

MULTIFUNCTIONAL MOLECULES BINDING TO TCR AND USES THEREOF

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/172,468 filed on April 08, 2021, the entire contents of which are hereby incorporated by reference.

BACKGROUND

[0002] Currently available molecules designed to redirect T cells to promote tumor cell lysis for cancer immunotherapy typically target the CD3 epsilon (CD3e) subunit of the T cell receptor (TCR). However, there are limitations to this approach. Previous studies have shown that, e.g., low doses of anti-CD3e monoclonal antibody (mAb) can cause T cell dysfunction and exert immunosuppressive effects. In addition, anti-CD3e mAbs bind to all T cells and thus activate a large number of T cells. Such non-physiological massive activation of T cells by these anti-CD3e mAbs can result in the production of proinflammatory cytokines such as IFN-gamma, IL-1-beta, IL-6, IL-10 and TNF-alpha, causing a “cytokine storm” known as the cytokine release syndrome (CRS), which is also associated with neurotoxicity (NT). Thus, there is a need for improved T cell receptor-binding molecules that redirect T cells for cancer immunotherapy.

SUMMARY

[0002a] In some aspects, described herein is a multifunctional polypeptide molecule comprising a first polypeptide, a second polypeptide, and at least one cytokine polypeptide or a functional fragment or variant thereof, wherein the first polypeptide and the second polypeptide are non-contiguous, wherein: (i) the first polypeptide comprises a first portion of a dimerization module linked to (A) a TCRβV-binding moiety comprising a heavy chain variable domain (VH) and a light chain variable domain (VL), or a single domain antibody, or (B) a first portion of a TCRβV-binding moiety comprising a VH of the TCRβV-binding moiety, wherein the multifunctional polypeptide molecule further comprises a third polypeptide comprising a second portion of the TCRβV-binding moiety comprising a VL of the TCRβV-binding moiety, wherein the third polypeptide is non-contiguous with the first polypeptide and the second polypeptide; and (ii) the second polypeptide comprises a second portion of the dimerization module, wherein the at least one cytokine polypeptide or a functional fragment or variant thereof is covalently linked to the second polypeptide; and, wherein the at least one cytokine polypeptide or a functional fragment or variant thereof comprises a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3523, optionally, wherein the at least one cytokine polypeptide or a functional fragment or variant thereof comprises the sequence of SEQ ID NO: 3523.

[0002b] In another aspects, described herein is a multifunctional polypeptide molecule comprising a first polypeptide, a second polypeptide, and a third polypeptide, wherein the first polypeptide, the second polypeptide, and the third polypeptide are non-contiguous; and wherein: (i) the first polypeptide comprises, from N-terminus to C-terminus, a sequence having at least 90% sequence identity to the

sequence of SEQ ID NO: 1346 operatively linked to a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3649; (ii) the second polypeptide comprises, from N-terminus to C-terminus, a sequence having at least 90% sequence identity to any one sequence selected from the group consisting of SEQ ID NO: 2170, SEQ ID NO: 2193, SEQ ID NO: 2270, SEQ ID NO: 3540, and SEQ ID NO: 3542 operatively linked to a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3648, optionally, via the sequence of SEQ ID NO: 3308; and (iii) the third polypeptide comprises, from N-terminus to C-terminus, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1349 operatively linked to a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3644.

[0002c] In another aspects, described herein is a recombinant polynucleotide comprising a sequence encoding the multifunctional polypeptide molecule as described herein.

[0002d] In another aspects, described herein is a method of making the multifunctional polypeptide molecule as described herein comprising culturing a host cell comprising the recombinant polynucleotide as described herein under conditions suitable for expression of the multifunctional polypeptide molecule.

[0002e] In another aspects, described herein is a medicament comprising the multifunctional polypeptide molecule as described herein or the recombinant polynucleotide as described herein, and a pharmaceutically acceptable carrier, excipient, diluent, or stabilizer.

[0002f] In another aspects, described herein is a use of the multifunctional polypeptide molecule as described herein, the recombinant polynucleotide as described herein, or the medicament as described herein in the manufacture of a pharmaceutical composition for treating cancer in a subject in need thereof, wherein the pharmaceutical composition comprises a therapeutically effective amount of the multifunctional polypeptide molecule, the recombinant polynucleotide, or the medicament for treating cancer in the subject when administered to the subject.

[0002g] In another aspects, described herein is a pharmaceutical composition comprising the multifunctional polypeptide molecule as described herein, the recombinant polynucleotide as described herein, or the medicament as described herein for use in therapy.

[0003] In some aspects, described herein is a multifunctional polypeptide molecule comprising a first polypeptide, a second polypeptide, and at least one cytokine polypeptide or a functional fragment or a functional variant thereof, wherein the first polypeptide and the second polypeptide are non-contiguous, wherein (i) the first polypeptide comprises a first portion of a dimerization module linked to (A) a first TCR β V-binding moiety comprising a first heavy chain variable domain (VH) and a first light chain variable domain (VL), or a single domain antibody, or (B) a first portion of a first TCR β V-binding moiety comprising a VH of the first TCR β V-binding moiety, wherein when the first polypeptide comprises the first portion of the first TCR β V-binding moiety, the multifunctional polypeptide molecule further comprises a third polypeptide comprising a second portion of the first TCR β V-binding moiety comprising a VL of the first TCR β V-binding moiety, wherein the third polypeptide is non-contiguous with the first polypeptide and the second polypeptide; and (ii) the second polypeptide comprises a second portion of the

dimerization module; wherein (a) the multifunctional polypeptide molecule comprises a single TCR β V-binding moiety and the at least one cytokine polypeptide or a functional fragment or a functional variant thereof is covalently linked to the second polypeptide, or (b) the multifunctional polypeptide molecule further comprises a second TCR β V-binding moiety and the at least one cytokine polypeptide or a functional fragment or a functional variant thereof is covalently linked to the first polypeptide, the second polypeptide, the third polypeptide when the multifunctional polypeptide molecule further comprises the third polypeptide, or a combination thereof.

[00156] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising, from the N-terminus to the C-terminus, an anti-TCR $\nu\beta$ antibody heavy chain variable region operatively linked to an immunoglobulin heavy chain constant region; (ii) a second polypeptide comprising, from the N-terminus to the C-terminus, an IL-21 molecule or a functional fragment or a functional variant thereof operatively linked to an immunoglobulin heavy chain constant region; and (iii) a third polypeptide comprising, from the N-terminus to the C-terminus, an anti-TCR $\nu\beta$ antibody light chain variable region operatively linked to an immunoglobulin light chain constant region.

[00157] In some embodiments, the IL-21 molecule or a functional fragment or a functional variant thereof is operatively linked to the immunoglobulin heavy chain constant region via a linker, or a combination thereof.

[00158] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising the sequence of SEQ ID NO: 1346, and the sequence of SEQ ID NO: 3649; (ii) a second polypeptide comprising the sequence of SEQ ID NO: 2193, and the sequence of SEQ ID NO: 3648; and (iii) a third polypeptide comprising the sequence of SEQ ID NO: 1349, and the sequence of SEQ ID NO: 3644.

[00159] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising, from the N-terminus to the C-terminus, the sequence of SEQ ID NO: 1346 operatively linked to the sequence of SEQ ID NO: 3649; (ii) a second polypeptide comprising, from the N-terminus to the C-terminus, the sequence of SEQ ID NO: 2193 operatively linked to the sequence of SEQ ID NO: 3648; and (iii) a third polypeptide comprising, from the N-terminus to the C-terminus, the sequence of SEQ ID NO: 1349 operatively linked to the sequence of SEQ ID NO: 3644.

[00160] In some embodiments, the sequence of SEQ ID NO: 2193 is operatively linked to the sequence of SEQ ID NO: 3648 via the sequence of SEQ ID NO: 3308.

[00161] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising a sequence having at least 75% sequence identity to the sequence of SEQ ID NO: 3517; (ii) a second polypeptide comprising a sequence having at least 75% sequence identity to the sequence of SEQ ID NO: 3546; and (iii) a third polypeptide comprising a sequence having at least 75% sequence identity to the sequence of SEQ ID NO: 3518.

[00162] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising the sequence of SEQ ID NO: 3517; (ii) a second polypeptide comprising the sequence of SEQ ID NO: 3546; and (iii) a third polypeptide comprising to the sequence of SEQ ID NO: 3518.

[00163] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising an anti-TCR $\nu\beta$ antibody heavy chain variable region, and an immunoglobulin heavy chain constant region; and (ii) a second polypeptide comprising an anti-TCR $\nu\beta$ antibody light chain variable region, an immunoglobulin light chain constant region, and an IL-2 molecule or a functional fragment or a functional variant thereof.

activated/expanded with the indicated antibody (anti-TCR β V 6-5 v1 (plate coated), anti-CD3 ϵ (plate coated), anti-TCR β V 6-5 v1 (in solution), or anti-CD3 ϵ (in solution) and cultured with said antibody for the indicated number of days (1, 3, 5, or 7). **FIG. 95L** is a bar graph showing the level of secreted TNF- α by T cells activated/expanded with the indicated antibody (anti-TCR β V 6-5 v1 (plate coated), anti-CD3 ϵ (plate coated), anti-TCR β V 6-5 v1 (in solution), or anti-CD3 ϵ (in solution) and cultured with said antibody for the indicated number of days (1, 3, 5, or 7).

[00293]FIG. 96 is a FACS plot showing the showing the ability of MH3-2 to bind PBMCs from one of two donors when the PBMCs are either preincubated with TM23 or not (MH3-2 Alone).

[00294]FIG. 97 is a FACS plot showing the ability of MH3-2 to bind PBMCs from one of two donors when the PBMCs are either preincubated with TM23 or not (MH3-2 Alone).

[00295]FIG. 98A is a bar graph showing the polyfunctional strength index (PSI) of PBMC CD4 $^{+}$ T cells, CD4 $^{+}$ T cells expanded with anti-CD3 antibody, (CD3 Expanded T cells), and CD4 $^{+}$ T cells expanded with anti-TCRV β 6-5 antibody (Drug Expanded T cells). The Effector mediators are Granzyme B, IFN γ , MIP-1 α , perforin, TNF α , and TNF β . The Stimulatory mediators are IL-5. The Chemoattractive mediators are MIP-1b. **FIG. 98B** is a bar graph showing the polyfunctional strength index (PSI) of PBMC CD8 $^{+}$ T cells, CD8 $^{+}$ T cells expanded with anti-CD3 antibody, (CD3 Expanded T cells), and CD8 $^{+}$ T cells expanded with anti-TCRV β 6-5 antibody (Drug Expanded T cells). The Effector mediators are Granzyme B, IFN γ , MIP-1 α , perforin, and TNF β . The Chemoattractive mediators are MIP-1b and RANTES.

[00296]FIG. 99 is a schematic of the experimental design for the pharmacokinetic (PK) profile and dosing strategy of the multifunctional polypeptide molecule as described herein.

[00297]FIG. 100 shows Table 9, which depicts alignment of TCRBV amino acid sequences (SEQ ID NOS: 3457-3516, 3669-3673, 3522, 3674-3675, 3525, 3676-3687, 3538, 3688-3698, 3550-3639 and 3699-3790, respectively, in order of appearance). The alignment of TCRBV amino acid sequences in Table 9 underscores the diversity of TCR sequences. In particular, the TRBV sequences from different subfamilies are considerably different from each other.

[00298]FIG. 101 shows alignment of affinity matured humanized Antibody A-H VL sequences (SEQ ID NOS: 3377-3389, respectively, in order of appearance).

[00299]FIG. 102 shows alignment of affinity matured humanized Antibody A-H VH sequences (SEQ ID NOS: 3390-3436, respectively, in order of appearance).

[00300]FIG. 103A shows an exemplary embodiment (e.g., BKM0186) of multifunctional molecules comprising a TCR β V-binding moiety and a cytokine polypeptide (e.g., IL2 or IL2-C125A) as described herein. **FIG. 103B** shows an exemplary embodiment of multifunctional molecules comprising a TCR β V-binding moiety and a cytokine polypeptide as described herein. **FIGS. 103C, 103D, 103E, and 103F** show exemplary embodiments of multifunctional molecules comprising a first TCR β V-binding moiety, a second TCR β V-binding moiety, and two cytokine polypeptides as described herein. In some embodiments, the cytokine polypeptide comprises IL-2 or a functional fragment or a functional variant

thereof, IL2-C125A or a functional fragment or a functional variant thereof, IL-15 or a functional fragment or a functional variant thereof, IL-7 or a functional fragment or a functional variant thereof, IL-

[001129] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3517; (ii) a second polypeptide comprising a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3541; and (iii) a third polypeptide comprising a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3518.

[001130] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising the sequence of SEQ ID NO: 3517; (ii) a second polypeptide comprising the sequence of SEQ ID NO: 3541; and (iii) a third polypeptide comprising to the sequence of SEQ ID NO: 3518.

[001131] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 1346, and a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3649; (ii) a second polypeptide comprising a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3540, and a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3648; and (iii) a third polypeptide comprising a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 1349, and a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3644.

[001132] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising the sequence of SEQ ID NO: 1346, and the sequence of SEQ ID NO: 3649; (ii) a second polypeptide comprising the sequence of SEQ ID NO: 2193, and the sequence of SEQ ID NO: 3648; and (iii) a third polypeptide comprising the sequence of SEQ ID NO: 1349, and the sequence of SEQ ID NO: 3644.

[001133] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising, from the N-terminus to the C-terminus, a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 1346 operatively linked to a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3649; (ii) a second polypeptide comprising,

from the N-terminus to the C-terminus, a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 2193 operatively linked to a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3648; and (iii) a third polypeptide comprising, from the N-terminus to the C-terminus, a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 1349 operatively linked to a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3644.

[001134] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising, from the N-terminus to the C-terminus, the sequence of SEQ ID NO: 1346 operatively linked to the sequence of SEQ ID NO: 3649; (ii) a second polypeptide comprising, from the N-terminus to the C-terminus, the sequence of SEQ ID NO: 2193 operatively linked to the sequence of SEQ ID NO: 3648; and (iii) a third polypeptide comprising, from the N-terminus to the C-terminus, the sequence of SEQ ID NO: 1349 operatively linked to the sequence of SEQ ID NO: 3644.

[001135] In some embodiments, a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 2193 is operatively linked to a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3648 via a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3308.

[001136] In some embodiments, the sequence of SEQ ID NO: 2193 is operatively linked to the sequence of SEQ ID NO: 3648 via the sequence of SEQ ID NO: 3308.

[001137] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3517; (ii) a second polypeptide comprising a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3546; and (iii) a third polypeptide comprising a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the sequence of SEQ ID NO: 3518.

[001138] In some embodiments, the multifunctional polypeptide molecule comprises: (i) a first polypeptide comprising the sequence of SEQ ID NO: 3517; (ii) a second polypeptide comprising the sequence of SEQ ID NO: 3546; and (iii) a third polypeptide comprising to the sequence of SEQ ID NO: 3518.

			SRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTD KTSATVICRKNASISVRAQDRYSSSWSEWASVPC
	3544	Linker	SGGGSGGGSGGGGS
	3545	IL-12 alpha subunit	RNLPVATPDPMFPCLLHHSQNLRLRAVSNNMLQKARQTLEFYPTCTSE EIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASR KTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIR AVTIDRVMSYLNAS
	3308	linker	GGGGSGGGGS
	3648	Human Fc Knob cys N297A	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK
Chain 3: TCRVβ light chain (CDRs underlined)	3518	Full Sequence	DIQMTQSPSFLSASVGDRTTITCKASQNVADRVVWHQKPGKAP KALIY <u>SSSHRYKGV</u> PSRFSGSGSGTEFTLTISSLQPEDFATYFCQQF <u>KSYPLTFGQGT</u> KTLEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	1349	anti-TCRVβ VL	DIQMTQSPSFLSASVGDRTTITCKASQNVADRVVWHQKPGKAP KALIY <u>SSSHRYKGV</u> PSRFSGSGSGTEFTLTISSLQPEDFATYFCQQF <u>KSYPLTFGQGT</u> KTLEIK
	3644	Immunoglobulin kappa constant C region	RTVAAPSVFIFPPSDEQLKSGTASVCLLNPFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
BLM0321 (IL-21)			
Chain 1: TCRVβ- Fc_Hole_Cys_ N297A heavy chain (CDRs underlined)	3517	Full Sequence	QVQLVQSGAEVKKPGSSVKVSCKASGHDFRLTYIHVWRQAPGQG LEWMGRVSAGSGNVKYNEKFKGRVTITADTSTSTAYMELSSLRS EDTAVYYCAV <u>SYYSYDVL</u> DYWGQGTITVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSR EEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLD DGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
	1346	anti-TCRVβ VH	QVQLVQSGAEVKKPGSSVKVSCKASGHDFRLTYIHVWRQAPGQG LEWMGRVSAGSGNVKYNEKFKGRVTITADTSTSTAYMELSSLRS EDTAVYYCAV <u>SYYSYDVL</u> DYWGQGTITVTVSS
	3649	Human IgG1 hole cys N297A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSN TKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVCTLPSSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
Chain 2: IL-21 – GS linker – IL15 – GS linker - Fc_Knob_Cys_ N297A	3546	Full Sequence	QGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEW SAFSCFQKAQLKSANTGNNERIINVSIIKKLRKPPSTNAGRRQKHR LTCPCDSYEKKPPKEFLERFKSLLQKMIHQHLSRTHGSEDSGGG GSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
	2193	IL-21	QGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEW SAFSCFQKAQLKSANTGNNERIINVSIIKKLRKPPSTNAGRRQKHR LTCPCDSYEKKPPKEFLERFKSLLQKMIHQHLSRTHGSEDS