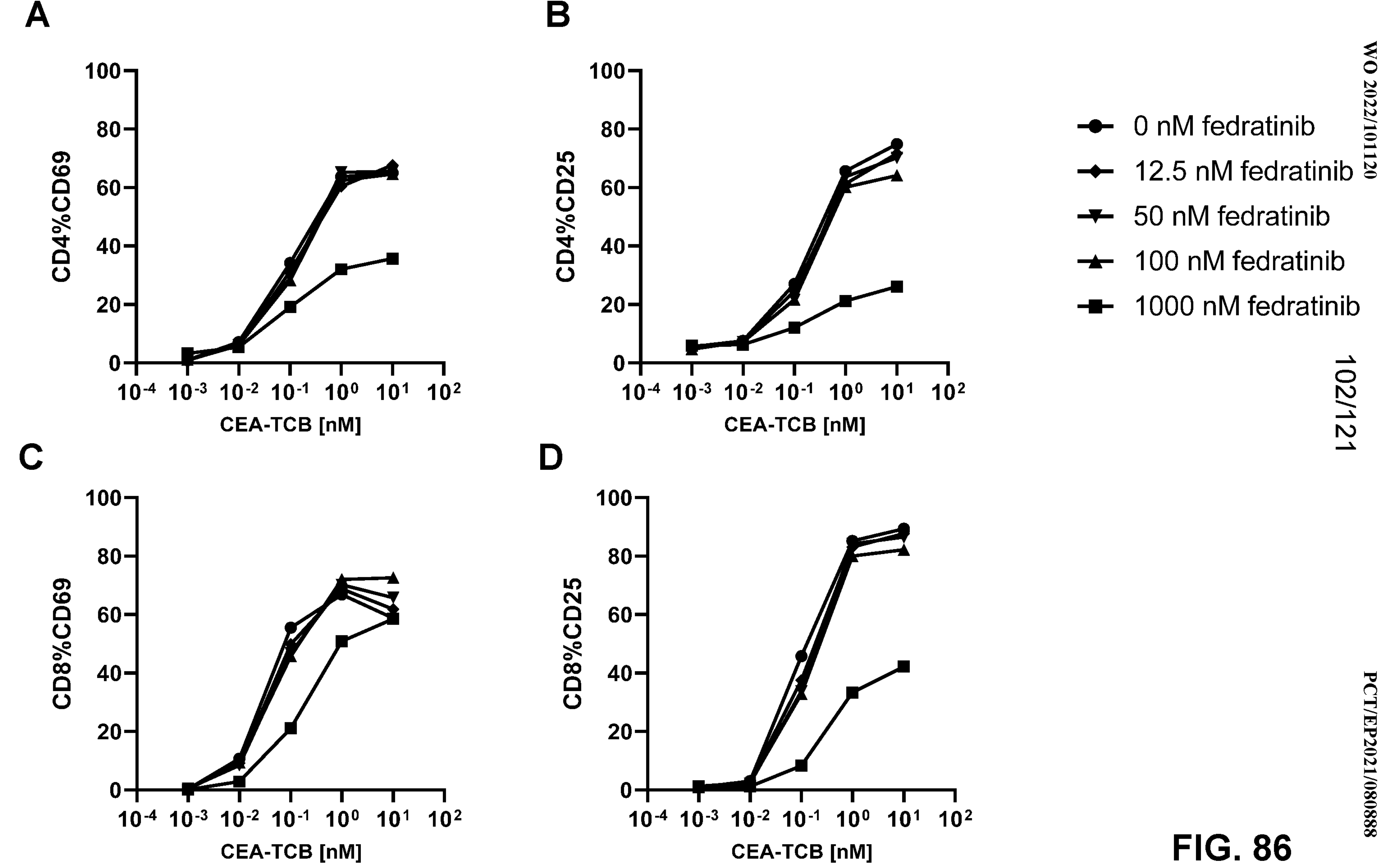


FIG. 86

A

IFN-γ (pg/mL)

CEA-TCB [nM]

B

IL-2 (pg/mL)

CEA-TCB [nM]

C

TNF-α (pg/mL)

CEA-TCB [nM]

D

IL-6 (pg/mL)

CEA-TCB [nM]

E

IL-8 (pg/mL)

CEA-TCB [nM]

0 nM fedratinib
12.5 nM fedratinib
50 nM fedratinib
100 nM fedratinib
1000 nM fedratinib

FIG. 87

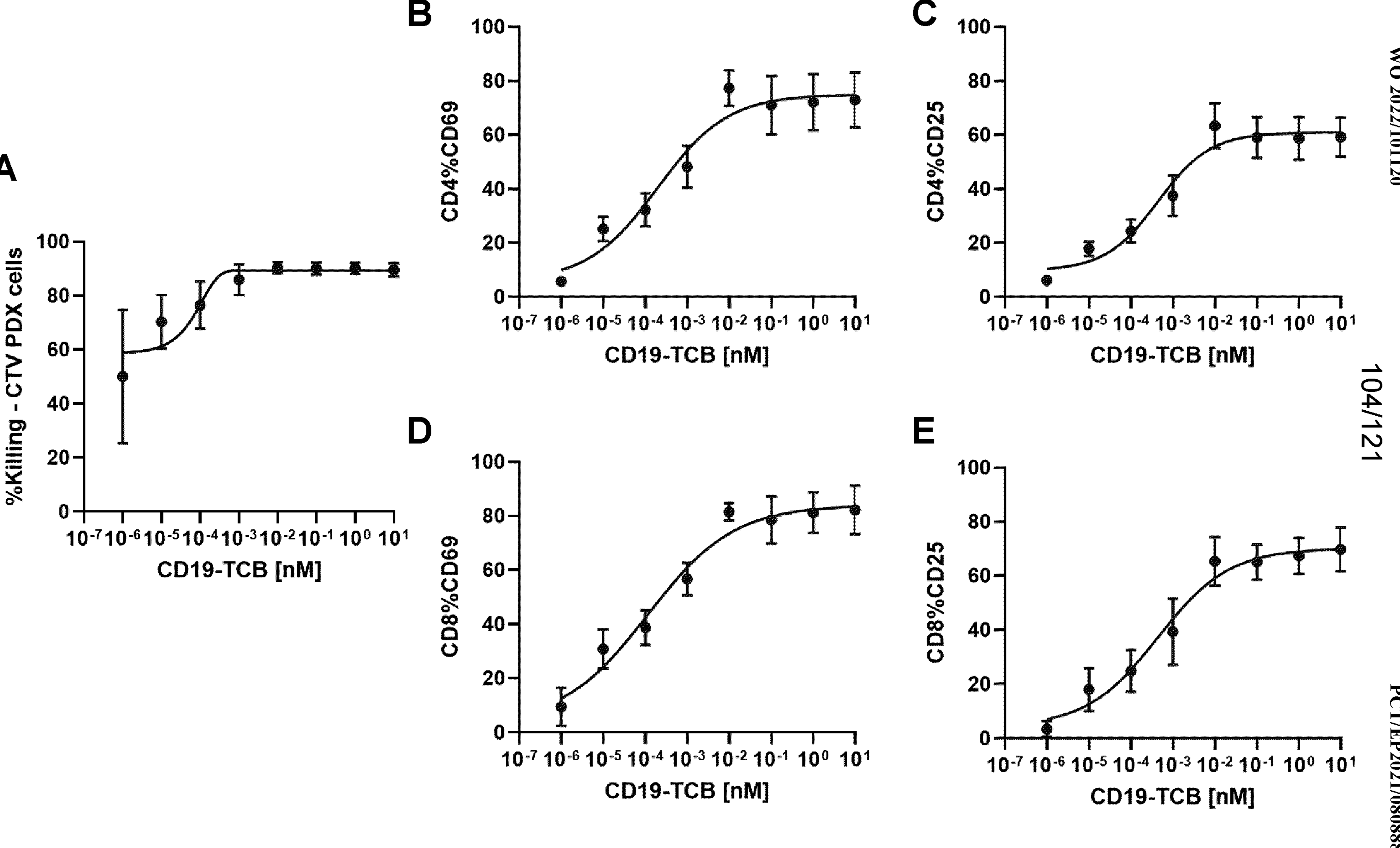


FIG. 88

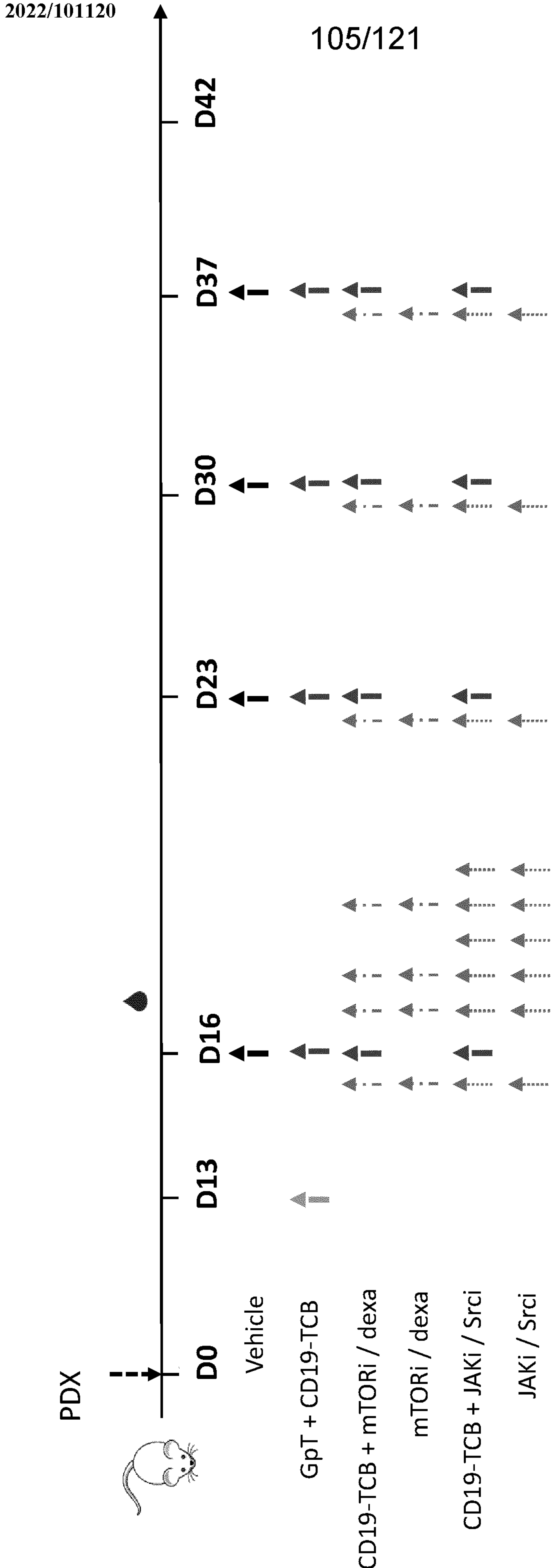


FIG. 89

FIG. 90

vehicle
ruxolitinib (JAK1/2i)
GpT + CD19-TCB
CD19-TCB + ruxolitinib (JAK1/2i)
CD19-TCB

ns
ns *
ns **

N=8
N=8
N=8
N=6
N=7

Tumor volume [mm^3]

days

FIG. 91

FIG. 92

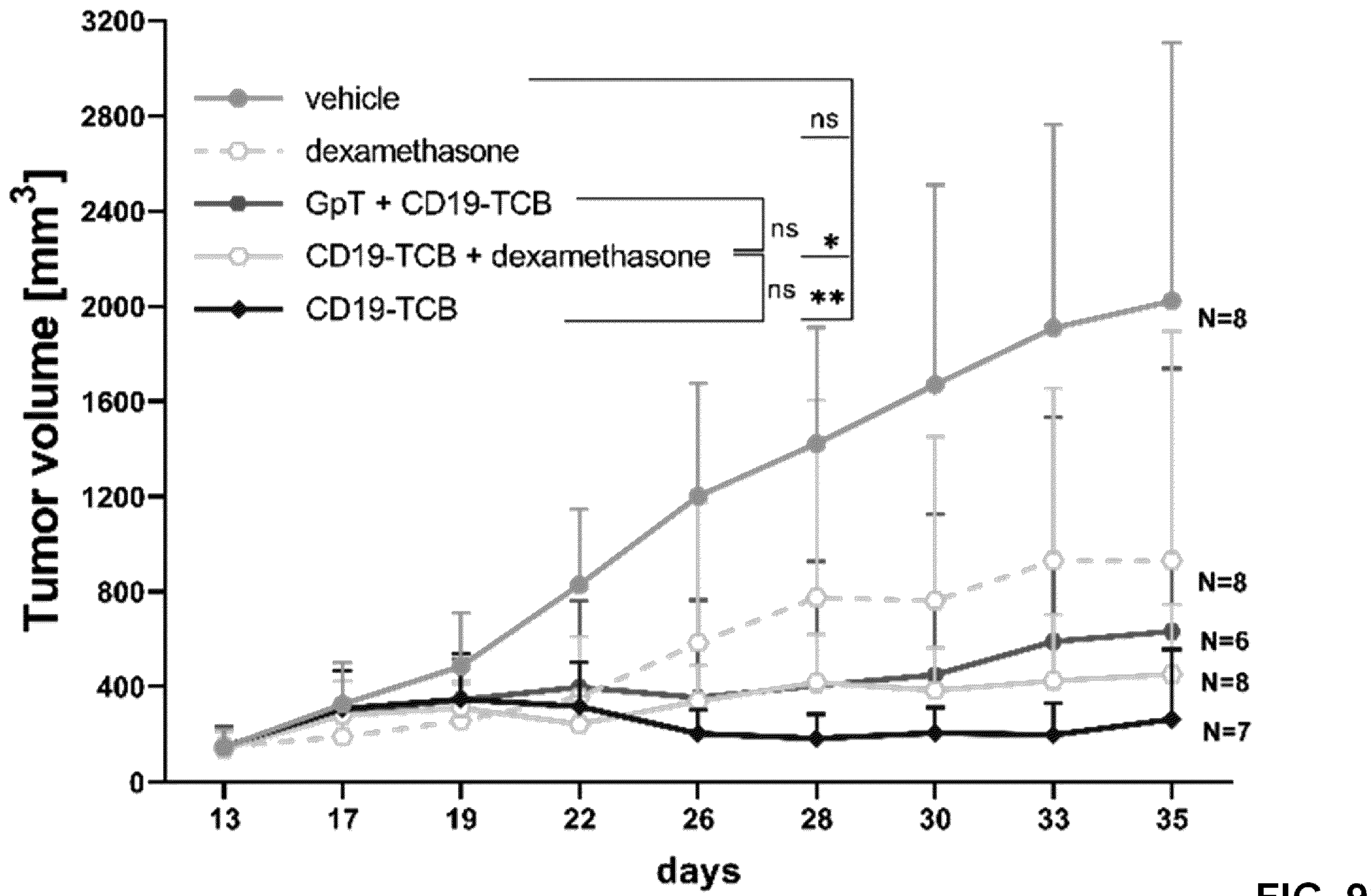
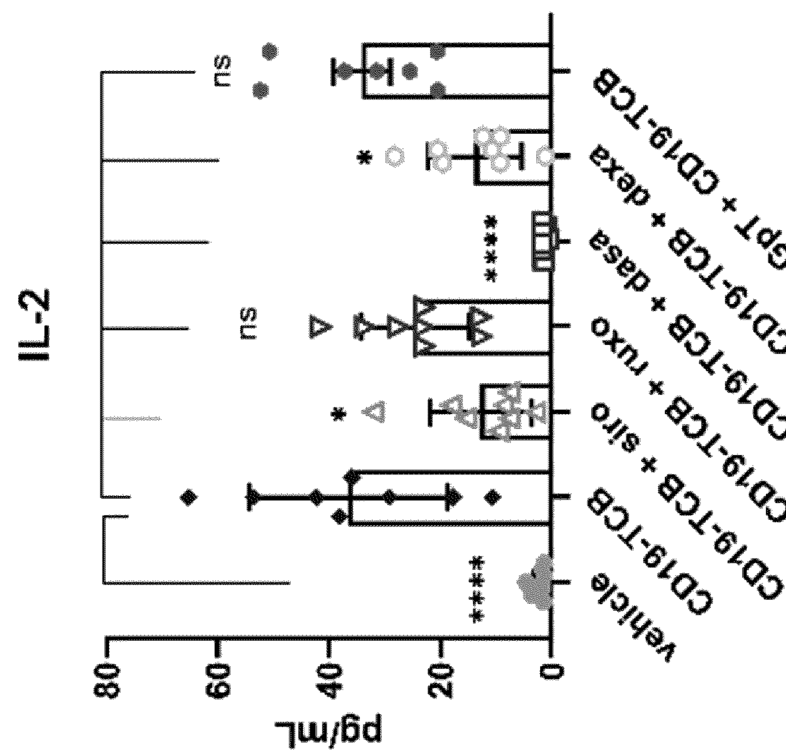


FIG. 93



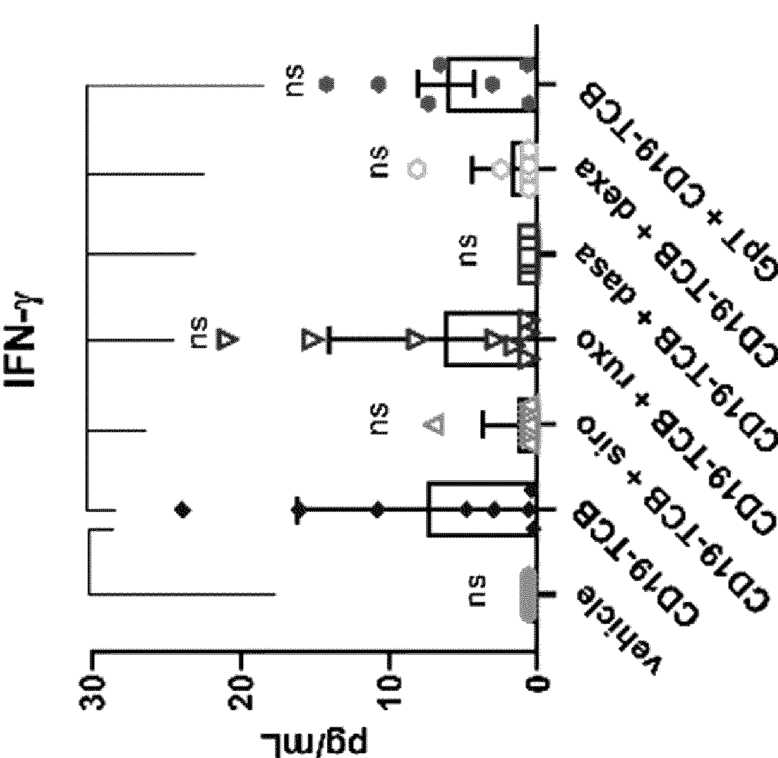
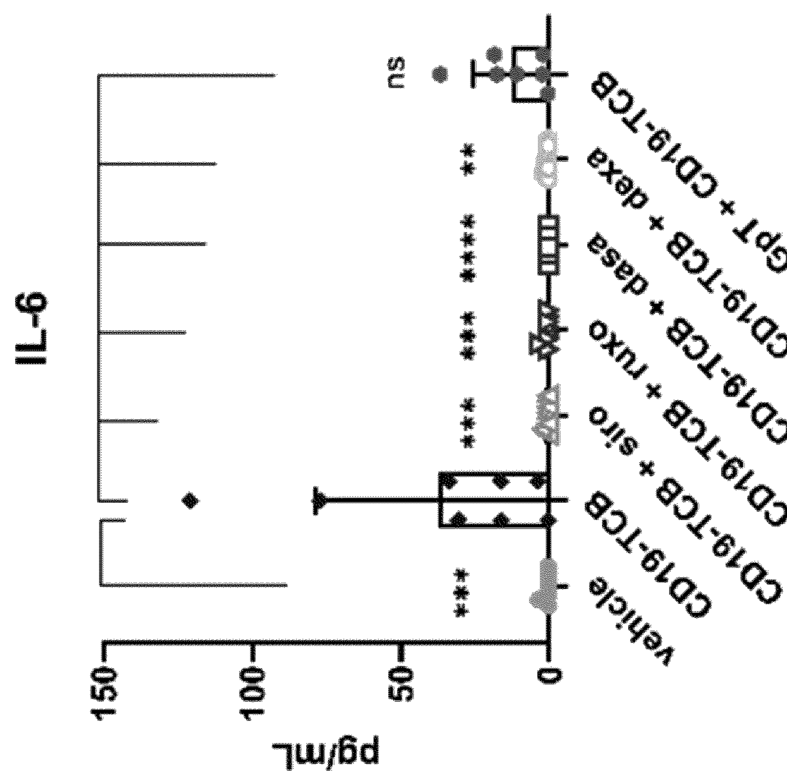


FIG. 94



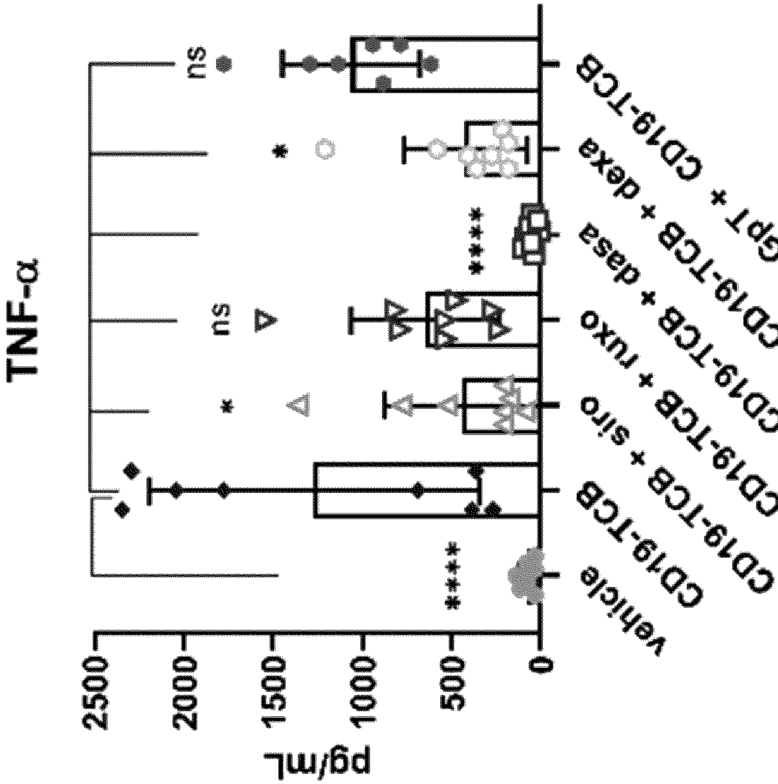


FIG. 94

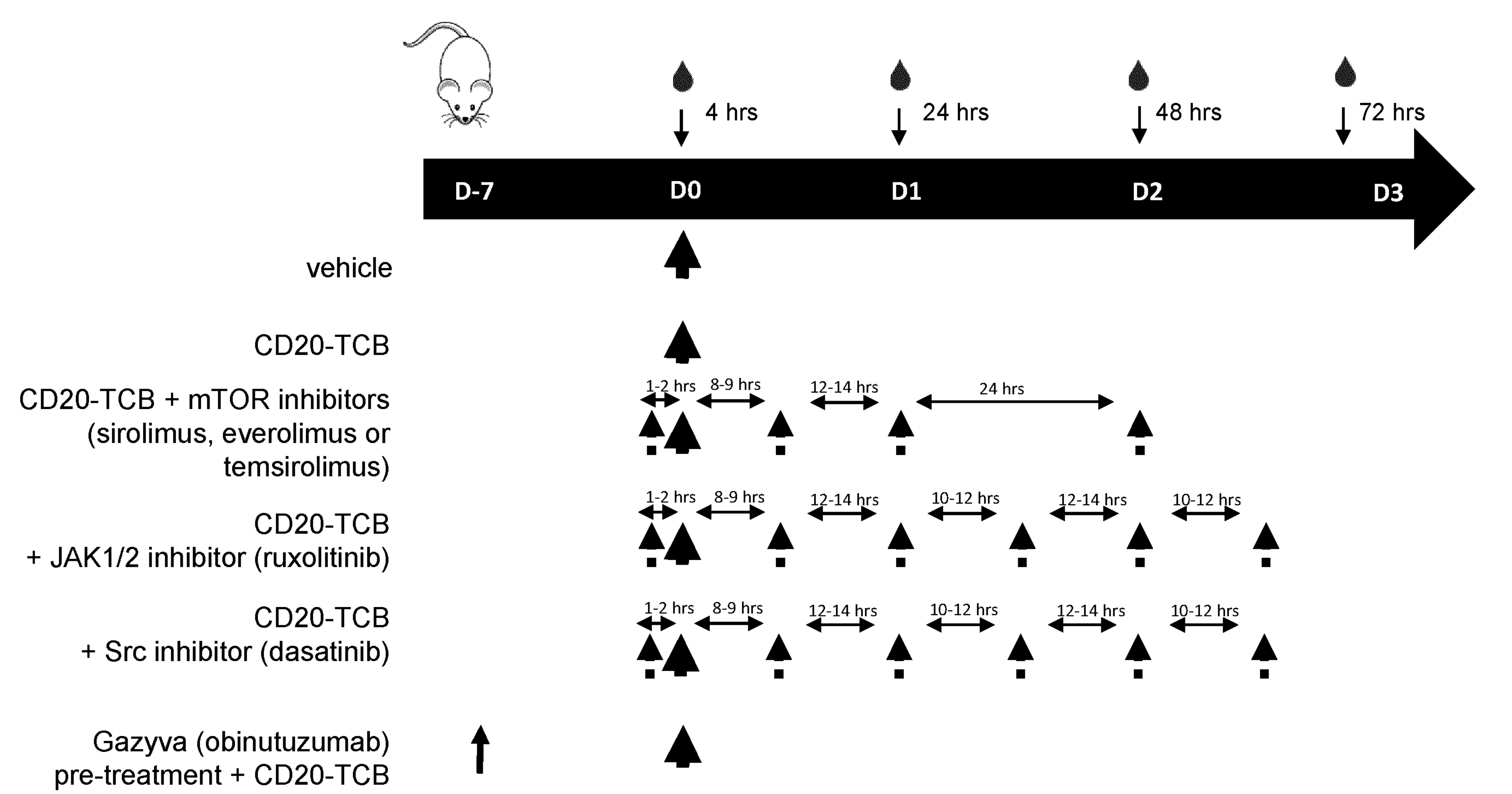


FIG. 95

C

		CD20-TCB	Gpt + CD20-TCB	CD20-TCB + 2 mg/kg sirolimus	CD20-TCB + 5 mg/kg sirolimus	CD20-TCB + 10 mg/kg sirolimus	CD20-TCB + 10 mg/kg temsiro-limus	CD20-TCB + 10 mg/kg everolimus	CD20-TCB + 30 mg/kg ruxolitinib	CD20-TCB + 60 mg/kg ruxoli-tinib	CD20-TCB + 10 mg/kg dasatinib	CD20-TCB + 50 mg/kg dasatinib
48 hrs	p value	0.487	0.001	0.001	0.169	0.009	0.256	0.176	0.209	>0.999	>0.999	>0.999
	summary	ns	**	**	ns	**	ns	ns	ns	ns	ns	ns
72 hrs	p value	0.292	0.007	0.007	0.048	0.012	0.379	0.019	0.697	>0.999	>0.999	>0.999
	summary	ns	**	**	*	*	ns	*	ns	ns	ns	ns

FIG. 96

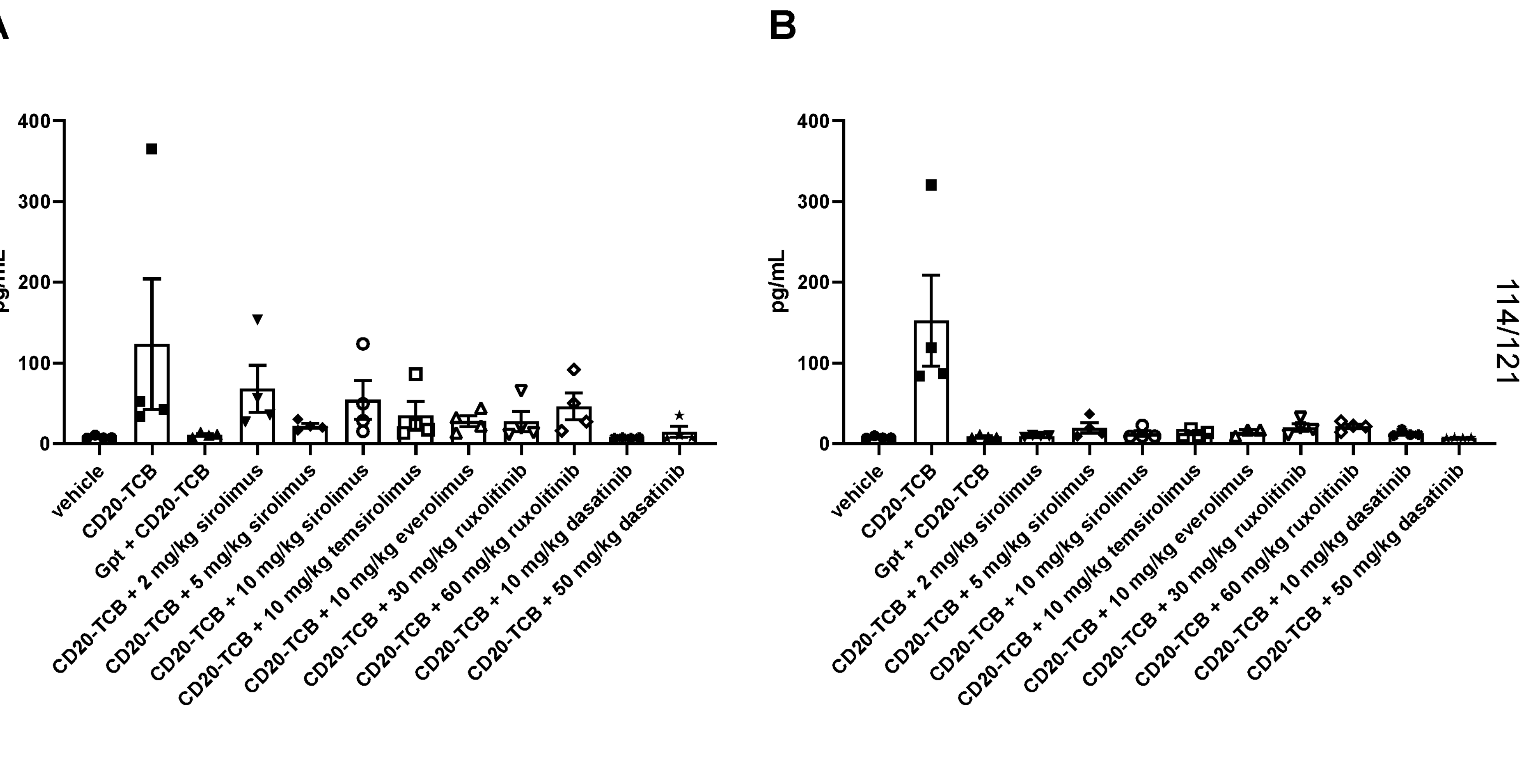


FIG. 97

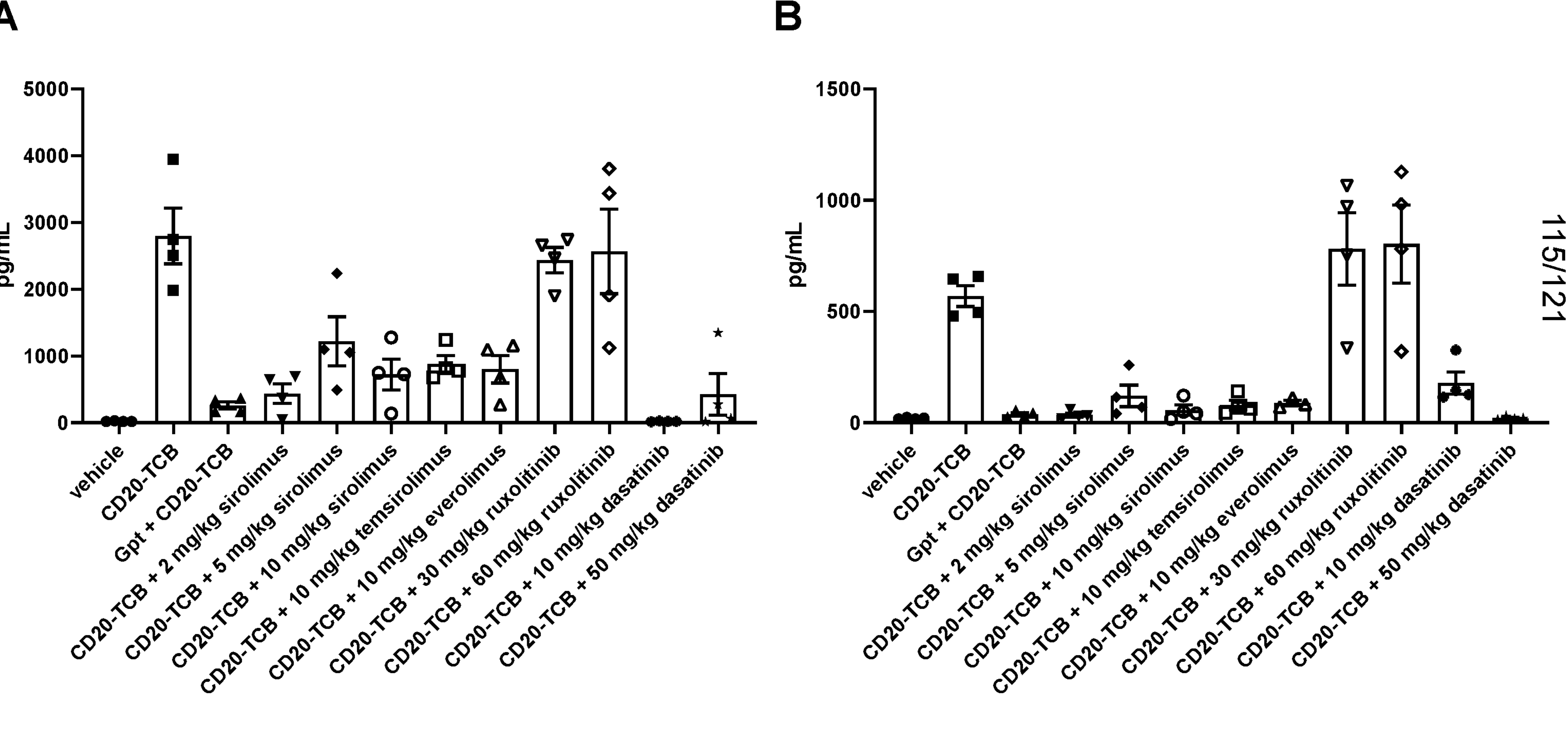


FIG. 98

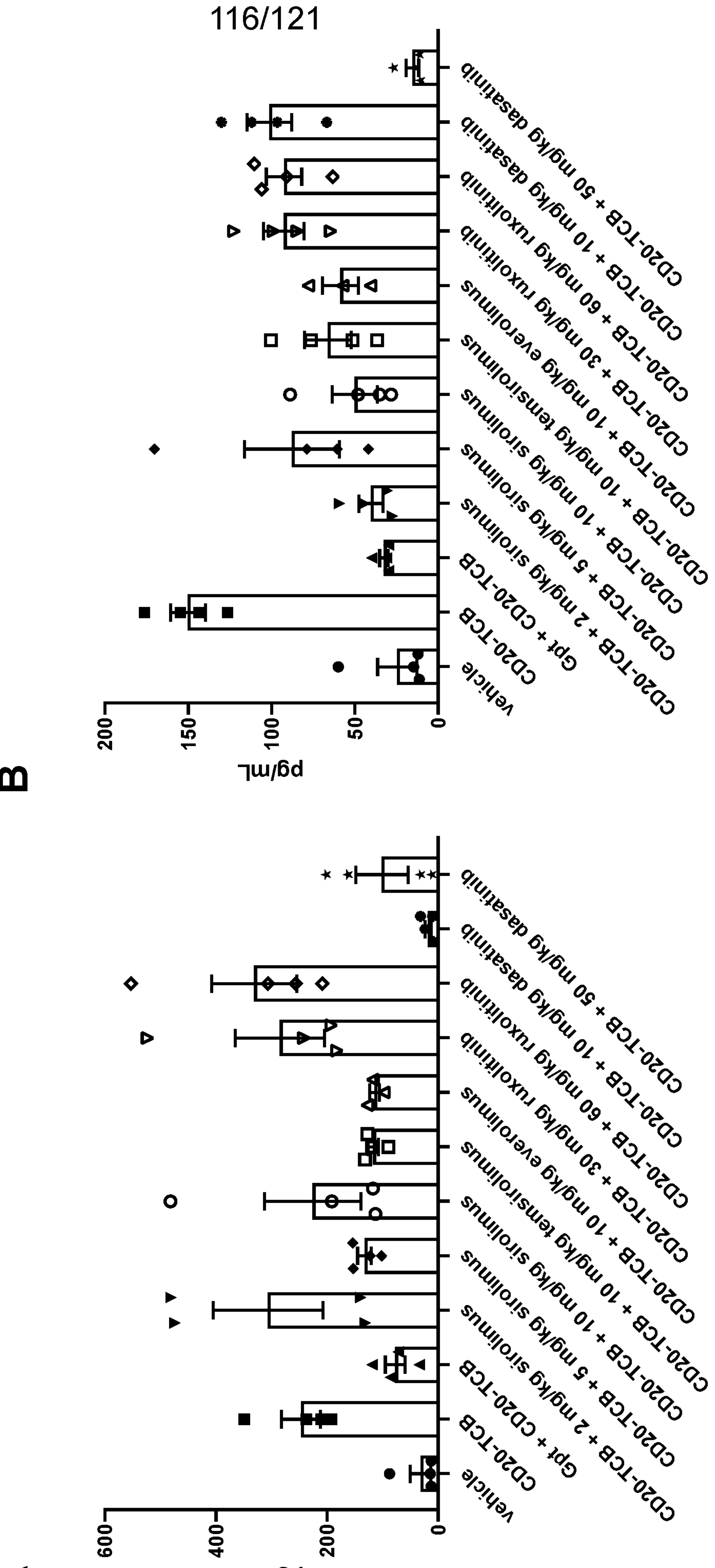


FIG. 99

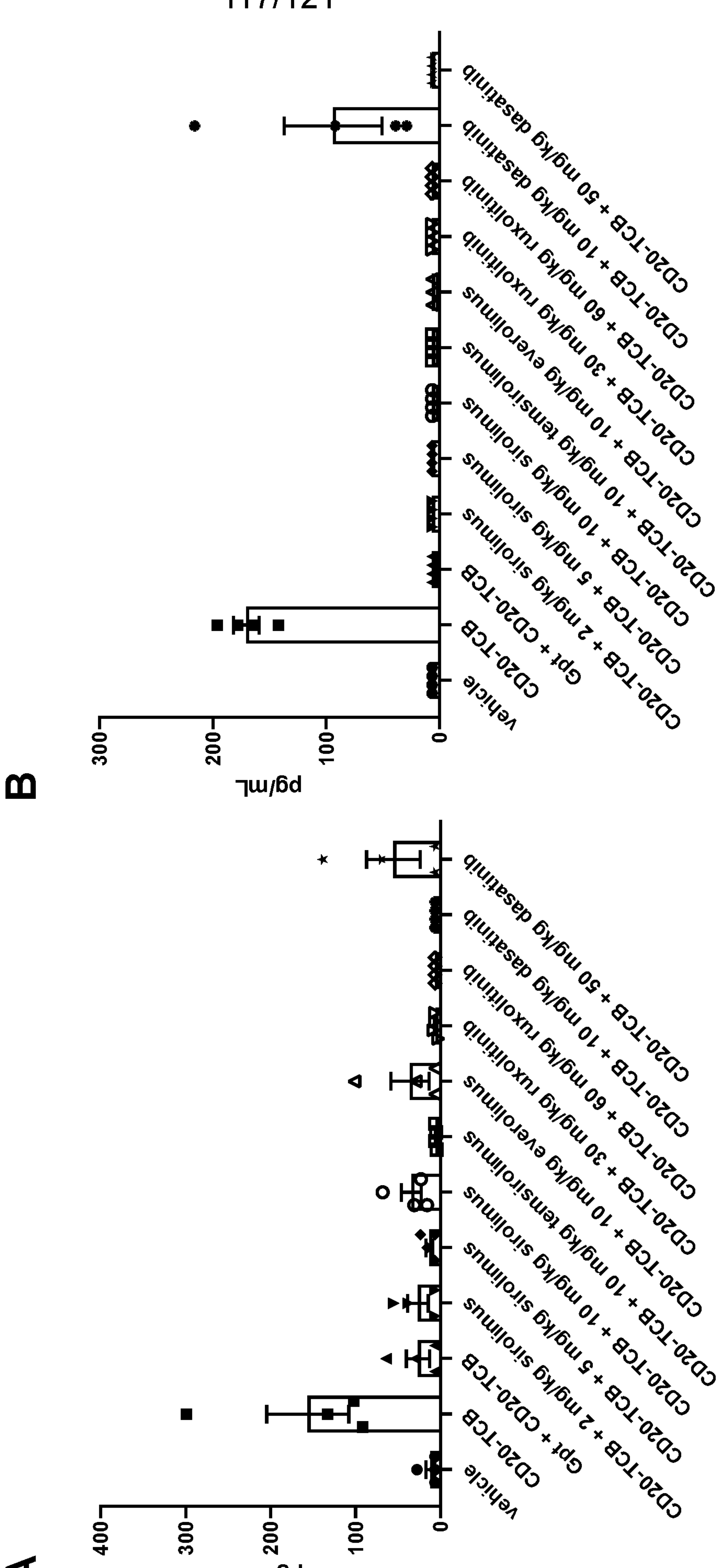


FIG. 100

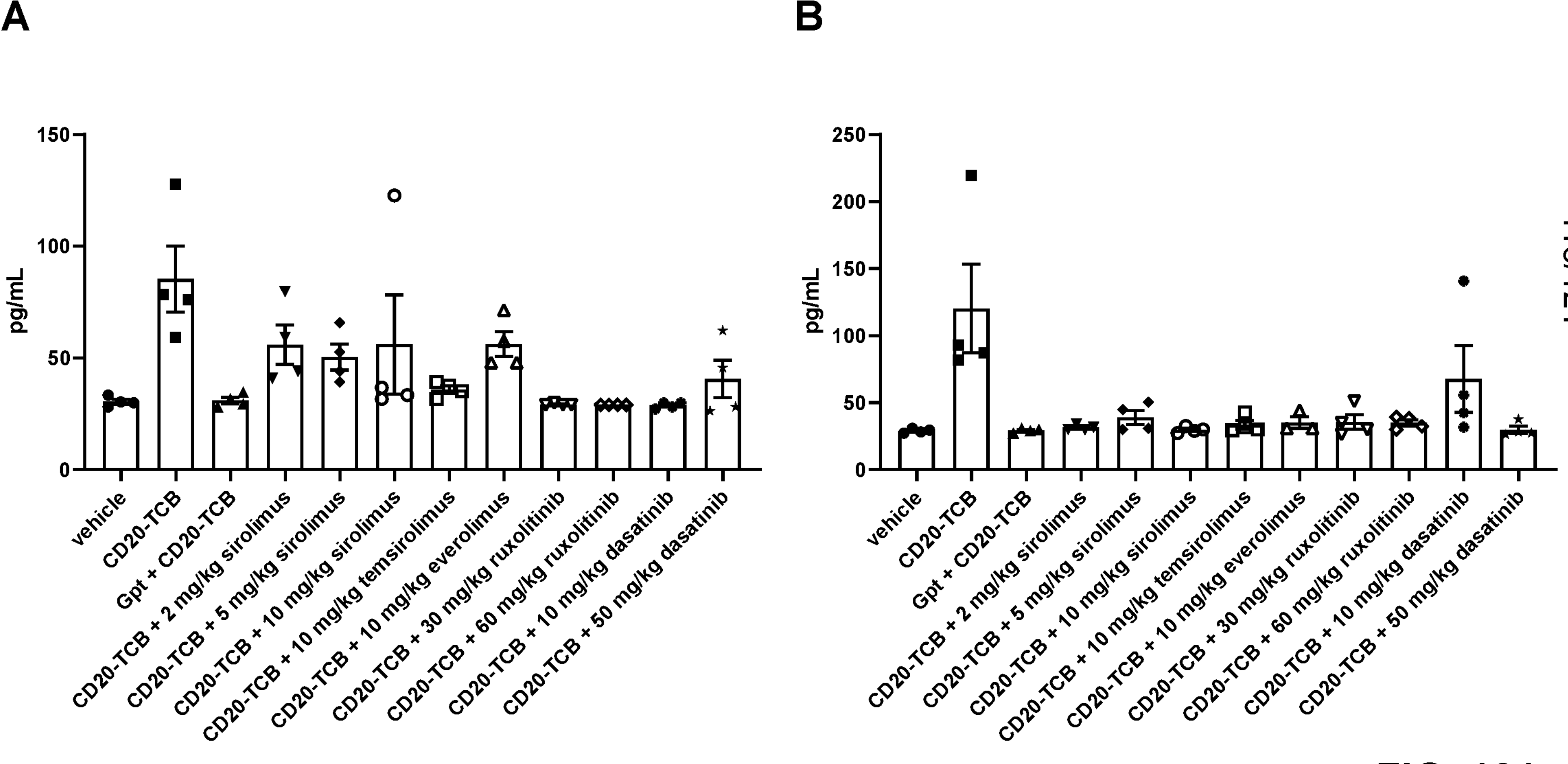


FIG. 101

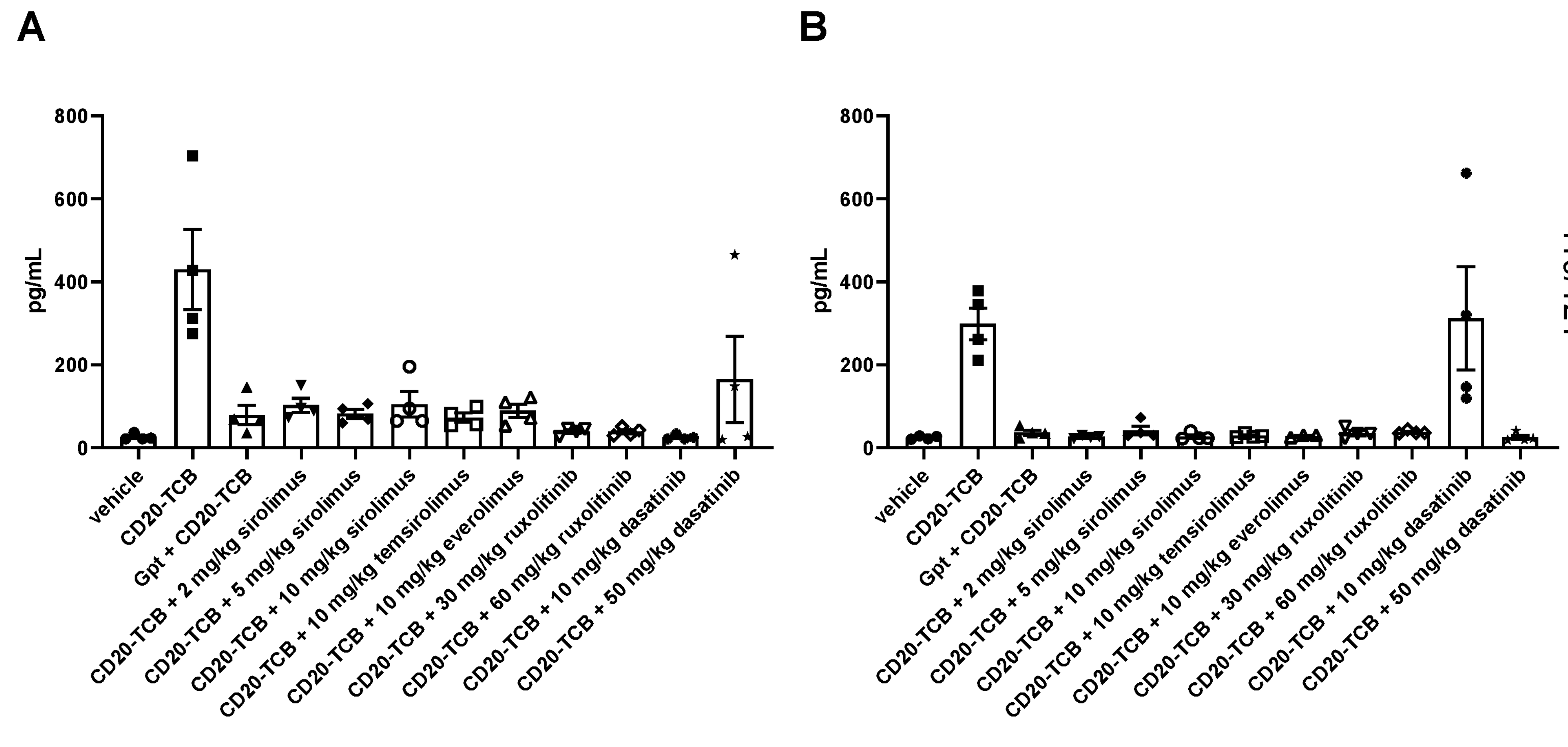


FIG. 102

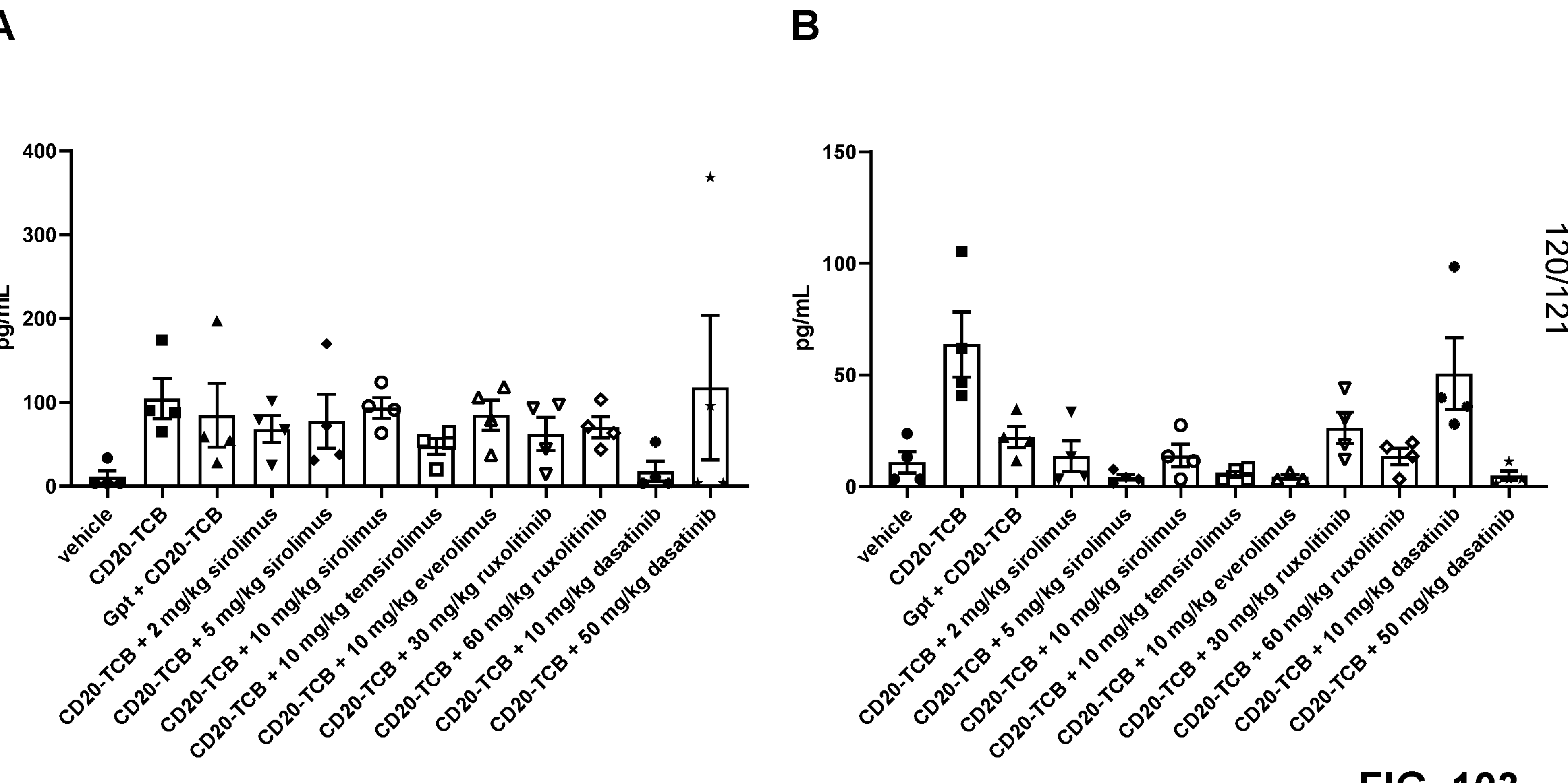


FIG. 103

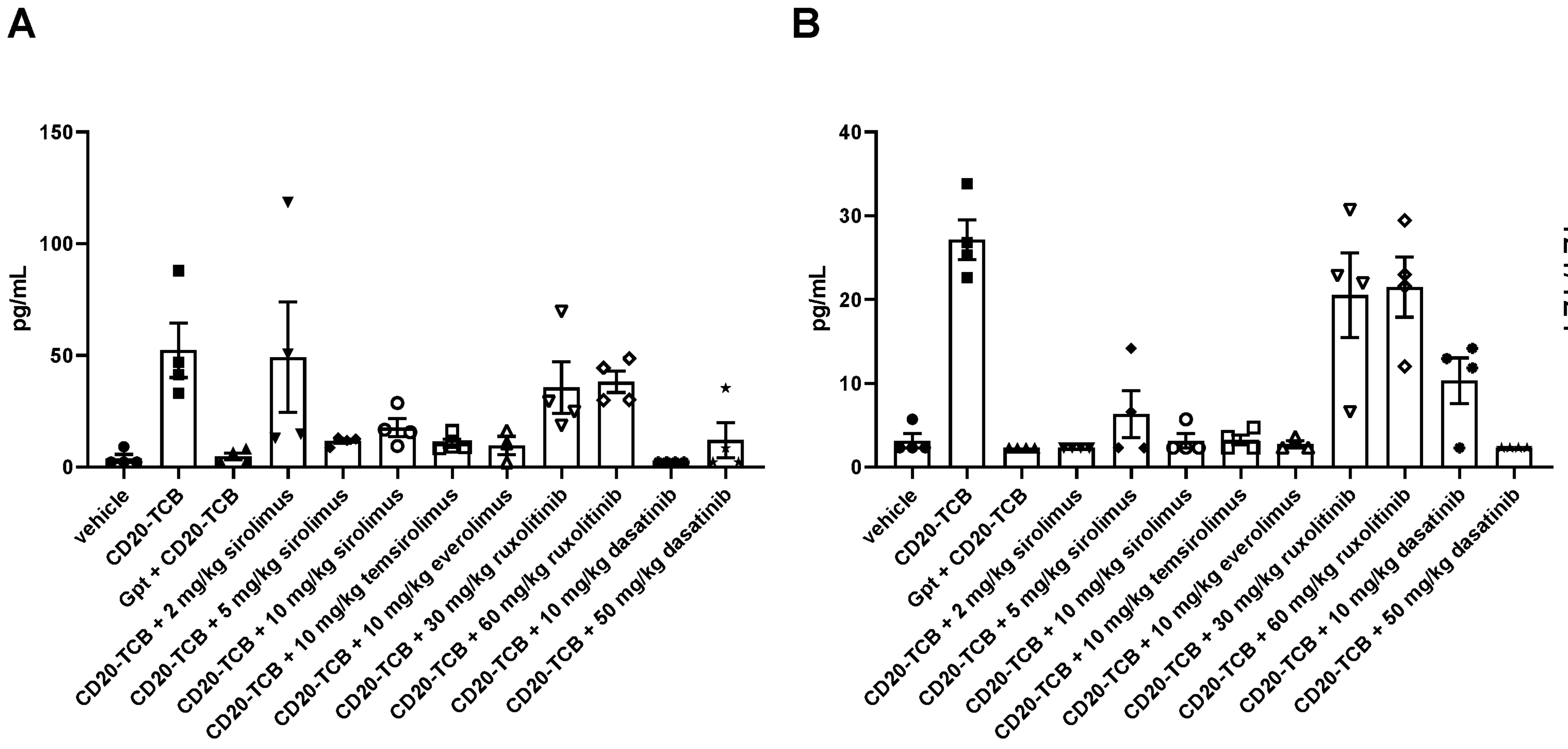


FIG. 104

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/080888

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 A61K31/00 A61K39/395 A61P35/00 A61K31/436
C07K16/28 A61K31/519 A61K35/17 A61P37/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61P A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2019/336504 A1 (GILL SAAR [US] ET AL) 7 November 2019 (2019-11-07)	1-18,50, 52,54,56
Y	paragraphs [0341], [0352], [0813], [0822], [0829], [0859], [0849], [0856], [0866], [0870], [0895], [1066]; example 1	1-56

X	US 2019/112380 A1 (CHAUDHARY PREET M [US]) 18 April 2019 (2019-04-18)	1-18, 50-52, 54,56
Y	paragraphs [0101], [0223], [0572], [0599], [0731]	1-56

	-/--	

[x] Further documents are listed in the continuation of Box C. [x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 February 2022	11/02/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Zellner, Eveline

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ROMAN H KHADKA ET AL: "Management of cytokine release syndrome: an update on emerging antigen-specific T cell engaging immunotherapies", IMMUNOTHERAPY, vol. 11, no. 10, 5 June 2019 (2019-06-05), pages 851-857, XP055770874, GB ISSN: 1750-743X, DOI: 10.2217/imt-2019-0074	1-7, 9-18,50, 52,54,56
Y	page 855, paragraph 4	1-56
X	HUARTE EDUARDO ET AL: "Prophylactic Itacitinib (INCB039110) for the Prevention of Cytokine Release Syndrome Induced By Chimeric Antigen Receptor T-Cells (CAR-T-cells) Therapy", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 134, 13 November 2019 (2019-11-13), page 1934, XP086672034, ISSN: 0006-4971, DOI: 10.1182/BLOOD-2019-128288	1-7, 10-13, 15,16, 18,52, 54,56
Y	page 1 - page 2	1-56
Y	US 2020/172627 A1 (BACAC MARINA [CH] ET AL) 4 June 2020 (2020-06-04) paragraphs [0020] - [0023], [0154], [0578]	1-54
Y	WO 2020/169698 A1 (F HOFFMANN-LA ROCHE AG [CH]; HOFFMANN-LA ROCHE INC [US]) 27 August 2020 (2020-08-27) page 29, line 6 - page 31, line 12 page 32, line 28 - line 32	1-56

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2021/080888

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2021/080888

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2019336504 A1	07-11-2019	AU 2017295886 A1	31-01-2019
		CA 3030837 A1	18-01-2018
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		EP 3436070 A2	06-02-2019
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		KR 20180130534 A	07-12-2018
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		WO 2017172981 A2	05-10-2017
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		BR 112019022558 A2	19-05-2020
		CA 3059010 A1	06-12-2018
		CN 110603266 A	20-12-2019
		EP 3630829 A1	08-04-2020
		JP 2020521791 A	27-07-2020
		KR 20200014304 A	10-02-2020
		TW 201902512 A	16-01-2019
		US 2020172627 A1	04-06-2020
		WO 2018220099 A1	06-12-2018
WO 2020169698 A1	27-08-2020	NONE	