TGF-BETA INHIBITORS AND USE THEREOF

RELATED APPLICATIONS

[1] This Application claims the benefit of and priority to US Provisional Applications 63/166,824 filed March 26, 2021; 63/202,260 filed June 3, 2021; 63/302,999 filed January 25, 2022; and 63/313,386 filed February 24, 2022, each entitled "TGF-BETA INHIBITORS AND USE THEREOF," the contents of which are expressly incorporated herein by reference in their entirety.

SEQUENCE LISTING

[1.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 1, 2022, is named 15094-0013-00304_SL.txt and is 251,422 bytes in size.

FIELD

[2] The instant application relates generally to TGFβ inhibitors and therapeutic use thereof, as well as related assays for diagnosing, monitoring, prognosticating, and treating disorders, including cancer.

BACKGROUND

[3] Transforming growth factor beta 1 (TGFβ1) is a member of the TGFβ superfamily of growth factors, along with two other structurally related isoforms, namely, TGFβ2 and TGFβ3, each of which is encoded by a separate gene. These TGFβ isoforms function as pleiotropic cytokines that regulate cell proliferation, differentiation, immunomodulation (e.g., adaptive immune response), and other diverse biological processes both in homeostasis and in disease contexts. The three TGFβ isoforms signal through the same cell-surface receptors and trigger similar canonical downstream signal transduction events that include the SMAD2/3 pathway.

[4] TGFβ has been implicated in the pathogenesis and progression of a number of disease conditions, such as cancer, fibrosis, and immune disorders. In many cases, such conditions are associated with dysregulation of the extracellular matrix (ECM). For these and other reasons, TGFβ has been an attractive therapeutic target for the treatment of immune disorders, various proliferative disorders, and fibrotic conditions. However, observations from preclinical studies, including in rats and dogs, have revealed serious toxicities associated with systemic inhibition of TGFβs *in vivo*, and to date, there are no TGFβ therapeutics available in the market which are deemed both safe and efficacious.

[5] Dose-limiting toxicities noted with inhibition of the TGFβ pathway have remained a major concern in the development of anti-TGFβ therapies. These include cardiovascular abnormalities, skin lesions, epithelial oral hyperplasia, and gingival bleeding (Vitsky 2009; Lonning 2011; Stauber 2014; Mitra 2020). Although many of these toxicities are either reversible or manageable, the cardiovascular lesions such as inflammation, hemorrhage or hyperplasia in the valves, aortic arch and associated arteries of the heart, are not reversible and therefore continue to be key safety issues when developing TGFβ inhibitors (Stauber 2014; Anderton 2011; Mitra 2020).

[6] Previously, Applicant described a class of monoclonal antibodies that have a novel mechanism of action to modulate growth factor signaling (see, for example, WO 2014/182676, the contents of which are herein incorporated by reference in their entirety). These antibodies were designed to exploit the fact that TGFβ1 is expressed as latent pro-protein complex comprised of prodomain and growth factor, which requires an activation step that releases the growth factor from the latent complex. Rather than taking the traditional approach of directly targeting the mature growth factor itself post-activation (such as neutralizing antibodies), the novel class of inhibitory antibodies specifically targeted the inactive pro-proprotein complex itself so as to preemptively block the activation step, upstream of ligand-receptor interaction. Without being bound by theory, it was reasoned that this unique mechanism of action should provide advantages for achieving both spatial and temporal benefits in that

[83] **FIG. 33** shows tumor depth of bladder samples.

[84] **FIG. 34** shows CD8 density in a melanoma sample.

[85] **FIGs. 35A-c** show exemplary analysis of MDSC by signal filtering.

[86] **FIGs. 36A-C** shows identification of tumor MDSC populations in various solid cancer samples.

[87] **FIGs. 37A-C** shows analysis of gMDSC and mMDSC populations in various solid cancer samples.

[88] **FIG. 38** shows a schematic of an exemplary pathology analysis of tumor tissue sample.

[89] **FIG. 39** shows a schematic of an exemplary pathology analysis of tumor tissue sample.

[90] **FIG. 40** shows a schematic of an exemplary TGFβ inhibitor treatment regimen.

[91] **FIG. 41** illustrates identification of three binding regions (Region 1, Region 2, and Region 3) following statistical analyses. Region 1 overlaps with a region called "Latency Lasso" within the prodomain of proTGFβ1, while Regions 2 and 3 are within the growth factor domain. FIG. 41 discloses SEQ ID NO: 172.

[92] **FIG. 42** depicts various domains and motifs of proTGFβ1, relative to the three binding regions involved in Ab6 binding. Sequence alignment among the three isoforms is also provided. FIG. 42 discloses SEQ ID NOS 142, 173-174, 138, 175-176, 141, and 177-178, respectively, in order of appearance.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

Definitions

[93] In order that the disclosure may be more readily understood, certain terms are first defined. These definitions should be read in light of the remainder of the disclosure and as understood by a person of ordinary skill in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. Additional definitions are set forth throughout the detailed description.

[94] *Advanced cancer, advanced malignancy*: The term "advanced cancer" or "advanced malignancy" as used herein has the meaning understood in the pertinent art, e.g., as understood by oncologists in the context of diagnosing or treating subjects/patients with cancer. Advanced malignancy with a solid tumor can be *locally advanced* or *metastatic*. The term "locally advanced cancer" is used to describe a cancer (e.g., tumor) that has grown outside the organ it started in but has not yet spread to distant parts of the body. Thus, the term includes cancer that has spread from where it started to nearby tissue or lymph nodes. By contrast, "metastatic cancer" is a cancer that has spread from the part of the body where it started (the primary site) to other parts (e.g., distant parts) of the body.

[95] *Affinity:* Affinity is the strength of binding of a molecule (such as an antibody) to its ligand (such as an antigen). It is typically measured and reported by the equilibrium dissociation constant (K_D). In the context of antibody-antigen interactions, K_D is the ratio of the antibody dissociation rate ("off rate" or K_{off}), how quickly it dissociates from its antigen, to the antibody association rate ("on rate" or K_{on}) of the antibody, how quickly it binds to its antigen. For example, an antibody with an affinity of ≤ 5 nM has a K_D value that is 5 nM or lower (i.e., 5 nM or higher affinity) determined by a suitable *in vitro* binding assay. Suitable *in vitro* assays can be used to measure K_D values of an antibody for its antigen, such as Biolayer Interferometry (BLI) and Solution Equilibrium Titration (e.g., MSD-SET). In a preferred embodiment, affinity is measured by surface plasmon resonance (e.g., Biacore®). An antibody with a suitable affinity in a surface plasmon resonance assay may have, e.g., a K_D of at most about 1 nM, e.g., at most about 0.5 nM, e.g., at most about 0.5, 0.4, 0.3, 0.2, 0.15 nM, or less.

Additional examples of antibodies or antigen-binding fragments thereof encompassed by the present disclosure are provided in PCT/US2019/041390, PCT/US2019/041373, PCT/US2021/012930, the contents of each of which are herein incorporated by reference in their entirety.

Table 1. Complementary determining regions of the heavy chain of exemplary antibodies, as determined using the numbering scheme described in Lu et al.

Ab	H-CDR1	H-CDR2	H-CDR3
Ab4	FTFSSYSMN (SEQ ID NO: 81)	YISSSSSTIYYADSVKG (SEQ ID NO: 82)	ARGVLDYGDMLDP (SEQ ID NO: 83)
Ab5	FTFSSFSMD (SEQ ID NO: 80)	YISPDASTIYYADSVKG (SEQ ID NO: 84)	ARGVLDYGDMLDP (SEQ ID NO: 83)
Ab6	FTFSSFSMD (SEQ ID NO: 80)	YISPSADTIYYADSVKG (SEQ ID NO: 76)	ARGVLDYGDMLMP (SEQ ID NO: 3)
Ab21	FTFSSFSMD (SEQ ID NO: 80)	YISPDASTIYYADSVKG (SEQ ID NO: 84)	ARGVLDYGDMLDP (SEQ ID NO: 83)
Ab22	FTFGSFSMN (SEQ ID NO: 88)	YIHSDASTIYYADSVKG (SEQ ID NO: 86)	ARGVLDYGDMLDP (SEQ ID NO: 83)
Ab23	FTFSSFSMN (SEQ ID NO: 87)	YISPSADTIYYADSVKG (SEQ ID NO: 76)	ARGVLDYGDMLDP (SEQ ID NO: 83)
Ab24	FTFSSFAMY (SEQ ID NO: 179)	YISPDASTIYYADSVKG (SEQ ID NO: 84)	ARGVLDYGDMLDP (SEQ ID NO: 83)
Ab25	FTFGSFSMD (SEQ ID NO: 180)	YISPDASTIYYADSVKG (SEQ ID NO: 84)	ARGVLDYGDMLDP (SEQ ID NO: 83)
Ab26	FTFSSFSMD (SEQ ID NO: 80)	YISPDASTIYYADSVKG (SEQ ID NO: 84)	ARGVLDYGDMLDP (SEQ ID NO: 83)
Ab27	FTFSFYAMN (SEQ ID NO: 90)	YISPDASTIYYADSVKG (SEQ ID NO: 84)	ARGVLDYGDMLDP (SEQ ID NO: 83)
Ab28	FTFSSFSMD (SEQ ID NO: 80)	YISPDASTIYYADSVKG (SEQ ID NO: 84)	VRGVLDYGDMLDP (SEQ ID NO: 91)
Ab29	FTFSSFAMN (SEQ ID NO: 92)	YISPDASTIYYAGSVKG (SEQ ID NO: 93)	VRAVLDYGDMLDP (SEQ ID NO: 94)
Ab30	FTFSSFSMD (SEQ ID NO: 80)	YISPDASTIYYADSVKG (SEQ ID NO: 84)	ARGTLDYGDMLDP (SEQ ID NO: 95)
Ab31	FTFSSFSMD (SEQ ID NO: 80)	YISPDASTIYYADSVKG (SEQ ID NO: 84)	ARAVLDYGDMLDP (SEQ ID NO: 96)
Ab32	FTFSSFSMN (SEQ ID NO: 87)	YISPSADTIYYADSVKG (SEQ ID NO: 76)	ARGVWDMGDMLDP (SEQ ID NO: 97)
Ab33	FTFSSFSMN (SEQ ID NO: 87)	YISPSADTIYYADSVKG (SEQ ID NO: 76)	AHGVLDYGDMLDP (SEQ ID NO: 98)
Ab34	FTFAFYSMN (SEQ ID NO: 99)	YISPDASTIYYADSVKG (SEQ ID NO: 84)	ARGVLDYGDMLDP (SEQ ID NO: 83)

Table 2. Complementary determining regions of the light chain of exemplary antibodies, as determined using the Kabat numbering scheme or the numbering system of Lu et al.

residues joined by peptide bonds and are used to link one or more antigen binding portions. Such linker polypeptides are well known in the art (see, *e.g.*, Holliger, P. et al., (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448; Poljak, R.J. et al., (1994) Structure 2:1121-1123). Exemplary linkers include, but are not limited to, ASTKGPSVFPLAP (SEQ ID NO: 44), ASTKGP (SEQ ID NO: 45); TVAAPSVFIFPP (SEQ ID NO: 46); TVAAP (SEQ ID NO: 47); AKTTPKLEEGEFSEAR (SEQ ID NO: 48); AKTTPKLEEGEFSEARV (SEQ ID NO: 49); AKTTPKLGG (SEQ ID NO: 50); SAKTTPKLGG (SEQ ID NO: 51); SAKTTP (SEQ ID NO: 52); RADAAP (SEQ ID NO: 53); RADAAPTVS (SEQ ID NO: 54); RADAAAAGGPGS (SEQ ID NO: 55); RADAAAA(G4S)4 (SEQ ID NO: 56); SAKTTPKLEEGEFSEARV (SEQ ID NO: 57); ADAAP (SEQ ID NO: 58); ADAAPTVSIFPP (SEQ ID NO: 59); QPKAAP (SEQ ID NO: 60); QPKAAPSVTLFPP (SEQ ID NO: 61); AKTTPP (SEQ ID NO: 62); AKTTPPSVTPLAP (SEQ ID NO: 63); AKTTAP (SEQ ID NO: 64); AKTTAPSVYPLAP (SEQ ID NO: 65); GGGGSGGGGSGGGGS (SEQ ID NO: 66); GENKVEYAPALMALS (SEQ ID NO: 67); GPAKELTPLKEAKVS (SEQ ID NO: 68); GHEAAAVMQVQYPAS (SEQ ID NO: 69); TVAAPSVFIFPPTVAAPSVFIFPP (SEQ ID NO: 70); and ASTKGPSVFPLAPASTKGPSVFPLAP (SEQ ID NO: 71).

[557] "Label" and "detectable label" or "detectable moiety" mean a moiety attached to a specific binding partner, such as an antibody or an analyte, *e.g.*, to render the reaction between members of a specific binding pair, such as an antibody and an analyte, detectable, and the specific binding partner, *e.g.*, antibody or analyte, so labeled is referred to as "detectably labeled." Thus, the term "labeled binding protein" as used herein, refers to a protein with a label incorporated that provides for the identification of the binding protein. In an embodiment, the label is a detectable marker that can produce a signal that is detectable by visual or instrumental means, *e.g.*, incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (*e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.*, ^{18}F. ^{11}C, ^{13}N, ^{15}O, ^{68}Ga, ^{18}F, ^{89}Zr, ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm); chromogens; fluorescent labels (*e.g.*, FITC, rhodamine, and lanthanide phosphors); enzymatic labels (*e.g.*, horseradish peroxidase, luciferase, and alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, and epitope tags); and magnetic agents, such as gadolinium chelates. Representative examples of labels commonly employed for immunoassays include moieties that produce light, *e.g.*, acridinium compounds, and moieties that produce fluorescence, *e.g.*, fluorescein. Other labels are described herein. In this regard, the moiety itself may not be detectably labeled but may become detectable upon reaction with yet another moiety. Use of "detectably labeled" is intended to encompass the latter type of detectable labeling.

[558] In some embodiments, the binding affinity of an antibody, or antigen binding portion thereof, to an antigen (*e.g.*, protein complex), such as presenting molecule-proTGFβ1 complexes, is determined using BLI (e.g., an Octet® assay). A BLI (e.g., Octet®) assay is an assay that determines one or more a kinetic parameters indicative of binding between an antibody and antigen. In some embodiments, an Octet® system (FortéBio®, Menlo Park, CA) is used to determine the binding affinity of an antibody, or antigen binding portion thereof, to presenting molecule-proTGFβ1 complexes. For example, binding affinities of antibodies may be determined using the FortéBio Octet® QKe dip and read label free assay system utilizing bio-layer interferometry. In some embodiments, antigens are immobilized to biosensors (*e.g.*, streptavidin-coated biosensors) and the antibodies and complexes (*e.g.*, biotinylated presenting molecule-proTGFβ1 complexes) are presented in solution at high concentration (50 µg/mL) to measure binding interactions. In some embodiments, the binding affinity of an antibody, or antigen binding portion thereof, to a presenting molecule-proTGFβ1 complex is determined using the protocol outlined herein.

[761] The present disclosure provides a TGFβ inhibitor (e.g., TGFβ1-selective inhibitor such as Ab6) for use in the treatment of a TGFβ-related disorder involving matrix stiffening and remodeling (e.g., as described herein) in a patient, wherein the treatment comprises administration of a composition comprising the TGFβ inhibitor (e.g., TGFβ1 inhibitor) which has been selected, at least in part, on the basis of its immune safety profile. A suitable immune safety profile of the TGFβ inhibitor is characterized in that i) it does not trigger unacceptable levels of cytokine release (e.g., within 2.5-fold of control); ii) it does not promote unacceptable levels of platelet aggregation; or both in field-accepted cell-based assay(s) and/or in *in vivo* assay(s) (such as those described herein).

Diseases involving proteases

[762] Activation of TGFβ from its latent complex may be triggered mechanically by integrin in a force-dependent manner, and/or by proteases. Evidence suggests that certain classes of proteases may be involved in the process, including but are not limited to Ser/Thr proteases such as Kallikreins, chemotrypsin, elastases, plasmin, thrombin, as well as zinc metalloproteases of MMP family, such as MMP-2, MMP-9 and MMP-13, and the Adam family of proteases, such as Adam10 and Adam17. MMP-2 degrades the most abundant component of the basement membrane, Collagen IV, raising the possibility that it may play a role in ECM-associated TGFβ1 regulation. MMP-9 has been implicated to play a central role in tumor progression, angiogenesis, stromal remodeling and metastasis, including in carcinoma, such as breast cancer. Thus, protease-dependent activation of TGFβ1 in the ECM may be important for treating ECM-associated diseases such as fibrosis and cancer.

[763] Kallikreins (KLKs) are trypsin- or chymotrypsin-like serine proteases that include plasma Kallikreins and tissue Kallikreins. The ECM plays a role in tissue homeostasis acting as a structural and signaling scaffold and barrier to suppress malignant outgrowth. KLKs may play a role in degrading ECM proteins and other components which may facilitate tumor expansion and invasion. For example, KLK1 is highly upregulated in certain breast cancers and can activate pro-MMP-2 and pro-MMP-9. KLK2 activates latent TGFβ1, rendering prostate cancer adjacent to fibroblasts permissive to cancer growth. KLK3 has been widely studied as a diagnostic marker for prostate cancer (PSA). KLK3 may directly activate TGFβ1 by processing plasminogen into plasmin, which proteolytically cleaves LAP, thereby causing the TGFβ1 growth factor to be released from the latent complex. KLK6 may be a potential marker for Alzheimer's disease.

[764] Moreover, data previously provided by Applicant indicated that such proteases may be a Kallikrein. Thus, the disclosure encompasses the use of an isoform-specific, context-independent inhibitor of TGFβ1 in a method for treating a disease associated with Kallikrein or a Kallikrein-like protease. In some embodiments, the TGFβ1 inhibitor is Ab6, or derivatives thereof.

[765] Known activators of TGFβ1, such as plasmin, TSP-1 and αVβ6 integrin, all interact directly with LAP. It is postulated that proteolytic cleavage of LAP may destabilize the LAP-TGFβ interaction, thereby releasing active TGFβ1 (the growth factor domain) from the latent complex. It has been suggested that the region containing the amino acid stretch 54-LSKLRL-59 (SEQ ID NO: 171) is important for maintaining TGFβ1 latency. Thus, agents (*e.g.*, antibodies) that stabilize the interaction, or block the proteolytic cleavage of LAP may prevent TGFβ1 activation.

[766] Many of these proteases associated with pathological conditions (*e.g.*, cancer) function through distinct mechanisms of action. Thus, targeted inhibition of particular proteases, or combinations of proteases, may provide therapeutic benefits for the treatment of conditions involving the protease-TGFβ axis. Accordingly, it is contemplated that inhibitors (*e.g.*, TGFβ1 antibodies) that selectively inhibit protease-induced activation of TGFβ1 may be advantageous in the treatment of such diseases (*e.g.*, cancer). Similarly, selective inhibition of TGFβ1 activation by one protease over another protease may also provide therapeutic benefit, depending on the condition being treated.