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(54) Title: ANTI-FAS LIGAND (FASL) ANTIBODIES IN THE TREATMENT OF SJS/TEN DISEASES

(57) Abstract: The present invention refers to the use of antagonists of human Fas ligand (FasL; also named CD95L or Apo 1L), more particularly to the use of antibodies specifically directed against the soluble FasL for the prevention and/or treatment of SJS and/or TEN skin diseases.

WO 2024/200287 A1

Anti-Fas Ligand (FasL) Antibodies in the Treatment of SJS/TEN Diseases

Description

The present invention refers to the use of antagonists of human Fas ligand (FasL; also named CD95L or CD178 or Apo1L), particularly of antagonists of the soluble form of the human Fas ligand (sFasL). More particularly, the present invention refers to the use of antibodies against FasL, particularly of sFasL, for the prevention and/or
5 treatment of SJS and/or TEN skin diseases (in the following SJS/TEN).

Background of the invention

Stevens-Johnson-Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) are
10 severe cutaneous conditions caused by adverse reaction to specific drugs or medical substances. Both skin conditions are characterized by wide-spread erythema, epidermal necrosis, blistering and detachment of skin sections. Almost all patients with SJS and TEN have also mucosal involvement in eyes, mouth and genitals. These syndromes are considered as the same disease with different
15 spectrums of severity. SJS represents the less severe disease spectrum and is defined by the detachment of less than 10% of the body surface area. TEN represents the more severe disease spectrum involving skin detachment greater than 30% of the body surface area. Overlapping SJS/TEN is defined as 10-30% skin detachment.

20

The extensive skin detachment in SJS/TEN causes significant morbidity and mortality. The reported mortality rate in patients with SJS/TEN is estimated between 4,8% and 14,8%, respectively. Thus, SJS/TEN are considered dermatological emergencies and early recognition and successful therapy is considered lifesaving.

25

Although SJS/TEN is a distressing disease, there is no standard treatment for these cutaneous conditions and no approved drugs. Patients are usually treated in

emergency with several drugs, such as cyclosporine, steroids, anti-TNF, IVIg or plasmapheresis (Chang HH et al., Biomedicines, 2022). None of these drugs, however, has been shown to have any beneficial impact in controlled study. Most patients receive only supportive care.

5

The clinical manifestation of skin exfoliation occurring in SJS/TEN is mainly due to an extensive induction of keratinocytes apoptosis and necroptosis. However, the mechanism responsible for the enhancement in SJS/TEN remains unclear.

10 Fas (or FasR) is a member of the TNF-receptor superfamily which, upon binding with Fas ligand (FasL), triggers apoptosis in many cell systems (Sharma *et al*, 2000). Fas-FasL interaction is involved in the pathomechanisms of several immune-inflammatory and infectious conditions. The implication of Fas-FasL pathway, for example, has been shown to be a critical mediator of keratinocytes apoptosis and
15 acantholysis in pemphigus (Lotti et al., 2018).

FasL exists in membrane-bound form (mFasL) and soluble form (sFasL). The soluble Fas ligand (sFasL) is generated and released by proteolytical cleavage of the extracellular domain of mFasL expressed at the plasma membrane (mFasL) by
20 zinc-regulated matrix metalloproteases (MMPs) and metalloproteinase, ADAM10. The soluble form (sFasL) displays both apoptotic and non-apoptotic activities.

The Fas/Fas ligand system plays a critical role in immune cell homeostasis, prevention of autoimmunity and progression of cancer. For maintaining homeostasis
25 in the immune system, the membrane-bound mFasL expressed on cytotoxic lymphocytes, binds to Fas receptor (Fas) and induces lysis of infected cells, hyperproliferative T lymphocytes as well as cancer cells via apoptosis. Cell-to-cell interactions between activated immune cells carrying membrane-bound FasL and target cells carrying membrane-bound Fas are required to maintain a homeostasis
30 in the immune system.

In addition to its role in the physiological regulation of the immune system, the Fas-FasL interaction and the signalling pathway triggered thereby have been shown to

be implicated in the pathogenesis and progression of various malignancies and immune-inflammatory and infectious conditions.

Recent studies have further demonstrated that that the Fas/Fas Ligand (FasL) system also plays a crucial role in the development of SJS/TEN based on the following evidence:

- (i) FasL levels in sera (i.e. the soluble form of FasL, sFasL) of patients with SJS/TEN are increased, particularly before the development of skin detachment and/or mucosal lesions (Viard et al, 1998; Abe et al., 2003, Chang et al., 2004, Murata et al, 2008);
 - (ii) Peripheral Blood Mononuclear Cells (PBMC) stimulated with sera of patients with SJS/TEN and/or with drug causing the SJS/TEN diseases release FasL (Abe et al. 2003);
 - (iii) sera from SJS/TEN patients induce keratinocyte cell death in culture (Abe et al. 2008); and
 - (iv) FasL is released from keratinocytes in SJS/TEN and causes cell death in neighboring keratinocytes (Abe et al. 2015).
- Therefore, it is crucial to develop inhibitors targeting FasL which do not affect the surveillance and homeostasis pathway regulated by the Fas/FasL system.

Antagonists of the Fas receptor (or FasR) are known in the art. WO 2010/102792 A2 discloses binding members directed to human Fas (or FasR) and in particular antibodies against human Fas, for use in inhibiting the Fas-mediated apoptosis and treating disease associated therewith, including Stevens-Johnson syndrome (SJS) and Toxic epidermal necrolysis (TEN). These Fas inhibitors, however, cannot distinguish between sFasL and mFasL-mediated pathways.

Furthermore, antagonists of the Fas receptor may lead to undesired effects triggered by the inhibition of the Fas/FasL pathway and its role in the physiological regulation of the immune system. This risk may include causing and/or increasing a dysfunction of immunological homeostasis which may lead to the development

and/or progression of a lymphoproliferative disorder, an autoimmunity disorder and/or cancer. Further, a dysfunction of the Fas/FasL pathway by inhibiting and/or blocking the Fas receptor may lead to compromising immune privileged sites, such as the eyes (ocular immune privilege) and the testes, placenta and nervous system.

5

Viard et al. (Science, vol. 282, no. 5388, 1998, p. 490-493) discloses the effective treatment of TEN with IVIG, which shows a Fas inhibitory activity due to the presence of naturally occurring anti-Fas antibodies contained within the IVIG preparation. IVIG is, however, an agent comprising a large multitude of antibodies with different specificities. Further, the quality of IVIG is heterogenous from batch to batch.

It is an object of the present invention to provide a therapeutic agent for preventing and treating SJS/TEN. In view of the above evidence on the crucial role of FasL in SJS/TEN, the development of a new drug which blocks FasL, and, in particular, which selectively blocks sFasL in the patient sera, would allow for prevention of keratinocyte apoptosis and subsequent cell detachment and acantholysis, thereby blocking the formation of severe skin lesions in SJS/TEN.

It is a further object of the present invention to provide an agent capable of selectively inhibiting the interaction between Fas and the soluble form of FasL (sFasL) for use in the prevention and treatment of SJS/TEN by selectively blocking the sFasL released in the sera of patients with SJS/TEN.

25 **Summary of the invention**

The present disclosure provides a monoclonal antibody or an antigen-binding fragment thereof specific for human Fas ligand protein (FasL), particularly specific for sFasL, as active agent for treating patients with Stevens-Johnson-Syndrome (SJS) and/or toxic epidermal necrolysis (TEN) (SJS/TEN). The antibody is characterised by the amino acid sequences of the variable regions (CDRs) of the heavy chain variable (VH) and at least one light chain variable (VL) regions, respectively, as defined in SEQ ID Nos 1-9 disclosed herein. The use of the antibody

is effective for the treatment of SJS/TEN due to its high binding affinity to human FasL, particularly to the soluble form of human FasL.

5 The therapeutic utility of the antibody or an antigen-binding fragment disclosed herein is based on a combination of high binding affinity and selectivity for the soluble form of human FasL. In particular, the antibody or an antigen-binding fragment thereof disclosed herein specifically targets the pathological form sFasL in the patient's sera while it does not effectively bind to the homeostatic form mFasL.

10 A particular embodiment of the present invention is directed to a monoclonal antibody or an antigen-binding fragment thereof comprising a VH region having complementary determining regions CDR H1, CDR H2 and CDR H3 as assigned in SEQ ID Nos 1, 3 and 5 and a VL region having complementary determining regions CDR L1, CDR L2 and CDR L3 as assigned in SEQ ID Nos 7-9. In a more preferred
15 embodiment of the invention the antibody has an IgG heavy chain constant region, preferably an IgG1 or IgG4 heavy chain constant region.

A further aspect of the invention relates to a nucleic acid molecule encoding a monoclonal antibody as disclosed herein or an antigen-fragment thereof for the use
20 in the treatment of SJS/TEN.

Still a further aspect of the invention is a pharmaceutical composition comprising the antibody or antigen-binding fragment or the nucleic acid molecule disclosed herein together with one or more pharmaceutical acceptable carriers for the use in the
25 treatment of SJS/TEN.

Embodiments of the invention

In the following, specific embodiments of the invention are disclosed as follows:

30

1. A monoclonal antibody or an antigen-binding fragment thereof specific for human Fas ligand protein (FasL) comprising at least one heavy chain variable (VH) region and at least one light chain variable (VL) region, wherein said antibody or an antigen-binding fragment is selected from:

(i) an antibody or an antigen-binding fragment comprising a VH region having complementary determining regions (CDRs) of the heavy chain CDR H1, CDR H2 and CDR H3 as follows:

5

(a₁) CDR H1: Arg His Gly Ile Thr (SEQ ID NO: 1) or

(a₂) CDR H1: Ser His Gly Ile Ser (SEQ ID NO: 2),

(b₁) CDR H2: Trp Ile Asn Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Val Gln Gly

10 (SEQ ID NO: 3) or

(b₂) CDR H2: Trp Ile Asn Ala Tyr Ser Gly Asn Thr Asn Tyr Ala Gln Lys Leu Gln Gly
(SEQ ID NO: 4),

(c₁) CDR H3: Glu Thr Met Val Arg Gly Val Pro Leu Asp Tyr (SEQ ID NO: 5) or

15 (c₂) CDR H3: Glu Thr Met Val Arg Gly Val Pro Cys Asp Tyr (SEQ ID NO: 6),

and complementary determining regions (CDRs) of the light chain CDR L1, CDR L2 and CDR L3 as follows:

20 (a₃) CDR L1: Arg Ala Ser Gln Ser Val Ser Ser Ser Tyr Leu Ala (SEQ ID NO: 7),

(b₃) CDR L2: Gly Ala Ser Ser Arg Ala Thr (SEQ ID NO: 8),

(c₃) CDR L3: Gln Gln Tyr Gly Ser Ser Pro Trp Thr (SEQ ID NO: 9); or

(ii) an antibody or an antigen-binding fragment competing with the antibody or
25 antigen-binding fragment of (i) in the binding to human Fas ligand protein (FasL);

for use in a method for the prevention and/or treatment of toxic epidermal necrolysis (TEN) and/or Stevens-Johnson-Syndrome (SJS).

30 2. The monoclonal antibody or an antigen-binding fragment thereof of embodiment 1 for the use of embodiment 1 comprising at least one heavy chain variable (VH) region and at least one light chain variable (VL) region, wherein said antibody or an antigen-binding fragment is selected from: an antibody or an antigen-binding

fragment comprising a VH region having complementary determining regions (CDRs) of the heavy chain CDR H1, CDR H2 and CDR H3 as follows:

(a₁) CDR H1: Arg His Gly Ile Thr (SEQ ID NO: 1),

5 (b₁) CDR H2: Trp Ile Asn Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Val Gln Gly (SEQ ID NO: 3),

(c₁) CDR H3: Glu Thr Met Val Arg Gly Val Pro Leu Asp Tyr (SEQ ID NO: 5);

10 and complementary determining regions (CDRs) of the light chain CDR L1, CDR L2 and CDR L3 as follows:

(a₃) CDR L1: Arg Ala Ser Gln Ser Val Ser Ser Ser Tyr Leu Ala (SEQ ID NO: 7),

(b₃) CDR L2: Gly Ala Ser Ser Arg Ala Thr (SEQ ID NO: 8),

(c₃) CDR L3: Gln Gln Tyr Gly Ser Ser Pro Trp Thr (SEQ ID NO: 9).

15

3. The antibody or antigen-binding fragment thereof of embodiment 1 or 2 for the use of embodiment 1, wherein the VL region of the antibody comprises the amino acid sequence:

20 EIVLTQSPGTL~~SL~~SPGERATL~~SC~~RASQSVSSSYLAWYQQKPGQAPRLLIYGASSR
ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGGTKVEIKRT
VAAPSVFIFP (SEQ ID NO: 10)

and the VH region of the antibody comprises the amino acid sequence:

25

QVQLVQSGAEVKKPGASVKVSCKASGYIFIRHGITWWRQAPGQGLEWMGWINA
YNGNTNYAQKVQGRVTMTTDKSTSTAYMELRSLRSDDAVYYCARETMVRGVP
LDYWGQGTLVTVSSASTKGPSVFPLA (SEQ ID NO: 11), or

30 QVQLVQSGAEVKKPGASVKVSCKASGYIFISHGISWWRQAPGQGLEWMGWINA
YSGNTNYAQKLQGRVTMTTDRSTSTAYMELRSLRSDDTAVYYCARETMVRGVP
CDYWGQGTLVTVSSASTKGPSVFPLA (SEQ ID NO: 12).

4. The antibody or antigen-binding fragment thereof of any one of embodiments 1-3 for the use of embodiment 1, wherein the VL region of the antibody comprises the amino acid sequence:

5 EIVLTQSPGTL~~SL~~SPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSR
ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK
(SEQ ID NO: 13),

and the VH region of the antibody comprises the amino acid sequence:

10

QVQLVQSGAEVKKPGASVKVSCKASGYIFIRHGITWWRQAPGQGLEWMGWINA
YNGNTNYAQKVQGRVTMTTDKSTSTAYMELRSLRSDDAVYYCARETMVRGVP
LDYWGQGTLVTVSS (SEQ ID NO: 14)

or

15 QVQLVQSGAEVKKPGASVKVSCKASGYIFISHGISWWRQAPGQGLEWMGWINA
YSGNTNYAQLQGRVTMTTDRSTSTAYMELRSLRSDDTAVYYCARETMVRGVP
CDYWGQGTLVTVSS (SEQ ID NO: 15)

20 5. The antibody or antigen-binding fragment thereof of any one of embodiments 1-4 for the use of embodiment 1 recognizing the same epitope on human FasL as the antibody of embodiment 1 (i) or 2 (i).

25 6. The antibody or antigen-binding fragment thereof of any one of embodiments 1-5 for the use of embodiment 1, wherein the antibody is selected from a partially or fully human antibody, a chimeric antibody and/or a humanized antibody and wherein the antigen-binding fragment thereof is selected from a Fab, Fab' and/or F(ab')₂ and/or a single chain Fv fragment.

30 7. The antibody or antigen-binding fragment thereof of any one of embodiments 1-6 for the use of embodiment 1, wherein the antibody has an IgG heavy chain constant region, preferably an IgG1 or IgG4 heavy chain constant region.

8. A nucleic acid molecule encoding a monoclonal antibody or an antigen-fragment thereof of any one of embodiments 1-7 for the use of embodiment 1.
9. A nucleic acid molecule of embodiment 8 for the use of embodiment 1, which is a DNA vector or an RNA molecule.
10. The antibody or antigen-binding fragment thereof of any one of embodiments 1-7 or the nucleic acid molecule of any one of embodiments 8-9 for the use of embodiment 1 in a monotherapy.
11. The antibody or antigen-binding fragment thereof of any one of embodiments 1-7 or the nucleic acid molecule of any one of embodiments 8-9 for the use of embodiment 1 in combination with at least one further active ingredient effective against toxic epidermal necrolysis (TEN) and/or Stevens-Johnson syndrome (SJS).
12. The antibody or antigen-binding fragment thereof of any one of embodiments 1-7 or the nucleic acid molecule of any one of embodiments 8-9 for the use of embodiment 11, wherein the further active ingredient is selected from at least one of steroids, cyclosporine, IVIg, TNF inhibitors and/or plasmapheresis.
13. The antibody or antigen-binding fragment thereof of any one of embodiments 1-7 or the nucleic acid molecule of any one of embodiments 8-9 for the use of embodiment 1 in human therapy.
14. A pharmaceutical composition comprising the antibody or antigen-binding fragment of any one of embodiments 1-7 or the nucleic acid molecule of any one of embodiments 8-9 together with one or more pharmaceutical acceptable carriers for the use of embodiment 1.
15. The antibody or antigen-binding fragment thereof of any one of embodiments 1-7 or the nucleic acid molecule of any one of embodiments 8-9 or the pharmaceutical composition of embodiment 14, which is administered systemically and/or locally.

Detailed description

The present invention refers to the treatment of SJS/TEN diseases, associated with keratinocyte apoptosis by administering a FasL antagonist. In a preferred embodiment, the invention refers to the treatment of SJS/TEN diseases in a human subject who additionally suffers from a dysfunction of immunological homeostasis and/or has an increased risk of developing a dysfunction of immunological homeostasis.

10 The term “immunological homeostasis” particularly refers to an adequate and/or balanced functioning of the immune system of the subject to be treated. The term “dysfunction” particularly refers to a disturbance of the immune system, e.g., of the functionality of immune cells carrying membrane-bound FasL, and more particularly to the capability of those immune cells to interact with target cells carrying a Fas
15 receptor.

In general, FasL antagonists may be selected from anti FasL antibodies or an antigen-binding fragment thereof, particularly humanized or human anti FasL antibodies or an antigen-binding fragment thereof, nucleic acid effector molecules
20 of Fas expression such as antisense molecules or molecules capable of RNA interference such as siRNA molecules, soluble Fas receptor molecules, antagonistic FasL muteins, and low molecular weight chemical compounds inhibiting the Fas-FasL interaction. FasL antagonists prevent keratinocyte apoptosis and subsequent cell-cell detachment (acantholysis).

25

In a very preferred embodiment, the anti FasL antibody may be selected from an antibody or an antigen-binding fragment which shows a selective high affinity binding to the soluble form of FasL, while it does not substantially inhibit the binding of mFasL to Fas. Selective binding to sFasL allows the targeted inhibition of sFasL
30 without substantially inhibiting mFasL. Thus, interactions of mFasL with Fas receptor are not substantially affected, thereby avoiding undesired effects on immunological homeostasis. In certain embodiments, the undesired effects on

immunological homeostasis include an inhibition of the function of activated immune cells carrying mFasL.

The present invention thus relates to the use of at least one anti-FasL compound
5 able to inhibit the biological effects of FasL, particularly of sFasL. The expression
“*inhibiting the biological effects of FasL*” or the expression “*inhibiting the biological
effects of sFasL*” used herein relates to compounds which can fully or at least
substantially inhibit or neutralize the biological effects of FasL or sFasL and in
particular of sFasL. For example, the inhibitory or neutralizing effect may be based
10 on suppressing the binding of FasL, particularly sFasL, to its natural receptor,
thereby suppressing the caused signal transmissions. This can be achieved e.g., by
using antibodies binding to FasL/sFasL per se or soluble receptors mimicking
Fas/sFasL or antagonistic FasL/sFasL muteins, thus blocking the binding of
FasL/sFasL to its cellular receptors. Alternatively, interfering with Fas or FasL/sFasL
15 expression by siRNA will block Fas/FasL system likewise.

One aspect of the present invention relates to the use of anti-FasL antibodies or an
active fragment thereof as therapeutic effective agent in the prevention and/or
treatment of SJS/TEN. In a preferred embodiment, the present invention relates to
20 the use of anti-sFasL antibodies or an active fragment thereof as therapeutic
effective agent in the prevention and/or treatment of SJS/TEN.

In the context of the present invention, the term “prevention of SJS/TEN” means the
treatment of the diseases at diagnosis, e.g. in an early stage in order to prevent the
25 progression of the diseases to a more severe spectrum of severity. The term
“treatment of SJS/TEN” means that the SJS/TEN diseases is managed to lessen
and/or ameliorate the symptoms of the disease, preferably to the point of curing the
disease.

30 The development of an active agent, in particular an antibody, which specifically
blocks sFasL allows a targeted therapeutic approach of SJS and TEN. Moreover,
this therapeutic approach minimizes the risks of triggering or enhancing a
dysfunction of immunological homeostasis associated with the Fas/mFasL system.

The antibodies are preferably chimeric, humanized or human anti-FasL antibodies, preferably anti-sFasL antibodies. Further, the antibodies may be monovalent or multivalent and may comprise modifications such as different glycosylation pattern, or modification of the Fc region to alteration of the antibody dependent cellular cytotoxicity (ADCC) and the complement-dependent cytotoxicity (CDC). The antigen-binding fragment or derivative of the anti-FasL antibody, preferably anti-sFasL antibodies of the invention may be a recombinant single chain antibody or single chain variable fragment. In the very preferred embodiment of the invention, the antibodies are human FasL-antibodies, more preferably human anti-sFasL antibodies. If desired, the antibodies may be conjugated to effector molecules, e.g., cytostatic, cytotoxic and/or radioactive compounds.

In a first aspect, the present invention is directed to a monoclonal antibody or an antigen-binding fragment thereof specific for human Fas ligand protein (FasL), preferably specific for the soluble form of human Fas ligand protein (sFasL) comprising at least one heavy chain variable (VH) region and at least one light chain variable (VL) region, wherein the antibody or the antigen-binding fragment comprises a VH region having complementary determining regions (CDRs) of the heavy chain CDR H1, CDR H2 and CDR H3 as follows:

- (a₁) CDR H1: Arg His Gly Ile Thr (SEQ ID NO: 1) or
- (a₂) CDR H1: Ser His Gly Ile Ser (SEQ ID NO: 2),
- (b₁) CDR H2: Trp Ile Asn Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Val Gln Gly (SEQ ID NO: 3) or
- (b₂) CDR H2: Trp Ile Asn Ala Tyr Ser Gly Asn Thr Asn Tyr Ala Gln Lys Leu Gln Gly (SEQ ID NO: 4), and
- (c₁) CDR H3: Glu Thr Met Val Arg Gly Val Pro Leu Asp Tyr (SEQ ID NO: 5) or
- (c₂) CDR H3: Glu Thr Met Val Arg Gly Val Pro Cys Asp Tyr (SEQ ID NO: 6),

and complementary determining regions (CDRs) of the light chain CDR L1, CDR L2 and CDR L3 as follows:

- (a₃) CDR L1: Arg Ala Ser Gln Ser Val Ser Ser Ser Tyr Leu Ala (SEQ ID NO: 7),
5 (b₃) CDR L2: Gly Ala Ser Ser Arg Ala Thr (SEQ ID NO: 8), and
(c₃) CDR L3: Gln Gln Tyr Gly Ser Ser Pro Trp Thr (SEQ ID NO: 9);

for use in a method for the prevention and/or treatment of toxic epidermal necrolysis (TEN) and/or Stevens-Johnson syndrome (SJS).

10

In a preferred embodiment the invention relates to a monoclonal antibody or an antigen-binding fragment thereof specific for human Fas ligand protein (FasL) comprising at least one heavy chain variable (VH) region having complementary determining regions (CDRs) of the heavy chain CDR H1, CDR H2 and CDR H3 as
15 follows:

- (a₁) CDR H1: Arg His Gly Ile Thr (SEQ ID NO: 1),
(b₁) CDR H2: Trp Ile Asn Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Val Gln Gly (SEQ ID NO: 3)
20 (c₁) CDR H3: Glu Thr Met Val Arg Gly Val Pro Leu Asp Tyr (SEQ ID NO: 5),

and at least one light chain variable (VL) region having complementary determining regions (CDRs) of the light chain CDR L1, CDR L2 and CDR L3 as follows:

- 25 (a₃) CDR L1: Arg Ala Ser Gln Ser Val Ser Ser Ser Tyr Leu Ala (SEQ ID NO: 7),
(b₃) CDR L2: Gly Ala Ser Ser Arg Ala Thr (SEQ ID NO: 8), and
(c₃) CDR L3: Gln Gln Tyr Gly Ser Ser Pro Trp Thr (SEQ ID NO: 9);

for use in a method for the prevention and/or treatment of toxic epidermal necrolysis
30 (TEN) and/or Stevens-Johnson syndrome (SJS).

Further, the invention relates to a monoclonal antibody or an antigen-binding fragment which competes with the monoclonal antibodies or antigen-binding

fragment disclosed above for the binding to human Fas ligand protein (FasL), particularly for selectively inhibiting the soluble form of human Fas ligand protein (sFasL). Preferably, the antibody competes with the monoclonal antibody having heavy chain CDR sequences H1- H3 comprising the amino acid sequences of SEQ ID Nos 1-5 and light chain CDR sequences L1-L3 comprising the amino acid sequences of SEQ ID Nos 7-9 as described above. In a very preferred embodiment, the antibody competes with the monoclonal antibody having heavy chain CDR sequences H1-H3 of SEQ ID Nos 1, 3 and 5 and light chain CDR L1-L3 of SEQ ID Nos 7-9 as described above.

10

In certain embodiments, a competing antibody of the present invention binds the same or an overlapping epitope on the human FasL, particularly the human sFasL, as the monoclonal antibody having CDRs as defined in SEQ ID Nos 1-9. Competition may be determined by standard assays in the art that can quantify binding affinity, relative and absolute, of the binding proteins, particularly antibody to human FasL with respect to the given reference antibody.

15

A determination of quantitative binding of FasL antibody to FasL protein can be conducted by one of various surface plasmon resonance (SPR) measuring platforms. Examples include Biacore and Forte Octet. Recombinant target protein, FasL (e.g., Acro Bio FAL-H5241) is tethered to a capture chip and FasL antibody is flowed over the chip while changes in molecular interactions are recorded in real time. On-rates (k_a ; $M^{-1} s^{-1}$), off-rates (k_d ; s^{-1}) can be calculated by adding and removing FasL from the flow. A measure of binding affinity (K_D , μM) can be determined by dividing the off-rate by the on-rate. K_D values less than 10 μM are typically required for therapeutic use. K_D values of 10 nM to 1 pM are optimally used.

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In particular embodiments of the present invention, at least one amino acid of the above CDR1, CDR2 or CDR3 amino acid sequence of the VH and/or VL chain is replaced by another amino acid, while preserving structural integrity and epitope-binding of the antibody. These exchanges can be conservative (i.e., by a similar amino acid) or non-conservative.

30

In particular, at least one amino acid of the VH and VL CDR1, CDR2 or CDR3 sequences, may be replaced by a conservative amino acid substitution, i.e., a substitution of an amino acid by another amino acid with similar biochemical properties, for example a substitution of an aliphatic amino acid, e.g., Gly, Ala, Val, Leu, or Ile, for another aliphatic amino acid; a substitution of a basic amino acid, e.g. His, Lys or Arg, against another basic amino acid or against Met; a substitution of an acidic amino acid or an amide thereof, e.g., Asp, Glu, Asn or Gln, against another acidic amino acid or an amide thereof; a substitution of an aromatic amino acid, e.g., Phe, Tyr or Trp, against another aromatic amino acid. In certain preferred embodiments of the invention, 1, 2, 3, 4, or 5 amino acids of SEQ. ID NO: 1-9 are replaced by another conservative or non-conservative amino acid.

In a preferred embodiment, the present invention is directed to the use of anti-FasL human antibodies, or antigen-binding portions thereof, comprising a light chain variable region and/or a heavy chain variable region as described in US 7,262,277 (SEQ ID NO 2 and SEQ ID NO 10 or 18, respectively of US 7,262,277), the content of which is herein incorporated by reference. It has been surprisingly found that these antibodies disclosed in US 7,262,277 have a high target affinity and specificity for the soluble form of FasL, whereas they fail to bind or do not substantially bind to the membrane-bound form of FasL (mFasL).

This finding provides novel therapeutic uses for these antibodies in a more targeted treatment of SJS and/or TEN. In a preferred embodiment, the antibodies are particularly useful for administration to a subject affected by SJS/TEN who additionally suffers from a dysfunction of immunological homeostasis and/or has an increased risk of developing a dysfunction of immunological homeostasis. In even more particular embodiments, the present disclosure provides uses for the antibodies in subjects affected by SJS/TEN who additionally suffer from an immune system-related dysfunction or having an increased risk of developing an immune system-related dysfunction, e.g., an immuno-inflammatory disorder, an autoimmune disorder and/or cancer.

Accordingly, in a still further preferred embodiment of the invention the anti-FasL antibody, particularly the anti-sFasL antibody comprises a light chain variable region (VL) comprising a polypeptide with the amino acid sequence as follows:

5 EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSR
ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIKRT
VAAPSVFIFP (SEQ ID NO: 10),

and a heavy chain variable region (VH) comprising a polypeptide with the amino
10 acid sequence as follows:

QVQLVQSGAEVKKPGASVKVSCKASGYIFIRHGITWWRQAPGQGLEWMGWINA
YNGNTNYAQKVQGRVTMTTDKSTSTAYMELRSLRSDDAAVYYCARETMVRGVP
LDYWGQGTLVTVSSASTKGPSVFPLA (SEQ ID NO: 11)

15 or

QVQLVQSGAEVKKPGASVKVSCKASGYIFISHGISWWRQAPGQGLEWMGWINA
YSGNTNYAQKLQGRVTMTTDRSTSTAYMELRSLRSDDTAVYYCARETMVRGVP
CDYWGQGTLVTVSSASTKGPSVFPLA (SEQ ID NO: 12).

20 for use in the prevention and/or treatment of toxic epidermal necrolysis (TEN) and/or
Stevens-Johnson syndrome (SJS).

In a further preferred embodiment, the present disclosure relates to an anti-FasL
antibody, particularly the anti-sFasL antibody or antibody-binding fragment thereof,
25 wherein the light chain variable region (VL) of the antibody or antibody-binding
fragment thereof comprises a polypeptide with the amino acid sequence as follows:

EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSR
ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK
30 (SEQ ID NO: 13),

and wherein the heavy chain variable region (VH) of the antibody or antibody-binding fragment thereof comprises a polypeptide with the amino acid sequence as follows:

5 QVQLVQSGAEVKKPGASVKVSCKASGYIFIRHGITWVRQAPGQGLEWMGWINA
YNGNTNYAQKVQGRVTMTTDKSTSTAYMELRSLRSDDAVYYCARETMVRGVP
LDYWGQGTLVTVSS (SEQ ID NO: 14)

or

10 QVQLVQSGAEVKKPGASVKVSCKASGYIFISHGISWVRQAPGQGLEWMGWINA
YSGNTNYAQKLQGRVTMTTDRSTSTAYMELRSLRSDDTAVYYCARETMVRGVP
CDYWGQGTLVTVSS (SEQ ID NO: 15)

for use in the prevention and/or treatment of toxic epidermal necrolysis (TEN) and/or Stevens-Johnson syndrome (SJS).

15

The amino acid sequences SEQ ID NOs: 13, 14, and 15 differ from the amino acid sequences SEQ ID NOs: 10, 11 and 12 only in that the last 12 amino acids of the latter are no longer assigned to the VH or VL regions but to the respective constant regions.

20

In a particularly preferred embodiment, the anti-FasL antibody, particularly the anti-sFasL antibody of the disclosure comprises a light chain variable region (VL) comprising a polypeptide with the amino acid sequence as set forth in SEQ ID NO: 10 or in SEQ ID NO: 13 described above and a heavy chain variable region (VH) comprising a polypeptide with the amino acid sequence as set forth in SEQ ID NO: 25 11 or in SEQ ID NO: 14 described above.

In a preferred embodiment the anti-FasL antibody, particularly the anti-sFasL antibody, or an antigen-binding fragment thereof as described above can comprise 30 an VL amino acid sequence having an identity to SEQ ID NO: 10 or to SEQ ID NO: 13 of at least 85%, at least 90%, at least 95% or at least 99% and an VH amino acid sequence having an identity to SEQ ID NO: 11 or 12 or to SEQ ID NO: 14 or 15,

respectively, of at least 85%, at least 90%, at least 95% or at least 99% over the whole length of the protein, whereby the high binding affinity and selectivity to FasL and particularly to sFasL is maintained.

- 5 "Percent (%) amino acid sequence identity" with respect to a peptide or polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for
10 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.

The antibody according to the present invention for the use in the prevention and/or
15 treatment of SJS/TEN may be selected from a partially or fully human antibody, a chimeric antibody and/or a humanized antibody. Preferably, the antibody is a human antibody. Further, the antibody according to the present invention may be monospecific and/or bispecific. Alternatively, the antibody may be multivalent and multispecific. Further, the antigen-binding fragment of the antibody according to the
20 present invention may be selected from a Fab, Fab' and/or F(ab')₂ and/or a single chain Fv fragment.

The antibody may be of any suitable class. The term "class" refers to the type of constant domain or constant region possessed by its heavy chain. As used herein,
25 "constant domain" or "constant region" denotes the sum of the domains of an antibody other than the variable region. The constant region is not directly involved in binding of an antigen but exhibits various effector functions. The antibody of the present disclosure may be of any of the five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, or any subclass thereof (isotype), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different
30 classes of immunoglobulins are called α , δ , ϵ , γ and μ , respectively. According to the present disclosure, an antibody of the class IgG, IgA or IgM or a fragment thereof is particularly suitable.

In a very preferred embodiment of the invention, the antibody has an IgG heavy chain constant region, preferably an IgG1 or IgG4 heavy chain constant region. In a further preferred embodiment of the invention, the antibody has a kappa (κ) light chain. In a most preferred embodiment of the invention, the antibody has an IgG4 heavy chain constant region.

Thus, the most preferred antibody of the invention is a monoclonal IgG4 anti-FasL antibody and preferably a monoclonal IgG4 anti-sFasL antibody or an antigen-binding fragment thereof comprising a VH region having complementary determining regions CDR H1, CDR H2 and CDR H3 as assigned in SEQ ID Nos 1, 3 and 5 described above and a VL region having complementary determining regions CDR L1, CDR L2 and CDR L3 as assigned in SEQ ID Nos 7-9 described above. In a more preferred embodiment of the invention the antibody comprises a light chain variable region (VL) comprising a polypeptide with the sequence as assigned in SEQ ID NO: 10 or in SEQ ID NO: 13 described above and a heavy chain variable region (VH) comprising a polypeptide with the sequence as assigned in SEQ ID NO: 11 or in SEQ ID NOs: 14 described above.

In a more preferred embodiment of the invention the anti-FasL antibody, preferably the anti-sFasL antibody described above comprises an IgG4 heavy chain constant region comprising a light chain constant region (LC) having a polypeptide with the amino acid sequence as follows:

PSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD
SKDSTYSLSS TLTLKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC
(SEQ ID NO: 16),

or comprising a light chain constant region (LC) having a polypeptide with the amino acid sequence as follows

RTVAAPSVFI FPPSDEQLKS GTASVCLLN NFYPREAKVQ WKVDNALQSG
NSQESVTEQD SKDSTYSLSS TLTLKADYE KHKVYACEVT HQGLSSPVTK
SFNRGEC (SEQ ID NO: 18)

- 5 and comprising a heavy chain constant region (HC) comprising a polypeptide with the amino acid sequence as follows:

PCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS
GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPPCP
10 APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD
GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS
SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE
ALHNHYTQKS LSLSLGK (SEQ ID NO: 17),

15

or comprising a heavy chain constant region (HC) comprising a polypeptide with the amino acid sequence as follows

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
20 HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES
KYGPPCPPCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED
PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK
GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSRL TVDKSRWQEG
25 NVFSCSVMHE ALHNHYTQKS LSLSLGK (SEQ ID NO: 19).

In a still further embodiment, the invention relates to the use of the anti-hFas Ligand human antibody, or an antigen binding fragment thereof, produced by the hybridoma cell with accession number ATCC PTA-4017 and/or to the use of the anti-hFas
30 Ligand human antibody, or an antigen binding fragment thereof, produced by the hybridoma cell with accession number ATCC PTA-4018 as described in US 7,262,277 as antibodies 3E1 and 4G11, respectively for the prevention and/or treatment of SJS/TEN. In the most preferred embodiment of the invention is directed

to the use of the anti-hFas Ligand human antibody 3E1 (produced by the hybridoma cell with accession number ATCC PTA-4017), or an antigen binding fragment thereof for the prevention and/or treatment of SJS/TEN.

- 5 The hybridoma cell under Accession No. ATCC PTA-4017 and ATCC PTA-4018 have been deposited on 29 January 2002 with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 (USA).

10 In a finally further preferred aspect, the present invention refers to the use of a monoclonal antibody or an antigen-binding fragment thereof recognizing the same epitope of human FasL, preferably the same epitope of human sFasL as the antibodies described above.

The antibody or the antigen-binding fragment thereof of the present disclosure
15 specifically binds with high affinity to the soluble form of human FasL (sFasL). Further, the antibody or the antigen-binding fragment disclosed herein does not effectively bind to the membrane-bound form of FasL. The binding characteristics of the antibody allow selective targeting of sFasL without undesired side-effects resulting from binding to mFasL. In particular, administration of an antibody of the
20 present invention may lead to a reduced risk of triggering physiological and/or pathological processes mediated and/or triggered by inhibition of the mFasL pathway.

As used herein, the terms "binding" and "specific binding" refer preferably to the
25 selective binding of the antibody of the invention or fragment thereof to human sFasL. The measure of the binding strength of an antibody is referred to as affinity. Methods for determining such a binding and/or affinity using in vitro assays are known to the person skilled in the art. According to the present disclosure, detection with flow cytometry, immuno-histochemistry and/or fluorescence are described and
30 in particular preferred herein.

A still further preferred embodiment of the invention refers to a nucleic acid molecule encoding a monoclonal antibody or an antigen-fragment thereof as disclosed above

for the use in the prevention and/or treatment of SJS/TEN. The nucleic acid molecule may be a DNA vector or an RNA molecule typically formulated as a lipid nanoparticle encapsulation. The nucleic acid molecule encoding the antibody of the invention when injected into a patient, is incorporated into the cells, which express
5 the antibody into circulation. The use of DNA and RNA delivery technology in antibody therapy and, in particular, the use of mRNA encoded therapeutic antibodies, is a well-known therapeutic approach in the art (e.g., Van Hoecke and Roose, J Trans Med (2019) 17:54 and Deal et al., Vaccines (2021), 9, 2018).

10 In therapeutic applications, the anti-FasL antibodies of the invention, or antibody fragments thereof, are administered in an effective amount to a subject in need thereof, particularly to a human subject. The dose will depend on the specific type of antibody, the severity and stage of disease, and the route of administration.

15 In a preferred embodiment of the invention, the subjects affected by SJS/TEN in need of the anti-FasL antibodies of the invention are subjects which have a FasL concentration value in the serum elevated compared to a healthy subject.

Typically, the anti-FasL antibodies of the invention, or antibody fragments thereof,
20 are administered as a pharmaceutical composition comprising the active agent and a pharmaceutically acceptable carrier or excipient. Examples of suitable carriers and excipients for formulating antibodies or antibody fragments are well-known in the art. An effective dose of a medicament of the present invention may be in the range of 0.1 µg to 100 mg, up to a total dose of about 1 g depending upon the route of
25 administration.

Thus, a further aspect of the present invention is a pharmaceutical composition comprising an anti-FasL antibody or antigen-binding fragment thereof as described above together with one or more pharmaceutical acceptable carriers for use in a
30 method for the prevention and/or treatment of toxic epidermal necrolysis (TEN) and/or Stevens-Johnson syndrome (SJS). More preferably, the pharmaceutical composition comprises an anti-sFasL antibody or antigen-binding fragment thereof as described above together with one or more pharmaceutical acceptable carriers

for use in a method for the prevention and/or treatment of toxic epidermal necrolysis (TEN) and/or Stevens-Johnson syndrome (SJS)

Depending on the stage and the severity of the disorder, the pharmaceutical composition may be administered once or several times during the course of the disorder. For example, it may be administered once or several times daily, each second day, two times weekly or weekly for a suitable period of time. The pharmaceutical composition may be administered in a single treatment cycle consisting of one or several administrations or in several treatment cycles each consisting of one or several administrations. Each treatment cycle may have a duration of one day up to several weeks, months, or even years.

In certain embodiments, the pharmaceutical composition is administered parenterally, e.g., by subcutaneous, intramuscular or intravenous injection or by infusion. In further embodiments, the pharmaceutical composition is administered locally, e.g., topically, orally, nasally or intrapulmonary, for example by inhalation as an aerosol. Preferably, the composition is administered systemically.

The anti-FasL antibodies, preferably anti-sFasL antibodies or antibody fragments thereof may be used in a monotherapy or in a combination therapy. Thus, the anti-FasL antibodies or antibody fragments thereof may be administered alone or together with at least one further active agent effective in the treatment of SJS and/or TEN. In particular, the anti-FasL antibodies of the present invention may be used in combination with other drugs particularly selected from steroids, cyclosporine, IVIg, TNF-inhibitors or plasmapheresis.

Further, the present invention is explained in more detail by the following Figures and Examples.

Figure Legends

Figure 1: FasL concentration in SJS/TEN serum measured by ELISA. S01 to S08 are all serum from SJS/TEN patients. The dotted line is the normal value; 124 pg/ml or less.

- 5 Figure 2: HaCaT cell viability after treatment with serum. H0A is healthy serum and S0A-S0B are SJS/TEN serum. Serum concentrations were set at 1, 5, and 10%.

Figure 3 and Figure 3a: Cell death inhibitory effect by the PC111 antibody, including statistical analysis. The PC111 antibody showed a cell death inhibitory effect in a concentration-dependent manner. NS = no serum; PC111 antibody dose is expressed in $\mu\text{g/mL}$; the "PC111 0" dose is SJS/TEN serum only.

10

Figure 4 and Figure 4a: Cell death inhibitory effect by zVAD, including statistical analysis (Z-VAD-FMK, pan-caspase inhibitor), μM . NS = no serum. The "zVAD 0" dose is SJS/TEN serum only.

15

Figure 5 and Figure 5a: No induction of HaCaT cell death with serum of healthy patients, including statistical analysis.

- 20 Figure 6a: Summary of the experimental design and schedules of the in vivo studies. An acetaminophen-induced SJS/TEN mouse model was constructed using PBMCs from one patient and the causative drug, acetaminophen. Effects of the PC111 antibody were compared by dividing groups into whether or not PC111 was injected.

- 25 Figure 6b: Schematic representation of the experimental design of the in vivo studies. An acetaminophen-induced SJS/TEN mouse model was constructed using PBMCs (intravenous, i.v.) from one patient and the causative drug, acetaminophen, orally administered (p.o.) once a day for 14 days (vehicle group, n=6 animals). A further group of animals were treated as above, but they also received PC111 intravenously (i.v.) every 2 days (PC111 group, n=6 animals). Body weight, eye and skin aspects/changes were analyzed daily.
- 30

Figure 7a: Ocular manifestation at D14. The degree of hyperemia and edema of the ocular conjunctiva was evaluated and scored in a 0-3 scale. N/A = not available.

Figure 7b: Example of ocular manifestation at D14. The degree of hyperemia and edema of the ocular conjunctiva was evaluated and scored in a 0-3 scale. A third group of normal untreated age-matched animals was used as negative control (n=4 animals).

Figure 8: Histopathological findings for each of the tested mice groups. Scale bar, 200 μ m (upper pictures), 50 μ m (lower pictures).

Figure 9: TUNEL staining for counting dead epithelial cells. The TUNEL assay detected numerous dead epithelial cells in Vehicle group compared with PC111 treated group and control (Normal). Scale bar, 200 μ m (upper pictures), 50 μ m (lower pictures).

Figure 10: Ratio of positive cells for TUNEL staining to the total conjunctival cell count. The ratio of dead cells was significantly decreased in the PC111 treated group and in the non-treated control group. Significant differences were evaluated by Ordinary one-way ANOVA, followed by Tukey's multiple comparisons test (**, p<0.001; ****, p<0.0001). Analysis of the mean differences by Dunnett's multiple comparisons test.

Figure 11: Body weight analysis overtime. (A) Body weight overtime for the 3 animal groups. (B) Body weight variations over the baseline (day 1) during time. (C) Area Under the Curve (AUC) analysis on body weight curves, significant differences were evaluated by Ordinary one-way ANOVA, followed by Tukey's multiple comparisons test (**, p<0.01). (D) Analysis of the AUC mean differences by Tukey's multiple comparisons test.

Figure 12: Binding curve of FasL (sFasL) titrated against a constant concentration of PC111 at 15nM (A), the data points involved in higher-order oligomers are marked in orange. Binding isotherm of the curve in panel A with the 3 points marked in orange are removed to allow the fitting (B).

Figure 13: Membrane Proteome Array overview.

Figure 14: Membrane Proteome Array (MPA): Optimization of ligand concentrations for screening. Serial dilutions of each test ligand were assayed by incubation with known targets, and target binding was measured by flow cytometry. The optimal ligand concentration for screening was chosen based on a joint assessment of binding strength and background signal (left) and rate of high background events (right).

Figure 15: MPA screen results. Each ligand was tested for binding by flow cytometry against the MPA at the previously determined optimal concentration. Binding interactions confirmed in downstream validation studies are displayed and any proteins that did not pass validation were removed.

Examples

In order to evaluate the therapeutic effect of FasL antibody on SJS/TEN *in vitro* and *in vivo* experiments have been performed.

An anti-FasL human IgG4, kappa, monospecific bivalent antibody comprising a heavy chain variable (VH) region with complementary determining regions CDR H1, CDR H2 and CDR H3 according to SEQ ID Nos 1, 3 and 5 and a light chain variable (VL) region with complementary determining regions CDR L1, CDR L2 and CDR L3 according to SEQ IDs 7-9 was used. This antibody is internally and hereafter named “antibody PC111”.

1. In vitro Studies

1.1 Materials and Methods

1.1.1 Cell culture

HaCaT cells, a spontaneously immortalised adult keratinocyte cell line, were purchased from COSMO BIO (Tokyo, Japan) and cultured in CnT-PR (CELLnTEC, Stauffacherstrasse, Switzerland), in an incubator at 37 °C under 5% CO₂.

5 1.1.2 Generation of SJS/TEN model cell

To generate SJS/TEN model cell, HaCaT cells cultured in CnT-PR were treated with 1%, 5% and 10 % SJS/TEN serum for 24 hours. The PC111 antibody was added at the same time as serum. SJS/TEN patient sera were obtained from Niigata
10 University Hospital.

1.1.3 Cell toxicity assay

Cell viability or toxicity were evaluated using Live/Dead Cell Staining Kit II
15 (PromoCell, Sickingenstr, Germany). HaCaT cells were treated in 96-well plates then incubated at 37 °C in a 5% CO₂ incubator. Images were acquired using a Keyence BZ-X710 all-in-one fluorescence microscope (Keyence, Osaka, Japan). The number of live cell and dead cell were counted using ImageJ. Cell toxicity was calculated as following: number of dead cell/(number of dead cell and live cell).

20

FasL levels in SJS/TEN and healthy sera were measured with an enzyme-linked immunosorbent assay (ELISA) kit (R&D systems, Minneapolis, MN).

1.2 Results

25

1.2.1 FasL levels were high in SJS/TEN patient sera

Before performing the *in vitro* assays using serum samples from the patients with SJS/TEN, we measured FasL level in SJS/TEN serum by an enzyme-linked
30 immunosorbent assay (ELISA). FasL level was elevated in 4 out of 8 SJS/TEN serum (normal value: 124 pg/ml or less) (Figure 1). This experiment was performed only once.

1.2.2 Anti-FasL antibody suppress cell death from SJS/TEN sera

SJS/TEN patient serum and serum from a normal healthy subject were tested at 1%, 5% and 10% concentration for 24 hours to observe loss of cell viability.

5

We confirmed that SJS/TEN serum can induce cell death in HaCaT cells. Compared with healthy serum, SJS/TEN serum significantly induced cell death, even at low concentration of SJS/TEN serum (1%) (S0A 1%; $p=0.0027$, S0B 1%; $p=0.0024$, respectively.) (Figure 2). Viability decreased from 80-90% with normal serum to 50-
10 60% with patient serum. It was found that even a serum concentration of 1% can sufficiently induce cell death.

We then confirmed the efficacy of the PC111 antibody using serum S08 (as analysed in Figure 1), which had the highest FasL concentration, i.e. in the range
15 from 90 to 200 pg/mL (cf. Figure 1). The serum with the highest level of FasL was then used for a PC111 titration experiment. As a result, it was found that SJS/TEN serum S08 induced HaCaT cell death, and that the PC111 antibody suppressed the SJS/TEN serum S08-induced cell death in a dose-dependent manner (Figures 3 and 3a). Cell death was significantly suppressed when the concentration of the
20 PC111 antibody was 10-100 $\mu\text{g/ml}$ (PC111 10 $\mu\text{g/ml}$; $p=0.0025$, 100 $\mu\text{g/ml}$; $p=0.0159$, respectively). This experiment was repeated 3 times with similar results (see statistical analysis in Figure 3a).

In addition, we conducted an experiment using zVAD, a pan caspase inhibitor, as a
25 pathway comparison control for the PC111 antibody, and confirmed that it has a significant effect of suppressing apoptosis (zVAD 50 μM ; $p=0.0025$, 100 μM ; $p=1.57 \times 10^{-5}$) (Figures 4 and 4a). Healthy serum did not induce HaCaT cell death (Figures 5 and 5a). This experiment was repeated 3 times with same results (see statistical analysis in Figure 4a and Figure 5a).

30

Since cell death is suppressed by zVAD, it is thought that apoptosis, which is caspase-dependent cell death, has been mainly observed in this experimental system using serum of patients with SJS/TEN. Furthermore, the experimental

results suggest that the PC111 antibody has a therapeutic effect on SJS/TEN through suppressing apoptosis of keratinocytes due to Fas-FasL interaction.

In sum, the *in vitro* studies showed that SJS/TEN serum can cause cell death in HaCaT cells *in vitro* and that the PC111 antibody can dose dependently suppress apoptosis of HaCaT cells.

2. In vivo Studies

A validated mouse model of SJS/TEN that uses transfer of PBMCs from SJS/TEN patients (Saito et al. 2013) was used for evaluating the *in vivo* efficacy of the anti-FasL antibody PC111.

As reported in the following, the *in vivo* study results confirmed that the PC111 antibody according to the invention is an effective therapeutic agent for use in the treatment of SJS/TEN.

2.1 Methods

2.1.1 Mice

Immunocompromised NOD/Shi-scid, IL-2R γ null (NOG) mice at 6 weeks of age were purchased from In-Vivo Science Inc. (Tokyo, Japan). Mice were included in the experiments with n=6 in the PC111 treatment and vehicle groups and n=4 in the control or normal group. All the animal experiments were performed under the approval of the ethics committee for animal studies of Niigata University.

2.1.2 SJS/TEN mouse model using patients' PBMCs

The experimental design and schedules are summarised in Fig. 6a and Fig. 6b.

Peripheral Blood Mononuclear Cells (PBMCs) were obtained from a patient who had recovered from SJS/TEN. The patients received no systemic glucocorticoids at the

time of PBMC collection. PBMCs (2×10^6) were injected intravenously into the NOD-scid IL2rgamma(null) (NOG) mice, followed by oral administration of the causative drugs (acetaminophen, 1.5 mg/100 μ l).

- 5 The dosage used in the model was based on milligrams per kilogram of body weight, converted from the adult human normal dose. The drug was administered to the mice once daily. In addition, it was confirmed that the dosage was under the median lethal dose in mice. Drug dosage was estimated by dose conversion by body weight. Thus, PC111 antibody (100 μ g/100 μ l) or PBS (100 μ l) as control was administered
10 intravenously every second day from day 1 (D1, 100 μ l). Mice were observed for 14 days. Any changes of the skin, eyes, and mucosa, such as skin colour or mucous haemorrhage was checked daily. Body weight was daily registered. Endpoints were collected at day 14 (D14). General anaesthesia was carried out on day 14, and the ocular conjunctiva was dislocated and evaluated in detail for findings of hyperemia and edema. The mice were then sacrificed, and the eyeballs were collected as
15 specimens. Ocular lesions were investigated by means of histopathologic examination and immunohistochemical staining.

2.1.3 Immunohistochemistry

- 20 Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) is a method for detecting apoptotic cells with DNA fragmentation by labeling the terminal end of nucleic acids.

- 25 The TUNEL assay was performed according to the manufacturer's protocol (Takara Bio, Shiga, Japan). The ocular conjunctiva was observed macroscopically and the number of dead cells was macroscopically counted for all animals per group. Significant differences in the ratio of dead cells among groups were analysed by ordinary one-way ANOVA, followed by Tukey's multiple comparisons test. $p < 0.05$
30 was defined as a significant difference.

2.2 Results

Daily observations of skin manifestation were made throughout the experiment. No remarkable changes of skin were observed until D14. One mouse in the vehicle group died at day 12 (D12) of unknown cause.

- 5 The ocular conjunctiva was observed in detail by dislocating eyeballs before mice sacrifice on D14 (Figures 7a and 7b). In the control vehicle group (n=2), hyperemia of the ocular conjunctiva was noticeable (score of 2, in a 0-3 scale) and mild edema (score of 1, in a 0-3 scale) was also observed. In the PC111-treated group (n=3), only one mouse showed mild conjunctival hyperemia (score of 1, in a 0-3 scale),
10 without edema, while the other two PC111-treated mice showed neither hyperemia nor edema.

In a further step, the recovered eyeballs were evaluated histologically (Figure 8). Hematoxylin-eosin staining showed mild edema of the ocular conjunctiva in both
15 PBMCs treated groups. Dyskeratosis of epithelial cells, such as those seen in patients with SJS/TEN, were present in the vehicle group. Therefore, TUNEL staining was performed to objectively assess the number of dead cells (Figure 9).

The results showed a large number of dead cells in the epithelial cells in the vehicle
20 group and only a few in the PC111-treated group. The ratio of the number of TUNEL-positive cells to the total conjunctival cell count was calculated for all mice, and significant differences were evaluated by One-way ANOVA followed by Dunnett's multiple comparisons test (Figure 10). The results showed that the PC111-treated group had a significantly lower ratio of TUNEL-positive cells than the vehicle-treated
25 group ($p=0.0003$) and no difference with the untreated control group ($p=0.195$). In the present experiment, hyperemia and edema of the ocular conjunctiva were clearly suppressed and TUNEL staining showed that TUNEL-positive cells significantly decreased in the PC111 treatment group.

- 30 The inventors found that although the vehicle-treated group and the PC111-treated group displayed similar mean weight at day 1, the treatment with PC111 rapidly induced a recovery of the body weight to values comparable to those of the normal control group (Figure 11A).

Moreover, the AUC of the PC111-treated group is very similar to the AUC of the control group. On the contrary, the vehicle-treated group displays an overall lower AUC. No statistic significant differences between vehicle and PC111 were seen, but
5 a clear trend in AUC amelioration, probably due to the small number of animals (Figure 11C).

In sum, the data shown in Figure 11 provide valuable therapeutic conclusions, in particular in view of the rapid recovery of the PC111-treated group compared to the
10 vehicle-treated group. Evidence of the similarities in AUC between PC111 and the control group has a high therapeutic value. Moreover, these data indicate that blocking soluble FasL does not display acute toxicity effects, indeed the PC111 group is pretty similar to the untreated control group.

15 Thus, taken together the results of the *in vivo* model study, provide evidence that the PC111 antibody is effective to decrease inflammatory symptoms and in inhibiting epithelial cell apoptosis death of the epithelium in the posterior conjunctiva of the eye in the ocular conjunctiva mouse model of SJS/TEN.

20

3. Binding affinity and specificity of PC111

3.1 Surface Plasmon Resonance (SPR) Analysis of PC111 Biding Affinity to sFasL

25 The binding affinity of PC111 to sFasL was determined for 3 different batches of PC111 using different source sFasL and different SPR instrumentation. The average binding affinity (equilibrium dissociation constant; KD) of the 6 measurements shown in Table 1 was 238 pM with a range from 650 pM to 93 pM.

30 Table 1

sFasL Source	Instrument	Ka (M ⁻¹ s ⁻¹)	Kd (s ⁻¹)	KD (M)
Acro FAL-H5421	Forte Octet	4.09 x 10 ⁽⁵⁾	6.19 x 10 ⁽⁻⁵⁾	1.52 x 10 ⁽⁻¹⁰⁾

FAL-H5421	Biacore	$3.10 \times 10(5)$	$2.89 \times 10(-5)$	$9.31 \times 10(-11)$
Sigma S8689	Biacore	$8.13 \times 10(4)$	$1.13 \times 10(-5)$	$1.39 \times 10(-10)$
Acro FAL-H5421	Biacore	$1.87 \times 10(5)$	$4.49 \times 10(-5)$	$2.39 \times 10(-10)$
Acro FAL-H5421	Biacore	$1.67 \times 10(5)$	$1.1 \times 10(-4)$	$6.5 \times 10(-10)$
Acro FAL-H5421	Biacore	$1.15 \times 10(6)$	$1.78 \times 10(-4)$	$1.54 \times 10(-10)$

3.2 Flow-Induced Dispersion Analysis

5 In the following experiments the antibody PC111 was tested against FasL protein for binding studies. In these studies, a flow-Induced Dispersion Analysis (FIDA) was used which takes advantage of a dispersion phenomenon in a pressure driven flow which, following signal analysis, gives rise to an accurate assessment of molecular diffusivity and hydrodynamic radius (Rh). The change in the hydrodynamic radius of
10 a binder reflects the strength of the interaction.

3.2.1 *Methods and Materials*

Capillary: Coated capillary
15 *Detector:* 480 nm fluorescence detection of riboflavin
Indicator (labelled molecule) consumption per measurement: 39 nL
Analyte consumption per measurement: 12 μ L
Analysis time: 5.5 min per measurement
PC111-Alexa conc.: 100 nM
20 *FasL conc.:* 0.01 – 320 nM
Assay buffer: PBS pH 7.45, BSA 0.1% (1mg/ml)
Mixing principle: Pre-Mix (> 10 min)
Temperature: 25 °C

25 3.2.2 *Results*

PC111 was used as indicator at 15nM constant concentration and titrated FasL from 0.01 to 320 nM. The Rh of PC111 alone was measured 4.8nm and 6.8nm when in complex FasL. Since FasL is a homotrimer, PC111 might theoretically bind to FasL
30 in a 1FasL:3PC111 stoichiometry depending on the steric hindrance. Multiple stoichiometry combinations are possible.

PC111 was labelled and an increase in its Rh was detected upon sFasL addition (Figure 12A). When sFasL concentration reached the range of 10-80 nM a higher Rh is detected. The orange squared points in Figure 12A strongly suggest the presence of higher-order oligomers. When sFasL was >100nM the Rh of PC111 was back to 6.8nm suggesting a 1:1 stoichiometry. A fitting model that includes also the higher-order oligomers is not yet developed as it might be different for each system, therefore by removing the 3 points marked in orange in Figure 12A a standard 1:1 Excess Indicator fitting model was applied (Figure 12B). The dissociation constant between PC111 and sFasL in solution KD was 340 pM.

10

3.3 Membrane Proteome Array

A Membrane Proteome Array (MPA) technology was used to screen PC111 for binding targets against the human membrane proteome in order to determine antibody target specificity and potential off-target binding. The technology uses flow cytometry to directly detect antibody binding to membrane proteins expressed in unfixed cells (Figure 13). All target proteins have native conformations and appropriate post-translational modifications.

20 3.3.1 Determination of assay screening conditions

To optimize ligand concentrations and cell lines for screening, HEK-293T cells (ATCC CRL-3216) and QT6 cells (ATCC CRL-1708) were transfected with plasmids encoding known ligand targets, Protein A (binds antibody Fc; positive control), or vector alone (pUC; negative control) in 384-well cell-culture plates at a density of 18,000 cells/well. The transfected cells were then incubated in media composed of Corning DMEM, 10% FBS, 2 mM L-alanyl-L-glutamine, Pen Strep, MEM NEAA, and 10 mM HEPES for 36 hours at 37°C and 5% CO₂. After incubation, four four-fold dilutions of each ligand starting at 20 µg/ml were added in quadruplicate to transfected cells, and bound ligand was detected using a single dilution of a fluorescently labelled secondary antibody in a high-throughput immunofluorescence flow cytometry assay.

30

Average mean fluorescence intensity (MFI) values were determined for each test ligand dilution in each cell line using ForeCyt Software (Intellicyt) and plotted using Excel (Microsoft). The high background rate for each assayed condition was calculated as the percentage of positive events above a defined fluorescence threshold in cells transfected with negative control. Optimal screening concentrations and cell line for each test ligand (Table 2, below) were determined by the background signal (MFI), and high background rate in the vector control (Figure 14). Molecules are preferentially screened on HEK-293T cells at the highest concentration yielding an acceptably low background (< 50,000 MFI) and minimal high background rate (<1%). If no acceptable screening conditions are identified on HEK-293T cells, molecules are screened at the highest acceptable concentration on QT6 cells.

Table 2

Parameter	PC111
Screening cell line	HEK-293T
Blocking buffer	10% goat serum
Primary Ligand	
Initial concentration, mg/ml	7.4
Storage	-20°C
Lot Number	N/A
Expiration Date	N/A
Source	N/A
Screening concentration, µg/ml	20
Room temperature incubation time, min	60
Post-primary washes	PBS (without Ca ²⁺ , Mg ²⁺) diluted in diH ₂ O x 5
Secondary Antibody	
Target	Human IgG
Optimal Concentration, µg/ml	3.75 (1:400)
RT incubation time, min	30
Manufacturer	Jackson ImmunoResearch
Cat #	109-606-008
Antibody ID	AlexaFluor® 647-AffiniPure Goat F(ab') ₂ Anti-Human Fc
Post-secondary washes	PBS (without Ca ²⁺ , Mg ²⁺) diluted in diH ₂ O x 2

3.3.2. Membrane Proteome Array Screen

Plasmids containing cDNA clones of ~6,000 membrane proteins (representing over 94% of the human membrane proteome) were each transfected into HEK-293T cells (18,000 cells/well) in 384-well cell-culture plates (one unique cDNA-containing plasmid per well) and incubated at 37°C and 5% CO₂ in media composed of Corning DMEM, 10% FBS, 2 mM L-alanyl-L-glutamine, Penn/strep, MEM NEAA, and 10 mM HEPES. Each 384-well plate contained wells independently transfected with plasmids encoding GFP or a membrane-bound protein-A construct as controls for transfection efficiency and fluorescently labelled detection antibody binding respectively. After incubation for 36 hours, the cells were lifted using CellStripper and re-formatted into a two-dimensional matrix in a new 384-well plate by rows and columns using a JANUS Automated Workstation. Each well on the matrix plate contains 48 different overexpressed protein constituents, and each protein is represented in a unique combination of two different wells of the matrix plate, as it is contained within a “row” pool and a “column” pool. Thus, every protein in the MPA is tested twice for reactivity. Test ligands were added to Membrane Proteome Array matrix plates at pre-determined concentrations (Table 2, above) washed in 1× PBS and detected by flow cytometry using a fluorescently labelled antibody. All flow cytometry data was captured using ForeCyt Software (Intellicyt).

Test ligand targets were then identified by detecting binding to overlapping pooled matrix wells emanating from the same transfection plate, thereby allowing specific deconvolution. Individual targets displaying binding of greater than 3 standard deviations above background in both wells were selected for downstream validation experiments.

In order to present a single value for test ligand binding to each protein in the Membrane Proteome Array, the two-dimensional binding data was transformed using standard matrix deconvolution methods. Briefly, each point (representing an individual over-expressed protein) was converted to radians, followed by transformation using the formula $r \cdot \sin(2\theta)$ and plotted as Target Binding (Figure 15). Non-specific fluorescence was determined to be any value below the transformed

value of 3 standard deviations of the calculated background fluorescence. All data and analyses were conducted in Excel (Microsoft).

3.3.3 Target Validation

5

To validate any off-target interaction identified, cells were transfected with plasmids encoding the identified targets, protein A, or vector alone in 384-well format. After incubation for 36 hours at 37°C and 5% CO₂, four four-fold dilutions of each test ligand, starting at 20 µg/ml, were added to transfected cells followed by detection of
10 ligand binding using a high-throughput immunofluorescence flow cytometry assay (same conditions as described in Table 2, above).

No validation is shown if a molecule's known target was the only protein validated in the MPA screen, as the Assay Setup results already demonstrate specific
15 reactivity in a screen equivalent to the validation screen.

3.3.4 Results

During the MPA study, binding of PC111 to Fc-binding control, Protein A, was
20 identified in addition to Fc-receptors FCGR1A and FCGR2B. No binding of PC111 was detected against membrane-bound FASLG. No off-target binding was identified for PC111.

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- 15 14) Deal et al., "Advancements in mRNA Encoded Antibodies for Passive Immunotherapy", *Vaccines* (2021), 9, 2018

Claims

1. A monoclonal antibody or an antigen-binding fragment thereof specific for human Fas ligand protein (FasL) comprising at least one heavy chain variable (VH) region and at least one light chain variable (VL) region,
5 wherein said antibody or an antigen-binding fragment is selected from:

(i) an antibody or an antigen-binding fragment comprising a VH region having complementary determining regions (CDRs) of the heavy chain CDR H1, CDR H2 and CDR H3 as follows:

(a₁) CDR H1: Arg His Gly Ile Thr (SEQ ID NO: 1) or
10 (a₂) CDR H1: Ser His Gly Ile Ser (SEQ ID NO: 2),

(b₁) CDR H2: Trp Ile Asn Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Val Gln Gly (SEQ ID NO: 3) or

(b₂) CDR H2: Trp Ile Asn Ala Tyr Ser Gly Asn Thr Asn Tyr Ala Gln Lys Leu Gln Gly (SEQ ID NO: 4),

15 (c₁) CDR H3: Glu Thr Met Val Arg Gly Val Pro Leu Asp Tyr (SEQ ID NO: 5) or

(c₂) CDR H3: Glu Thr Met Val Arg Gly Val Pro Cys Asp Tyr (SEQ ID NO: 6),
and

complementary determining regions (CDRs) of the light chain CDR L1, CDR
20 L2 and CDR L3 as follows:

(a₃) CDR L1: Arg Ala Ser Gln Ser Val Ser Ser Ser Tyr Leu Ala (SEQ ID NO: 7),

(b₃) CDR L2: Gly Ala Ser Ser Arg Ala Thr (SEQ ID NO: 8),

(c₃) CDR L3: Gln Gln Tyr Gly Ser Ser Pro Trp Thr (SEQ ID NO: 9);

or

(ii) an antibody or an antigen-binding fragment competing with the antibody or antigen-binding fragment of (i) in the binding to human Fas ligand protein (FasL);

5 for use in a method for the prevention and/or treatment of toxic epidermal necrolysis (TEN) and/or Stevens-Johnson-Syndrome (SJS).

2. The monoclonal antibody or an antigen-binding fragment thereof of claim 1
10 for the use of claim 1 comprising at least one heavy chain variable (VH) region and at least one light chain variable (VL) region,

wherein said antibody or an antigen-binding fragment is selected from:

an antibody or an antigen-binding fragment comprising a VH region having complementary determining regions (CDRs) of the heavy chain CDR H1, CDR H2 and CDR H3 as follows:

15 (a₁) CDR H1: Arg His Gly Ile Thr (SEQ ID NO: 1);

(b₁) CDR H2: Trp Ile Asn Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Val Gln Gly (SEQ ID NO: 3);

(c₁) CDR H3: Glu Thr Met Val Arg Gly Val Pro Leu Asp Tyr (SEQ ID NO: 5);

20 and complementary determining regions (CDRs) of the light chain CDR L1, CDR L2 and CDR L3 as follows:

(a₃) CDR L1: Arg Ala Ser Gln Ser Val Ser Ser Ser Tyr Leu Ala (SEQ ID NO: 7),

(b₃) CDR L2: Gly Ala Ser Ser Arg Ala Thr (SEQ ID NO: 8),

(c₃) CDR L3: Gln Gln Tyr Gly Ser Ser Pro Trp Thr (SEQ ID NO: 9).

3. The antibody or antigen-binding fragment thereof of claim 1 or 2 for the use of claim 1, wherein the VL region of the antibody comprises the amino acid sequence

5 EIVLTQSPGTL~~SL~~SPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY
GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFG
QGTKVEIKRTVAAPSVFIFP (SEQ ID NO: 10)

and the VH region of the antibody comprises the amino acid sequence

10 QVQLVQSGAEVKKPGASVKVSCKASGYIFIRHGITWVRQAPGQGLEWMG
WINAYNGNTNYAQKVQGRVTMTTDKSTSTAYMELRSLRSDDAAVYYCAR
ETMVRGVPLDYWGQGTLVTVSSASTKGPSVFPLA (SEQ ID NO: 11)

or

15 QVQLVQSGAEVKKPGASVKVSCKASGYIFISHGISWVRQAPGQGLEWMG
WINAYSGNTNYAQKLQGRVTMTTDRSTSTAYMELRSLRSDDTAVYYCAR
ETMVRGVPCDYWGQGTLVTVSSASTKGPSVFPLA (SEQ ID NO: 12).

4. The antibody or antigen-binding fragment thereof of any one of claims 1-3 for the use of claim 1, wherein the VL region of the antibody comprises the amino acid sequence:

20 EIVLTQSPGTL~~SL~~SPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY
GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFG
QGTKVEIK (SEQ ID NO: 13),

25 and the VH region of the antibody comprises the amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYIFIRHGITWVRQAPGQGLEWMG
WINAYNGNTNYAQKVQGRVTMTTDKSTSTAYMELRSLRSDDAAVYYCAR
ETMVRGVPLDYWGQGTLVTVSS (SEQ ID NO: 14)

or

QVQLVQSGAEVKKPGASVKVSCKASGYIFISHGISWVRQAPGQGLEWMG
WINAYSGNTNYAQKLQGRVTMTTDRSTSTAYMELRSLRSDDTAVYYCAR
ETMVRGVPCDYWGQGTLVTVSS (SEQ ID NO: 15)

5

5. The antibody or antigen-binding fragment thereof of any one of claims 1-4 for the use of claim 1 recognizing the same epitope on human FasL as the antibody of claim 1 (i) or 2 (i).

10

6. The antibody or antigen-binding fragment thereof of any one of claims 1-5 for the use of claim 1, wherein the antibody is selected from a partially or fully human antibody, a chimeric antibody and/or a humanized antibody and wherein the antigen-binding fragment thereof is selected from a Fab, Fab' and/or F(ab')₂ and/or a single chain Fv fragment.

15

7. The antibody or antigen-binding fragment thereof of any one of claims 1-6 for the use of claim 1, wherein the antibody has an IgG heavy chain constant region, preferably an IgG1 or IgG4 heavy chain constant region.

20

8. A nucleic acid molecule encoding a monoclonal antibody or an antigen-fragment thereof of any one of claims 1-7 for the use of claim 1.

9. A nucleic acid molecule of claim 8 for the use of claim 1, which is a DNA vector or an RNA molecule.

25

10. The antibody or antigen-binding fragment thereof of any one of claims 1-7 or the nucleic acid molecule of any one of claims 8-9 for the use of claim 1 in a monotherapy.
- 5 11. The antibody or antigen-binding fragment thereof of any one of claims 1-7 or the nucleic acid molecule of any one of claims 8-9 for the use of claim 1 in combination with at least one further active ingredient effective against toxic epidermal necrolysis (TEN) and/or Stevens-Johnson syndrome (SJS).
- 10 12. The antibody or antigen-binding fragment thereof of any one of claims 1-7 or the nucleic acid molecule of any one of claims 8-9 for the use of claim 11, wherein the further active ingredient is selected from at least one of steroids, cyclosporine, IVIg, TNF inhibitors and/or plasmapheresis.
- 15 13. The antibody or antigen-binding fragment thereof of any one of claims 1-7 or the nucleic acid molecule of any one of claims 8-9 for the use of claim 1 in human therapy.
- 20 14. A pharmaceutical composition comprising the antibody or antigen-binding fragment of any one of claims 1-7 or the nucleic acid molecule of any one of claims 8-9 together with one or more pharmaceutical acceptable carriers for the use of claim 1.
- 25 15. The antibody or antigen-binding fragment thereof of any one of claims 1-7 or the nucleic acid molecule of any one of claims 8-9 or the pharmaceutical composition of claim 14, which is administered systemically and/or locally.

Figures

Figure 1

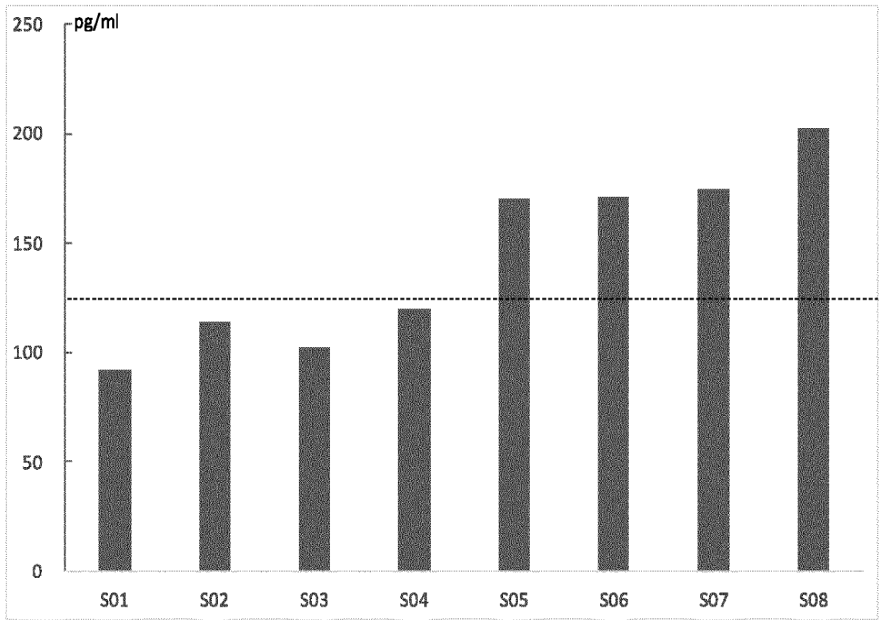


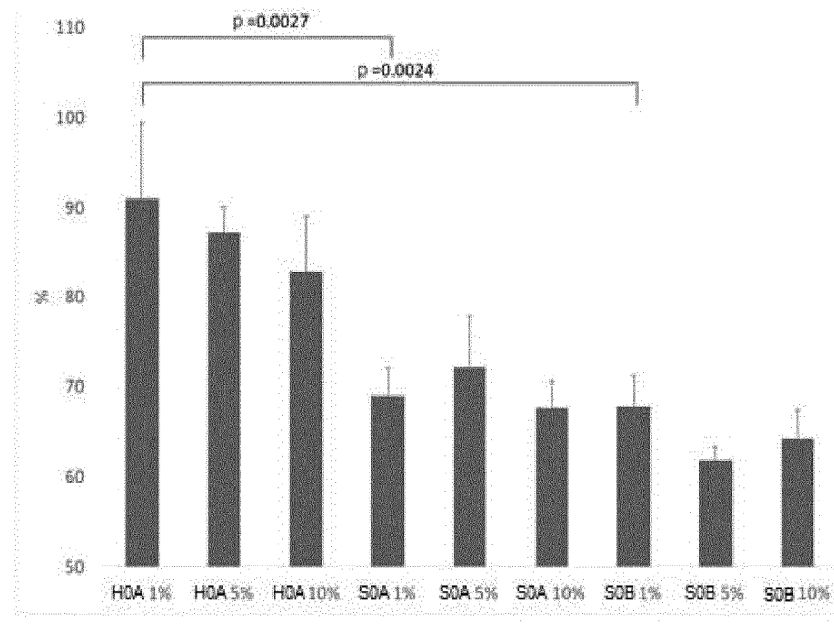
Figure 2

Figure 3

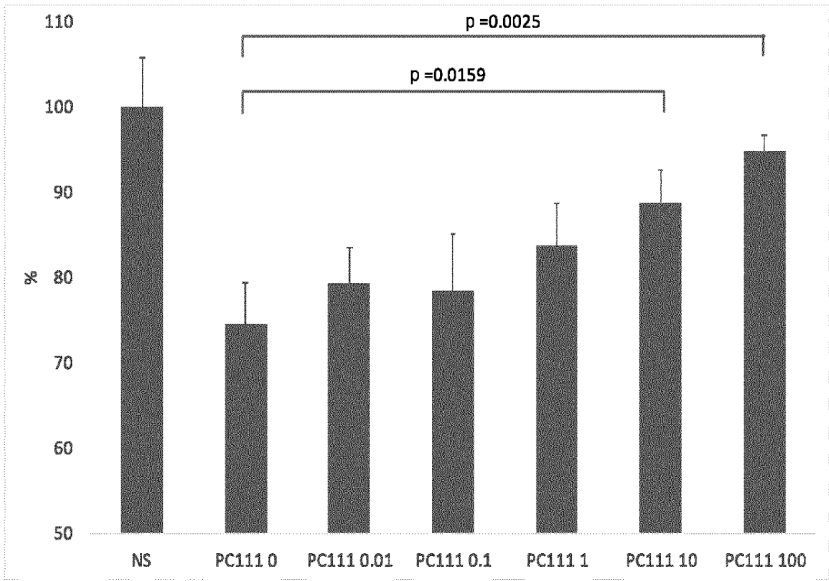


Figure 3a

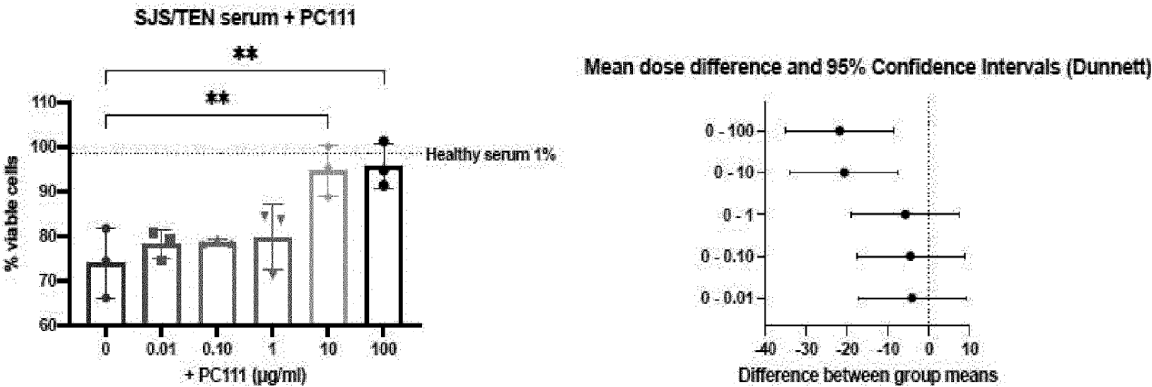


Figure 4

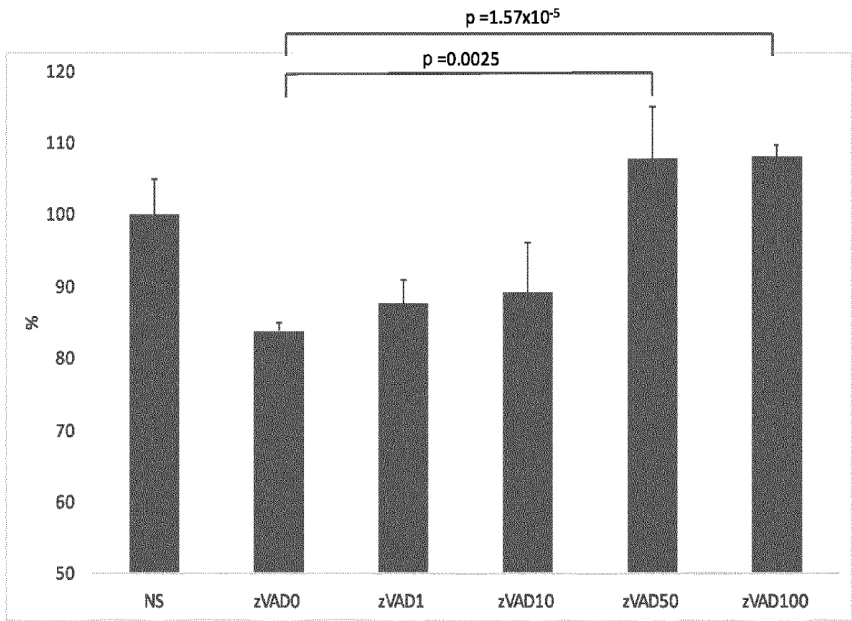
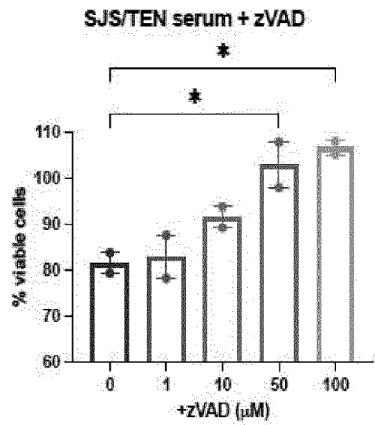


Figure 4a



Mean dose difference and 95% Confidence Intervals (Dunnett)

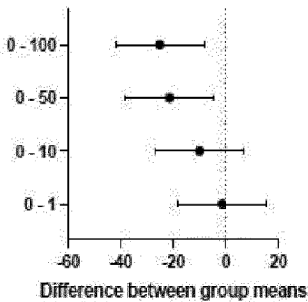


Figure 5

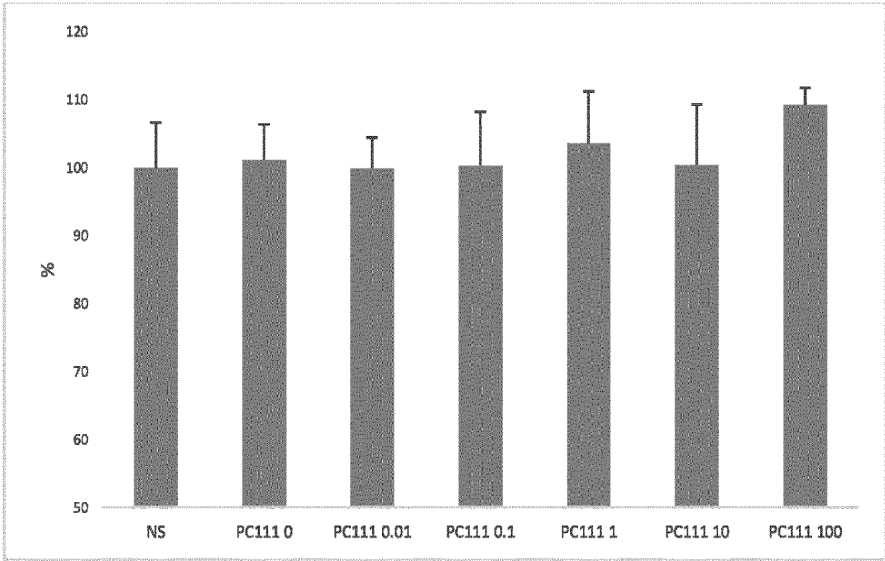
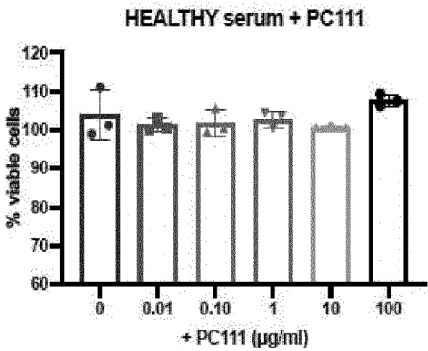


Figure 5a



Mean dose difference and 95% Confidence Intervals (Dunnett)

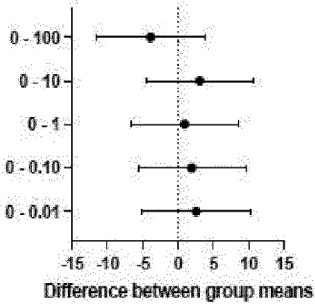


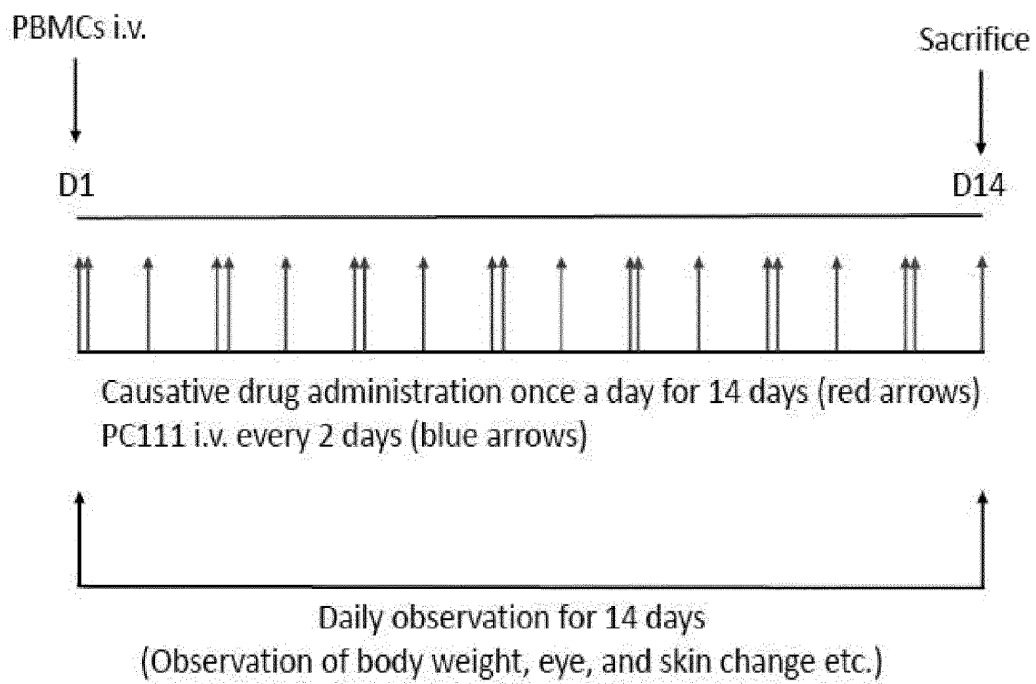
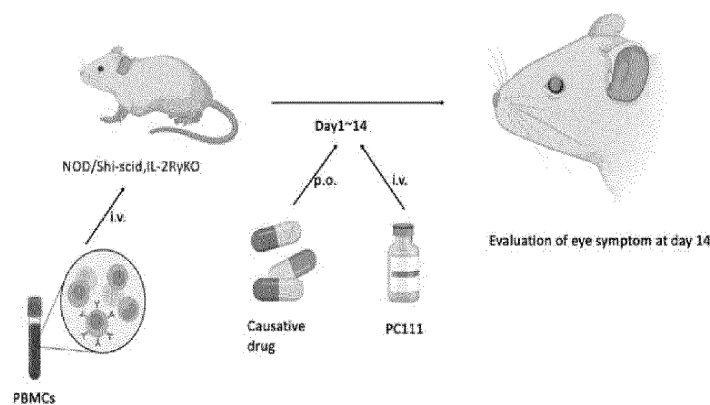
Figure 6a**Figure 6b**

Figure 7a

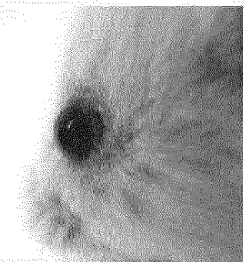
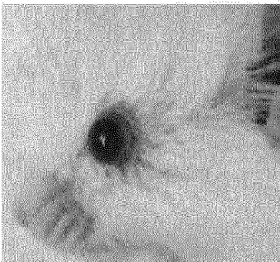
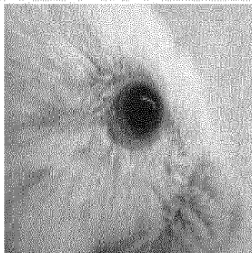
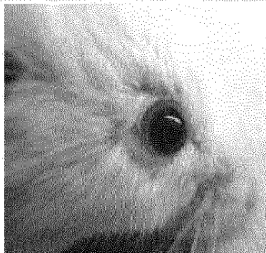
Control			
Hyperemia	2	2	N/A
Edema	1	1	Dead at D12
PC111			
Hyperemia	1	0	0
Edema	0	0	0

Figure 7b

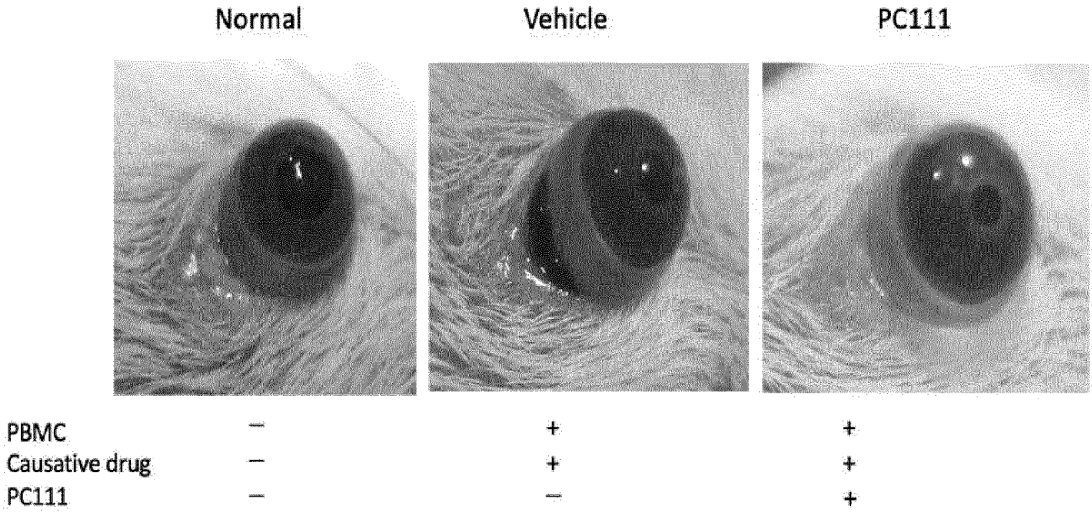


Figure 8

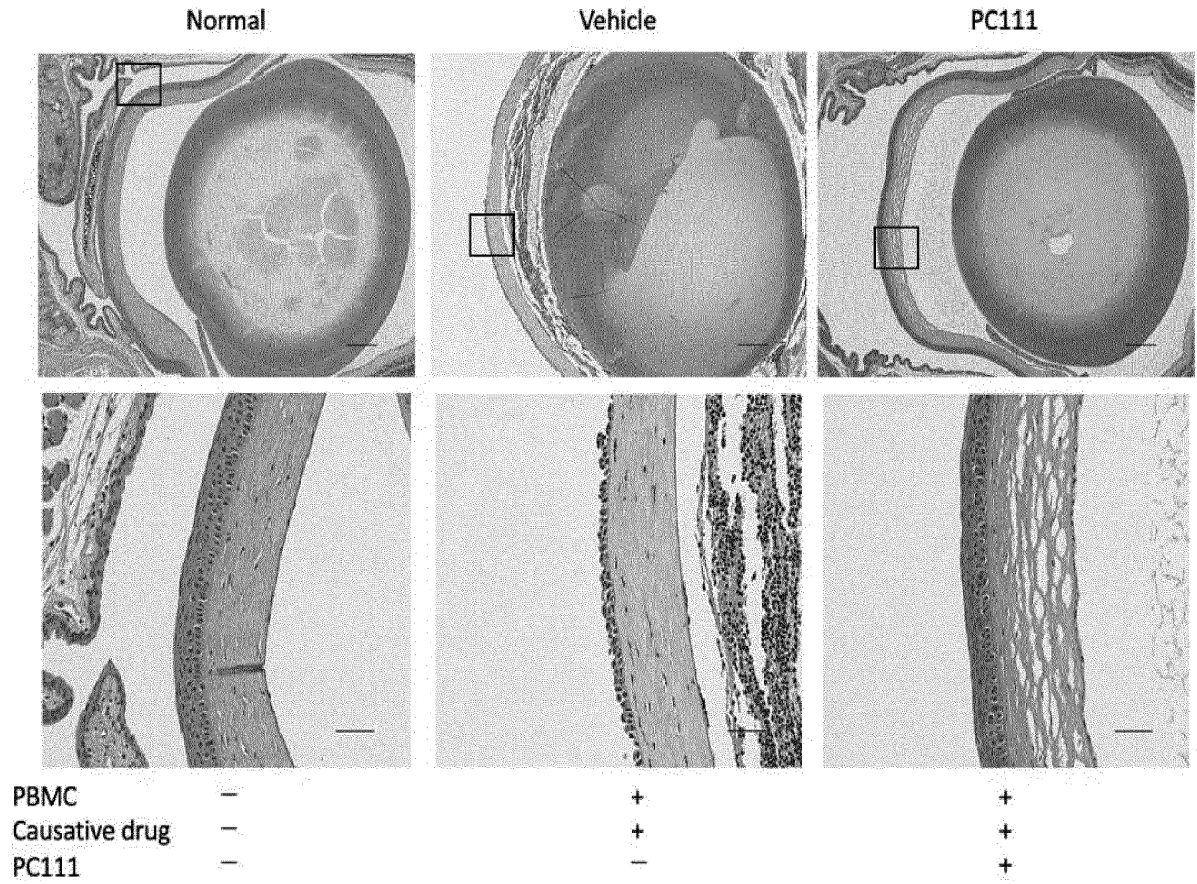


Figure 9

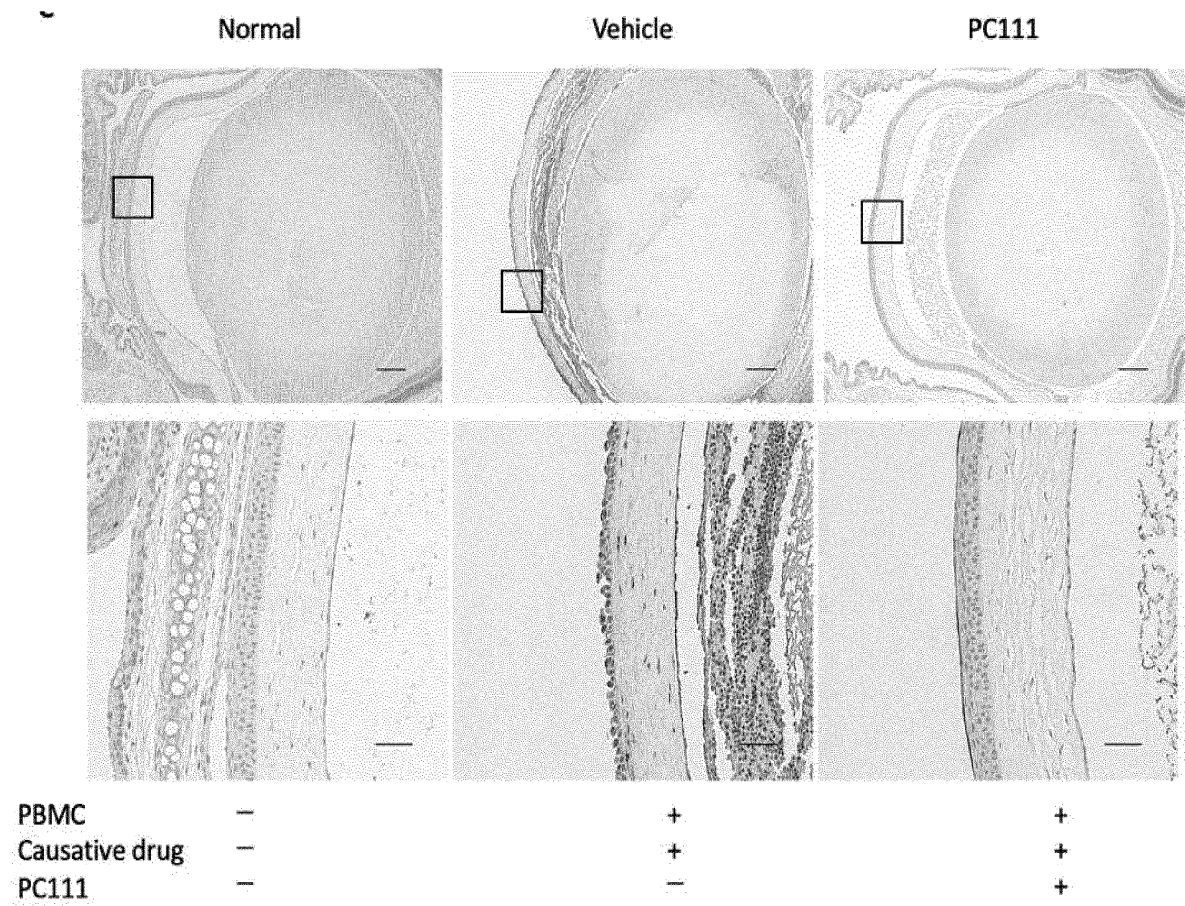


Figure 10

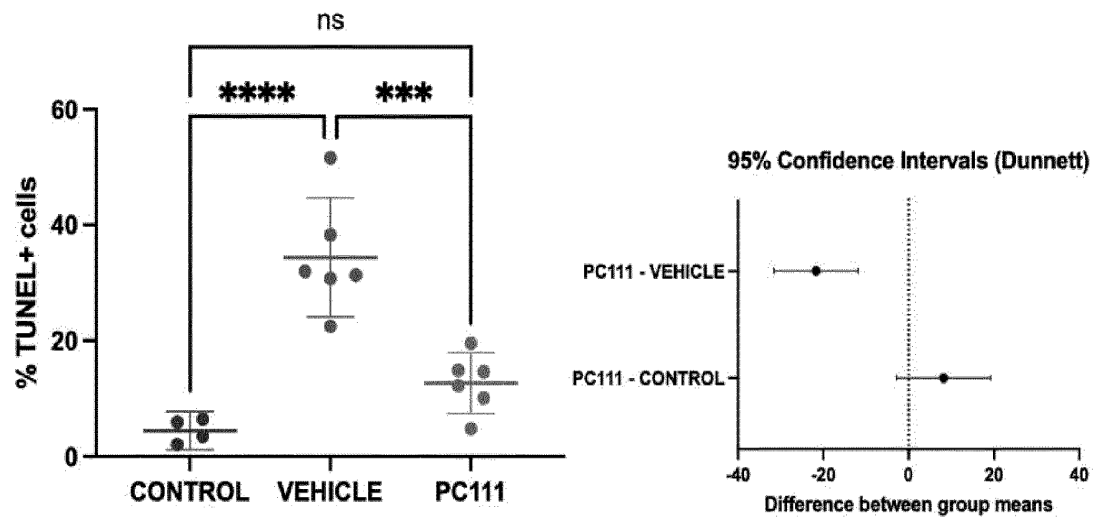


Figure 11

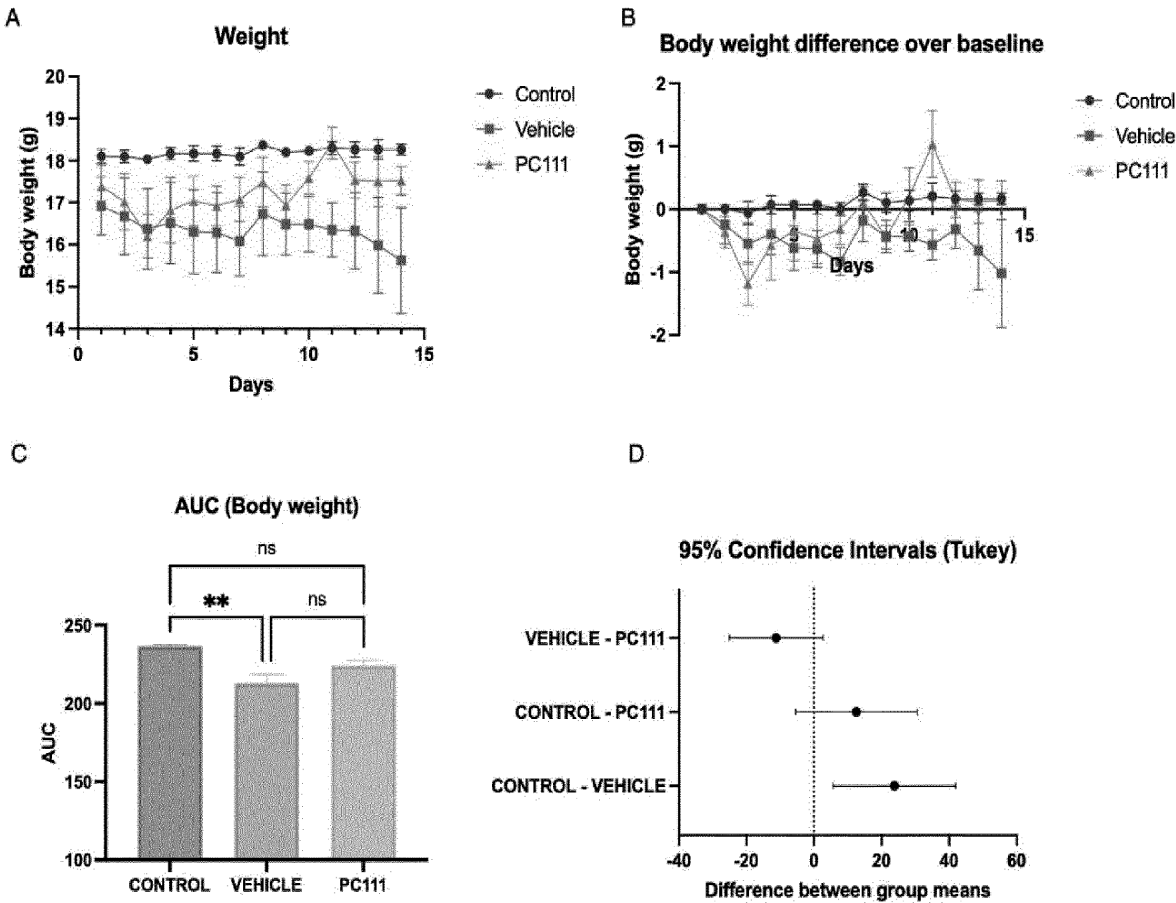


Figure 12

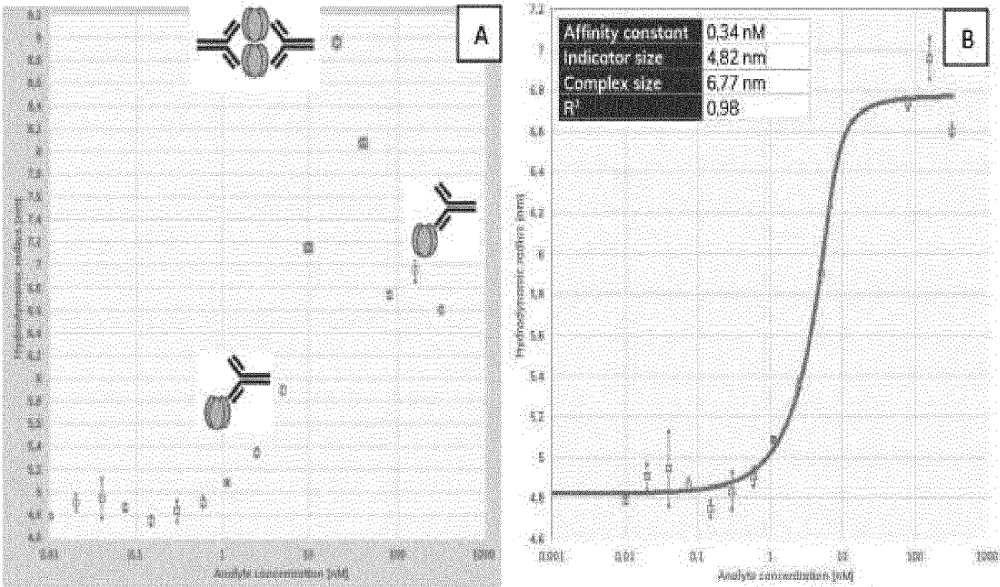


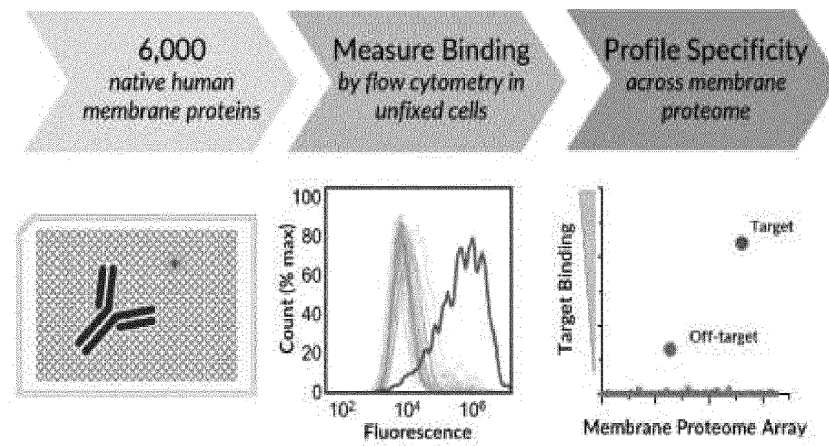
Figure 13

Figure 14

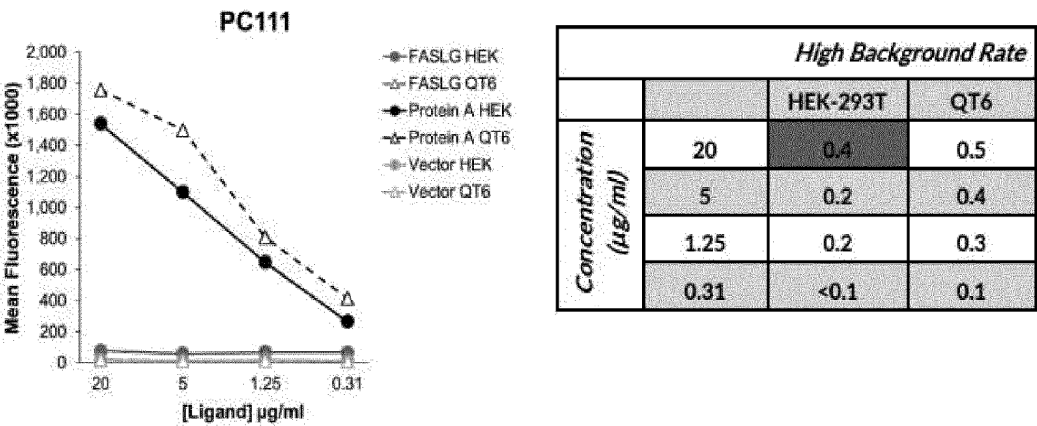
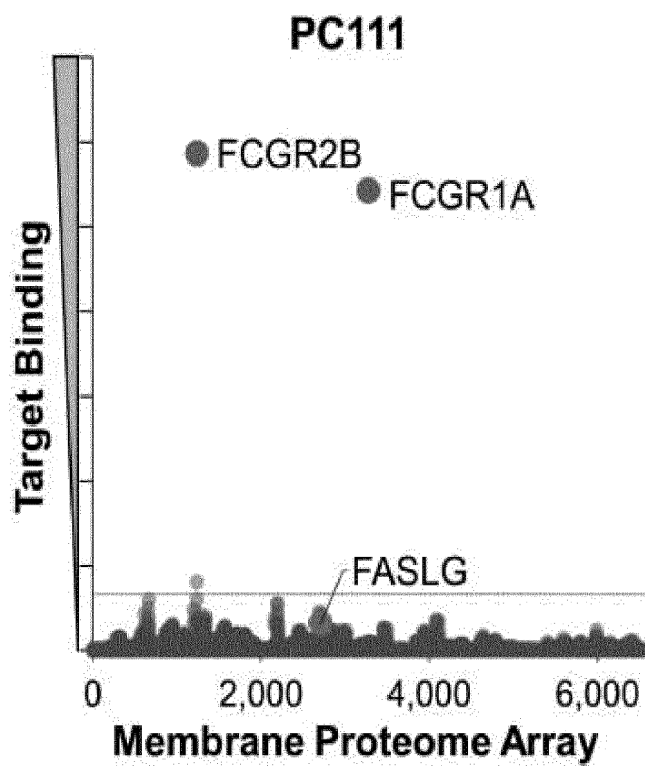


Figure 15

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/057817

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/28 A61P17/02 A61P27/02 A61K39/395
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)

C07K A61K A61P

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, Sequence Search, 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	WO 03/079750 A2 (LILLY CO ELI [US]; LANCASTER JOANNE SLOAN [US]) 2 October 2003 (2003-10-02) claims 1-38; examples 1-8; sequences 2,10,18 -----	1-15
X	WO 2010/066914 A2 (PINCELLI CARLO [IT]; MARCONI ALESSANDRA [IT]; PINCELL SRL [IT]) 17 June 2010 (2010-06-17) claims 1-7; figure 13B -----	1-15
X	WO 2010/102792 A2 (IMED AB [SE]; OSORIO LYDA [SE] ET AL.) 16 September 2010 (2010-09-16) claims 1-40 ----- - / - -	1-15



Further documents are listed in the continuation of Box C.



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

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search

21 May 2024

Date of mailing of the international search report

03/06/2024

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Authorized officer

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/057817

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	CHAVE T A ET AL: "Toxic epidermal necrolysis: current evidence, practical management and future directions", BRITISH JOURNAL OF DERMATOLOGY, JOHN WILEY, HOBOKEN, USA, vol. 153, no. 2, 12 July 2005 (2005-07-12), pages 241-253, XP071156902, ISSN: 0007-0963, DOI: 10.1111/J.1365-2133.2005.06721.X the whole document -----	1-15
T	ALBRAHIM LATIFAH ET AL: "Pemphigus vulgaris mimicking Steven-Johnson syndrome/toxic epidermal necrolysis: report of an unusual case", DERMATOLOGY REPORTS , 23 May 2023 (2023-05-23), XP093096973, ISSN: 2036-7392, DOI: 10.4081/dr.2023.9649 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10563021/pdf/dr-15-3-9649.pdf the whole document -----	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2024/057817

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. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)).
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2024/057817

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