

ANTI-KIR3DL3 ANTIBODIES AND USES THEREOF

Cross-Reference to Related Applications

This application claims the benefit of priority to U.S. Provisional Application Serial No. 62/910,594, filed on 04 October 2019; the entire contents of said application is incorporated herein in its entirety by this reference.

Statement of Rights

This invention was made with government support under grant number P50CA101942 awarded by The National Institutes of Health. The government has certain rights in the invention.

Sequence Listing

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 November 17, 2020, is named DFS-277 25 SL.txt and is 120,293 bytes in size.

Background of the Invention

Immune checkpoints, such as CTLA-4, PD-1, VISTA, B7-H2, B7-H3, PD-L1, B7-H4, B7-H6, ICOS, HVEM, PD-L2, CD160, gp49B, PIR-B, KIR family receptors, TIM-1, TIM-3, TIM-4, LAG-3, GITR, 4-1BB, OX-40, BTLA, SIRPalpha (CD47), CD48, 2B4 (CD244), B7.1, B7.2, ILT-2, ILT-4, TIGIT, butyrophilins, and A2aR, and many more, negatively regulate immune response progression based on complex and combinatorial interactions between numerous inputs. Inhibitors of immune checkpoints can modulate immune responses in some subjects, but immune checkpoint expression and interactions with natural binding partners vary between subjects and within tissues of a subject. A significant percentage of patients do not respond to this treatment and the many patients that do respond eventually develop resistance. Thus, there is a critical unmet need to find additional immune pathways that are non-redundant with the PD-1 pathway.

HERV-H LTR-associating 2 (HHLA2, also known as B7-H5, B7-H7) is a B7 family member that modulates T-cell functions. HHLA2 is broadly expressed in a variety of tumors (*e.g.*, solid and hematologic cancers including primary human renal cell carcinoma (RCC)) and antigen presenting cells and has been implicated as both an activating and inhibitory ligand for T cells. HHLA2 was identified as a specific ligand for TMIGD2 (CD28H, IGPR-1) and the HHLA2/TMIGD2 interaction selectively costimulates human T-cell growth and cytokine production via an AKT-dependent signaling cascade (Zhu *et al.* (2013) *Nat. Comm.* 4:2043; Janakiram *et al.* (2015) *Clin. Cancer Res.* 21:2359–2366). TMIGD2 expressed in naive T cells is an activating receptor for HHLA2 and transduces co-stimulatory signals following T cell antigen receptor (TCR) engagement. TMIGD2 is

AVS-3698HC [574.2.1C7.D2.6.5 heavy chain]

Region	Sequence Fragment	Residues	Length
HFR1	QVQLQQSGAELMKPGASVKISKAT (SEQ ID NO: 13)	1 - 25	25
CDR-H1	GYTFSNY (SEQ ID NO: 14)	26 - 32	7
HFR2	WIEWIKQRPBGHGLEWIGEI (SEQ ID NO: 15)	33 - 51	19
CDR-H2	LPGSGS (SEQ ID NO: 16)	52 - 57	6
HFR3	TNYNEKFKGKATFTAATSSNTAYMQLSSLTSEDSAVYYCAR (SEQ ID NO: 17)	58 - 98	41
CDR-H3	RVPDYYGSRYYFDY (SEQ ID NO: 18)	99 - 112	14
HFR4	WGQGTTLTVSS (SEQ ID NO: 19)	113 - 123	11

AVS-3698LC [574.2.1C7.D2.6.5 light chain]

Region	Sequence Fragment	Residues	Length
LFR1	NIVMTQSPKSMMSVGERVTLS (SEQ ID NO: 20)	1 - 23	23
CDR-L1	KASENVGIYVS (SEQ ID NO: 21)	24 - 34	11
LFR2	WYQQKPEQSPKLLIY (SEQ ID NO: 22)	35 - 49	15
CDR-L2	GASNRYT (SEQ ID NO: 23)	50 - 56	7
LFR3	GVPDRFTGSGSATDFTLTISVQAEDLADYHC (SEQ ID NO: 24)	57 - 88	32
CDR-L3	GQSYNYPFT (SEQ ID NO: 25)	89 - 97	9
LFR4	FGSGTKLEIK (SEQ ID NO: 26)	98 - 107	10

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3698	574.2.1C7.D2.6.5	AVS-3698HC.3	HC	GYTFSNY (SEQ ID NO: 14)	LPGSGS (SEQ ID NO: 16)	RVPDYYGSRYYFDY (SEQ ID NO: 18)
Protein sequence			Nucleotide sequence				
QVQLQQSGAELMKPGASVKISKATGYTFSNYWIEWIKQRPBGHGLEWIGEILPGSGSTNYNEFKGKATFTAATSSNTAYMQLSSLTSEDSAVYYCARRVPDYYGSRYYFDYWGQGTTLTVSS (SEQ ID NO: 27)			CAGGTTTCAGCTGCAGCAGTCTGGAGCTGAGCTGATGAAGCC TGGGGCCTCAGTGAAGATATCCTGCAAGGCTACTGGCTACAC ATTCAGTAATACTACTGGATAGAGTGGATAAAGCAGAGGCCTG GACATGGCCTTGAGTGGATTGGAGAGATTTTACCTGGAAGTG GTAGTACTAATACTACAATGAGAAGTTCAAGGGCAAGGCCACA TTCACTGCAGCTACATCCTCCAACACAGCCTACATGCAACTC AGCAGCCTGACATCTGAGGACTCTGCCGTCTATTACTGTGCA AGAAGGGTCCCTGATTACTACGGTAGTAGGTACTACTTTGAC TACTGGGGCCAAGGCACCACTCTCACAGTCTCTCTCA (SEQ ID NO: 28)				

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3698	574.2.1C7.D2.6.5	AVS-3698LCP.4	LC	KASENVGIYVS (SEQ ID NO: 21)	GASNRYT (SEQ ID NO: 23)	GQSYNYPFT (SEQ ID NO: 25)
Protein sequence			Nucleotide sequence				

NIVMTQSPKSMMSVGERVTL SCKASENVGIYVSWYQQKPEQ SPKLLIYGASNRYTGVDPDRFTG SGSATDFTLTISSVQAEDLADY HCGQSYNYPFTFGSGTKLEIK (SEQ ID NO: 29)	AACATTGTAATGACCCAATCTCCCAAATCCATGTCCATGTCA GTAGGAGAGAGGGTCACCTTGAGCTGCAAGGCCAGTGAGAA TGTGGGTATTTACGTATCCTGGTATCAACAGAAACCAGAGCA GTCTCCTAAACTACTAATATACGGGGCATCCAACCGATACAC TGGGGTCCCCGATCGCTTACAGGCAGTGGATCTGCAACAG ATTTCACTCTGACCATCAGCAGTGTGCAGGCTGAAGACCTTG CAGATTATCACTGTGGACAGAGTTACAACCTATCCATTACGT TCGGCTCGGGGACAAAGTTGGAAATCAAA (SEQ ID NO: 30)
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2A3 Heavy Chain Variable (vH) and Light Chain Variable (vL) DNA and Amino Acid Sequences*

AVS-3699HC [574.2.2A3.5.9 heavy chain]

Region	Sequence Fragment	Residues	Length
HFR1	EVQLQQSGPELVKPGASVKMSCKAS (SEQ ID NO: 31)	1 - 25	25
CDR-H1	GYTFTSY (SEQ ID NO: 32)	26 - 32	7
HFR2	VMHWLQKPGQGLEWIAIYI (SEQ ID NO: 33)	33 - 51	19
CDR-H2	NPYNDG (SEQ ID NO: 34)	52 - 57	6
HFR3	TKYNENFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCAR (SEQ ID NO: 35)	58 - 98	41
CDR-H3	PSMVTAAWFAY (SEQ ID NO: 36)	99 - 109	11
HFR4	WGQGTLLTVSA (SEQ ID NO: 37)	110 - 120	11

AVS-3699LC [574.2.2A3.5.9 light chain]

Region	Sequence Fragment	Residues	Length
LFR1	DVVMQTPLSLPVSLGDQASISC (SEQ ID NO: 38)	1 - 23	23
CDR-L1	RSSQNLAHSNGDITYLH (SEQ ID NO: 39)	24 - 39	16
LFR2	WYLQKPGQSPKLLIY (SEQ ID NO: 40)	40 - 54	15
CDR-L2	KVSNRFS (SEQ ID NO: 41)	55 - 61	7
LFR3	GVPDRFSGSGSTDFTLKISRVEADLGVIYFC (SEQ ID NO: 42)	62 - 93	32
CDR-L3	SQTTHVLWT (SEQ ID NO: 43)	94 - 102	9
LFR4	FGGGTKLEIK (SEQ ID NO: 44)	103 - 112	10

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3699	574.2.2A3.5.9	AVS-3699HC.1	HC	GYTFTSY (SEQ ID NO: 32)	NPYNDG (SEQ ID NO: 34)	PSMVTAAWFAY (SEQ ID NO: 36)
Protein sequence			Nucleotide sequence				
EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWLQKPGQGLEWIAIYNPYNDGTKYNENFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARPSMVTAAWFAYWGQGTLLTVSA (SEQ ID NO: 45)			GAGGTCCAGCTGCAGCAGTCTGGACCTGAGCTGGTAAAGCC TGGGGCTTCAGTGAAGATGTCCTGCAAGGCTTCTGGATACACATTCAGTAGCTATGTCATGCACTGGTTGAAGCAGAAGCCTGGGCAGGGCCTTGAGTGGATTGCATATATTAATCCTTACAA TGATGGTACTAAGTACAATGAGAACTTCAAAGGCAAGGCCACACTGACTTCAGACAAATCCTCCAGCACAGCCTACATGGA GCTCAGCAGCCTGACCTCTGAGGACTCTGCGGTCTATTACTGTGCAAGACCTAGTATGGTAACTGCCGCTGGTTTGCTTAC TGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 46)				

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3699	574.2.2A3.5.9	AVS-3699LCP.1	LC	RSSQNLAH SNGDTYLH (SEQ ID NO: 39)	KVSNRFS (SEQ ID NO: 41)	SQTTHVLW T (SEQ ID NO: 43)
Protein sequence			Nucleotide sequence				
DVVMTQTPLSLPVSLGDQASIS CRSSQNLAHSNGDTYLHWYLQ KPGQSPKLLIYKVSNRFSGVPD RFSGSGSGTDFTLKISRVEAEDL GVYFCSQTTHVLWTFGGGTKL EIK (SEQ ID NO: 47)			GATGTTGTGATGACCCAACTCCACTCTCCCTGCCTGTCAGT CTTGAGATCAAGCCTCCATCTCCTGCAGATCTAGTCAGAA CCTTGACACAGTAATGGAGACACCTATTACATTGGTACC TGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATCTACAAA GTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTTCAGTGG CAGTGGATCAGGGACAGATTTCACTCAAGATCAGCAGA GTGGAGGCTGAGGATCTGGGAGTTTATTTCTGCTCTCAAAC TACACATGTTTTGTGGACGTTTCGGTGGAGGCACCAAGCTGG AAATCAAA (SEQ ID NO: 48)				

8F7 Heavy Chain Variable (vH) and Light Chain Variable (vL) DNA and Amino Acid Sequences*

AVS-3700HC [574.2.8F7.1.7.4 heavy chain]

Region	Sequence Fragment	Residues	Length
HFR1	DVKLVESGGGLVKPGGSLKLSAAS (SEQ ID NO: 49)	1 - 25	25
CDR-H1	GFTFSSY (SEQ ID NO: 50)	26 - 32	7
HFR2	CMSWVRQTPEKRLEWVATI (SEQ ID NO: 51)	33 - 51	19
CDR-H2	SSGGNY (SEQ ID NO: 52)	52 - 57	6
HFR3	IYYPDVSVKGRFTISRDIKNTLYLQMSLKS EDTAMYYCTR (SEQ ID NO: 53)	58 - 98	41
CDR-H3	GGYDERYFDY (SEQ ID NO: 54)	99 - 108	10
HFR4	WGQGTILTVSS (SEQ ID NO: 55)	109 - 119	11

AVS-3700LC [574.2.8F7.1.7.4 light chain]

Region	Sequence Fragment	Residues	Length
LFR1	DIQMTQSPASLSASVGETVTITC (SEQ ID NO: 56)	1 - 23	23
CDR-L1	RASENIYSYLA (SEQ ID NO: 57)	24 - 34	11
LFR2	WYQQKQKGKSPQLLVY (SEQ ID NO: 58)	35 - 49	15
CDR-L2	NAKTLTE (SEQ ID NO: 59)	50 - 56	7
LFR3	GMPSRFSGSGTQFSLRINSLQPEDFGSYYC (SEQ ID NO: 60)	57 - 88	32
CDR-L3	QHHHGTPRT (SEQ ID NO: 61)	89 - 97	9
LFR4	FGGGTKLEIK (SEQ ID NO: 44)	98 - 107	10

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3700	574.2.8F7.1.7.4	AVS-3700HC.2	HC	GFTFSSY (SEQ ID NO: 50)	SSGGNY (SEQ ID NO: 52)	GGYDERYFDY (SEQ ID NO: 54)
Protein sequence			Nucleotide sequence				

DVKLVESGGGLVKPGGSLKLSC AASGFTFSSYCMSWVRQTPEKR LEWVATISSGGNYIYPDSVKG RFTISRDI AKNTLYLQMSSLKSE DTAMYYCTRGGYDERYFDYW GQGTILTVSS (SEQ ID NO: 62)	GACGTGAAGTTGGTGGAGTCTGGGGGAGGCTTAGTGAAAC CTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCTGGATTC ACTTTCAGTTCCTATTGCATGTCTTGGGTTCCGCCAGACTCCG GAGAAGAGGCTGGAGTGGGTGCGCAACCATTAGTAGTGGTG GTAATTACATCTACTATCCGGACAGTGTGAAGGGCCGATTC ACCATCTCCAGAGACATTGCCAAGAACACCCTGTACCTGCA AATGAGCAGTCTGAAGTCTGAGGACACAGCCATGTATTACT GTACAAGAGGGGGTTACGACGAGAGGTACTTTGACTACTGG GGCCAAGGCACCATTCTCACAGTCTCTCA (SEQ ID NO: 63)
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SR linked	AVS Linke d	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3700	574.2.8F7.1.7.4	AVS-3700LCP.3	LC	RASENIYSYL A (SEQ ID NO: 57)	NAKTLT E (SEQ ID NO: 59)	QHHHGTPR T (SEQ ID NO: 61)
Protein sequence			Nucleotide sequence				
DIQMTQSPASLSASVGETVTITC RASENIYSYLAWYQQKQKSP QLLVYNAKLTLEGMPSRFSGS SGTQFSLRINSLQPEDFGSYCQ HHHGTPRTFGGGTKLEIK (SEQ ID NO: 64)			GACATCCAGATGACTCAGTCTCCAGCCTCCCTTTCTGCTTCT GTGGGAGAACTGTCACCATCACATGTCGAGCAAGTGAAA ATATTTACAGTTATTTAGCATGGTATCAGCAGAAACAGGGA AAATCTCTCAGCTCCTGGTCTATAATGCAAAAACCTTAAC AGAAGGTATGCCATCAAGGTTCAAGTGCAGTGGATCAGGTA CACAGTTTTCTCTGAGGATCAATAGCCTGCAGCCTGAAGAT TTTGGGAGTTATTACTGTCAACATCATCATGGTACTCCTCGG ACGTTTCGGTGGAGGCACCAAGTTGGAAATCAAA (SEQ ID NO: 65)				

1G7 Heavy Chain Variable (vH) and Light Chain Variable (vL) DNA and Amino Acid Sequences*

AVS-3701HC [574.2.1G7.9.9 heavy chain]

Region	Sequence Fragment	Residues	Length
HFR1	ELKLEESGGGLVQPGGSMKLSVAS (SEQ ID NO: 66)	1 - 25	25
CDR-H1	GFTFSNY (SEQ ID NO: 67)	26 - 32	7
HFR2	WMNWVRQSPEKLEWIAEI (SEQ ID NO: 68)	33 - 51	19
CDR-H2	RLKSNNYA (SEQ ID NO: 69)	52 - 59	8
HFR3	THYAESVKGRFTISRDDSKSSVYLQMNNLRAEDTGIFYCTP (SEQ ID NO: 70)	60 - 100	41
CDR-H3	FYGSSFPY (SEQ ID NO: 71)	101 - 108	8
HFR4	WGQGTLVTVSA (SEQ ID NO: 37)	109 - 119	11

AVS-3701LC [574.2.1G7.9.9 light chain]

Region	Sequence Fragment	Residues	Length
LFR1	DIQMTQTSSLSASLGDRVTISC (SEQ ID NO: 72)	1 - 23	23
CDR-L1	RASQDISNYLN (SEQ ID NO: 73)	24 - 34	11
LFR2	WYQQKPDGTVKLLIY (SEQ ID NO: 74)	35 - 49	15
CDR-L2	YTSILQL (SEQ ID NO: 75)	50 - 56	7
LFR3	GVPPRFSGSGSGTDYSLTISNLEQEDIATYFC (SEQ ID NO: 76)	57 - 88	32
CDR-L3	QQGNTLPFT (SEQ ID NO: 77)	89 - 97	9
LFR4	FGAGTKVELK (SEQ ID NO: 78)	98 - 107	10

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3701	574.2.1G7.9.9	AVS-3701HC T.2	HC	GFTFSNY (SEQ ID NO: 67)	RLKSNNY A (SEQ ID NO: 69)	FYGSSFPY (SEQ ID NO: 71)
Protein sequence			Nucleotide sequence				
ELKLEESGGGLVQPGGSMKLSCV ASGFTFSNYWMNWVRQSPEKGL EWIAEIRLKSNNYATHYAESVKG RFTISRDDSKSSVYLQMNRLRAE DTGIFYCTPFYGSSFPYWGGQTLV TVSA (SEQ ID NO: 79)			GAACTGAAGCTTGAGGAGTCTGGAGGAGGCTTGGTGCAA CCTGGAGGATCCATGAAACTCTCTGTGTTCCTCTGGAT TCACTTTCAGTAACTACTGGATGAACTGGGTCCGCCAGTC TCCAGAGAAGGGGCTTGAATGGATTGCTGAAATTAGATT GAAATCTAATAATTATGCAACACATTATGCGGAGTCTGTG AAAGGGAGGTTCAACATCTCAAGAGATGATTCCAAAAGT AGTGTCTACCTACAGATGAACAACTTAAGAGCTGAAGAC ACTGGCATTCTTTACTGTACCCCTTCTACGGTAGTTCTTT TCCTTACTGGGGCCAAGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 80)				

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3701	574.2.1G7.9.9	AVS-3701LCP.2	LC	RASQDISN YLN (SEQ ID NO: 73)	YTSILQL (SEQ ID NO: 75)	QQGNTLPFT (SEQ ID NO: 77)
Protein sequence			Nucleotide sequence				
DIQMTQTSSLSASLGDRVITISC RASQDISNYLNWYQQKPDGTV KLLIYYTSILQLGVPPRFGSGS GTDYSLTISNLEQEDIATYFCQQ GNTLPFTFGAGTKVELK (SEQ ID NO: 81)			GATATCCAGATGACACAGACTACATCGTCCCTGTCTGCCTC TCTGGGAGACAGAGTCACCATCAGTTGCAGGGCAAGTCAG GACATTAGCAATTACTTAACTGGTATCAGCAGAAACCAGA TGGAAGTGTAACTCCTTATCTATTACACATCAATATTACA ATTAGGAGTCCCGCCAAGGTTCAAGTGGCAGTGGGTCTGGAA CAGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAGAT ATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCCGTT ACGTTTCGGTGCTGGGACCAAGGTGGAGCTGAAA (SEQ ID NO: 82)				

2D8 Heavy Chain Variable (vH) and Light Chain Variable (vL) DNA and Amino Acid Sequences*

AVS-3702HC [574.2.2D8.6.9 heavy chain]

Region	Sequence Fragment	Residues	Length
HFR1	DVQLVESGGGLVQPGGSRKLSAAS (SEQ ID NO: 83)	1 - 25	25
CDR-H1	GFTFSYF (SEQ ID NO: 84)	26 - 32	7
HFR2	GMHWVRRAPKGLWVAFI (SEQ ID NO: 85)	33 - 51	19
CDR-H2	SSDSTT (SEQ ID NO: 86)	52 - 57	6
HFR3	IYYTDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAIYYCAR (SEQ ID NO: 87)	58 - 98	41
CDR-H3	FYDGAMDY (SEQ ID NO: 88)	99 - 106	8
HFR4	WGQGTSTVST (SEQ ID NO: 89)	107 - 117	11

AVS-3702LC [574.2.2D8.6.9 light chain]

Region	Sequence Fragment	Residues	Length
LFR1	DIVMTQSQKFMSTSVGDRVSITC (SEQ ID NO: 90)	1 - 23	23

CDR-L1	KASQSVRTAVA (SEQ ID NO: 91)	24 - 34	11
LFR2	WYQQKPGQSPKVLII (SEQ ID NO: 92)	35 - 49	15
CDR-L2	LASNRHT (SEQ ID NO: 93)	50 - 56	7
LFR3	GVPDRFTGSGSGTDFLTISNVQSEDLADYFC (SEQ ID NO: 94)	57 - 88	32
CDR-L3	LQHWNYPLT (SEQ ID NO: 95)	89 - 97	9
LFR4	FGAGTKLELK (SEQ ID NO: 96)	98 - 107	10

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3702	574.2.2D8.6.9	AVS-3702HC.2	HC	GFTFSYF (SEQ ID NO: 84)	SSDSTT (SEQ ID NO: 86)	FYDGAMDY (SEQ ID NO: 88)
Protein sequence			Nucleotide sequence				
DVQLVESGGGLVQPGGSRKLSCAAS GFTFSYFGMHVRRRAPEKGLEWVAF ISSDSTTIYYTDTVKGRFTISRDNPKNT LFLQMTSLRSEDTAIYYCARFYDGAM DYWGQGTSTVTVST (SEQ ID NO: 97)			GATGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGC AGCCTGGAGGGTCCCGGAACTCTCCTGTGCAGCCTC TGGATTCACTTTCAGTTACTTTGGAATGCACTGGGTTC GTCGGGCTCCAGAGAAGGGGCTGGAGTGGGTCGCATT CATTAGTAGTGACAGTACTACCATCTACATATACAGAC ACAGTGAAGGGCCGATTACCATCTCCAGAGACAATC CCAAGAACACCCTGTTCTGCAAATGACCAGTCTAAG GTCTGAGGACACGGCCATATATTACTGTGCAAGATTT TACGACGGTGCTATGGACTACTGGGGTCAAGGAACCT CAGTCACCGTCTCCACA (SEQ ID NO: 98)				

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3702	574.2.2D8.6.9	AVS-3702LCP.5	LC	KASQSVRTAVA (SEQ ID NO: 91)	LASNRHT (SEQ ID NO: 93)	LQHWNYPLT (SEQ ID NO: 95)
Protein sequence			Nucleotide sequence				
DIVMTQSQKFMSTSVGDRVSITCKAS QSVRTAVAWYQQKPGQSPKVLIIYLA SNRHTGVPDRFTGSGSGTDFLTISNV QSEDLADYFCLQHWNYPLTFGAGTK LELK (SEQ ID NO: 99)			GACATTGTGATGACCCAGTCTCAAAAATTCATGTCCA CATCAGTAGGAGACAGGGTCAGCATCACCTGCAAGG CCAGTCAGAGTGTTCTGCTGTAGCCTGGTATCA ACAGAAACCAGGGCAGTCTCCTAAAGTATTGATTTAC TTGGCATCCAACCGGCACACTGGAGTCCCTGATCGCT TCACAGGCAGTGGATCTGGGACAGACTTCACTCTCAC CATTAGCAATGTGCAATCTGAAGACCTGGCAGATTAT TTCTGTCTTCAACATTGGAATTATCCTCTCACGTTCGG TGCTGGGACCAAGCTGGAGCTGAAA (SEQ ID NO: 100)				

2F11 Heavy Chain Variable (vH) and Light Chain Variable (vL) DNA and Amino Acid Sequences*

AVS-3703HC [574.2.2F11.2.7.4 heavy chain]

Region	Sequence Fragment	Residues	Length
HFR1	DVQLVESGGGLVQPGGSRKLSCAAS (SEQ ID NO: 83)	1 - 25	25
CDR-H1	GFTFSYF (SEQ ID NO: 84)	26 - 32	7
HFR2	GMHWVRRRAPEKGLEWVAFI (SEQ ID NO: 85)	33 - 51	19

CDR-H2	SSDSTT (SEQ ID NO: 86)	52 - 57	6
HFR3	IYYTDTVKGRTISRDNPKNTLFLQMTSLRSEDTAIYYCAR (SEQ ID NO: 87)	58 - 98	41
CDR-H3	FYDGAMDY (SEQ ID NO: 88)	99 - 106	8
HFR4	WGQGTSVTVST (SEQ ID NO: 89)	107 - 117	11

AVS-3703LC [574.2.2F11.2.7.4 light chain]

Region	Sequence Fragment	Residues	Length
LFR1	DIVMTQSQKFMSTSVGDRVSITC (SEQ ID NO: 90)	1 - 23	23
CDR-L1	KASQSVRTAVA (SEQ ID NO: 91)	24 - 34	11
LFR2	WYQQKPGQSPKVLII (SEQ ID NO: 92)	35 - 49	15
CDR-L2	LASNRHT (SEQ ID NO: 93)	50 - 56	7
LFR3	GVPDRFTGSGSGTDFLTISNVQSEDLADYFC (SEQ ID NO: 94)	57 - 88	32
CDR-L3	LQHWNYPLT (SEQ ID NO: 95)	89 - 97	9
LFR4	FGAGTKLELK (SEQ ID NO: 96)	98 - 107	10

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3703	574.2.2F11.2.7.4	AVS-3703HC T.2	HC	GFTFSYF (SEQ ID NO: 84)	SSDSTT (SEQ ID NO: 86)	FYDGAMDY (SEQ ID NO: 88)
Protein sequence			Nucleotide sequence				
DVQLVESGGGLVQPGGSRKLSKAAS GFTFSYFGMHWVRRRAPEKGLEWVAF ISSDSTTIYYTDTVKGRTISRDNPKNT LFLQMTSLRSEDTAIYYCARFYDGAM DYWGQGTSVTVST (SEQ ID NO: 97)			GATGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGC AGCCTGGAGGGTCCCGGAACTCTCCTGTGCAGCCTC TGGATTCACTTTCAGTTACTTTGGAATGCACTGGGTTC GTCGGGCTCCAGAGAAGGGGCTGGAGTGGGTTCGATT CATAGTAGTGACAGTACTACCATCTACTATACAGAC ACAGTGAAGGGCCGATTACCATCTCCAGAGACAATC CCAAGAACACCCTGTTCTGCAAATGACCAGTCTAAG GTCTGAGGACACGGCCATATATTACTGTGCAAGATTT TACGACGGTGCTATGGACTACTGGGGTCAAGGAACCT CAGTCACCGTCTCCACA (SEQ ID NO: 98)				

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3703	574.2.2F11.2.7.4	AVS-3703LC T.2	LC	KASQSVRTAVA (SEQ ID NO: 91)	LASNRHT (SEQ ID NO: 93)	LQHWNYPLT (SEQ ID NO: 95)
Protein sequence			Nucleotide sequence				
DIVMTQSQKFMSTSVGDRVSITCKA SQSVRTAVAWYQQKPGQSPKVLII LASNRHTGVPDRFTGSGSGTDFLT ISNVQSEDLADYFCLQHWNYPLTFG AGTKLELK (SEQ ID NO: 99)			GACATTGTGATGACCCAGTCTCAAAAATTCATGTCCAC ATCAGTAGGAGACAGGGTCAGCATCACCTGCAAGGCCA GTCAGAGTGTTCTGACTGCTGTAGCCTGGTATCAACAG AAACCAGGGCAGTCTCCTAAAGTATTGATTACTTGGC ATCCAACCGGCACACTGGAGTCCCTGATCGCTTCACAG GCAGTGGATCTGGGACAGACTTCACTCTCACCATTAGC AATGTGCAATCTGAAGACCTGGCAGATTATTTCTGTCTT CAACATTGGAATTATCCTCTACGTTCCGGTGCTGGGACC AAGCTGGAGCTGAAA (SEQ ID NO: 100)				

2H1 Heavy Chain Variable (vH) and Light Chain Variable (vL) DNA and Amino Acid Sequences*

AVS-3704HC [574.2.2H1.11.6 heavy chain]

Region	Sequence Fragment	Residues	Length
HFR1	DVQLVESGGGLVQPGGSRKLSAAS (SEQ ID NO: 83)	1 - 25	25
CDR-H1	GFTFSYF (SEQ ID NO: 84)	26 - 32	7
HFR2	GMHWVRRAPKLEWVAFI (SEQ ID NO: 85)	33 - 51	19
CDR-H2	SSDSTT (SEQ ID NO: 86)	52 - 57	6
HFR3	IYYTDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAIYYCAR (SEQ ID NO: 87)	58 - 98	41
CDR-H3	FYDGAMDY (SEQ ID NO: 88)	99 - 106	8
HFR4	WGQGTSTVST (SEQ ID NO: 89)	107 - 117	11

AVS-3704LC [574.2.2H1.11.6 light chain]

Region	Sequence Fragment	Residues	Length
LFR1	DIVMTQSQKFMSTSVGDRVSITC (SEQ ID NO: 90)	1 - 23	23
CDR-L1	KASQSVRTAVA (SEQ ID NO: 91)	24 - 34	11
LFR2	WYQQKPGQSPKVLII (SEQ ID NO: 92)	35 - 49	15
CDR-L2	LASNRHT (SEQ ID NO: 93)	50 - 56	7
LFR3	GVPDRFTGSGSGDFTLTISNVQSEDLADYFC (SEQ ID NO: 94)	57 - 88	32
CDR-L3	LQHWNYPLT (SEQ ID NO: 95)	89 - 97	9
LFR4	FGAGTKLELK (SEQ ID NO: 96)	98 - 107	10

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3704	574.2.2H1.11.6	AVS-3704HC T.1	HC	GFTFSYF (SEQ ID NO: 84)	SSDSTT (SEQ ID NO: 86)	FYDGAMDY (SEQ ID NO: 88)
Protein sequence			Nucleotide sequence				
DVQLVESGGGLVQPGGSRKLSAASGFTFSYFGMHVRRAPKLEWVAFISSDSTTIYYTDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAIYYCARFYDGAMDYWGQGTSTVST (SEQ ID NO: 97)			GATGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCGGAACTCTCCTGTGCAGCCTCTGGA TTCACTTTCAGTTACTTTGGAATGCACTGGGTTCGTCGGG CTCCAGAGAAGGGGCTGGAGTGGGTTCGATTCATTAGTA GTGACAGTACTACCATCTACTATACAGACACAGTGAAGG GCCGATTACCATCTCCAGAGACAATCCCAAGAACACCC TGTTCTGCAAATGACCAGTCTAAGGTCTGAGGACACGG CCATATATTACTGTGCAAGATTTTACGACGGTGCTATGG ACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCACA (SEQ ID NO: 98)				

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
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SR-14910	AVS-3704	574.2.2H1.11.6	AVS-3704LCP.5	LC	KASQSV RTAVA (SEQ ID NO: 91)	LASNRH T (SEQ ID NO: 93)	LQHWNYPL T (SEQ ID NO: 95)
Protein sequence			Nucleotide sequence				
DIVMTQSQKFMSTSVGDRVSITCK ASQSVRTAVAWYQQKPGQSPKVL IYLASNHRHTGVPDRFTGSGSGTDF TLTISNVQSEDLADYFCLQHWNYPL LTFGAGTKLELK (SEQ ID NO: 99)			GACATTGTGATGACCCAGTCTCAAAAATTCATGTCCACA TCAGTAGGAGACAGGGTCAGCATCACCTGCAAGGCCAG TCAGAGTGTTCTGACTGCTGTAGCCTGGTATCAACAGAA ACCAGGACAGTCTCCTAAAGTATTGATTACTTGGCATC CAACCGGCACACTGGAGTCCCTGATCGCTTCACAGGCAG TGGATCTGGGACAGACTTCACTCTCACCATTAGCAATGT GCAATCTGAAGACCTGGCAGATTATTTCTGTCTTCAACA TTGGAATTATCCTCTCACGTTTCGGTGCTGGGACCAAGCT GGAGCTGAAA (SEQ ID NO: 101)				

1D12 Heavy Chain Variable (vH) and Light Chain Variable (vL) DNA and Amino Acid Sequences*

AVS-3705HC [574.2.1D12.1.6 heavy chain]

Region	Sequence Fragment	Residues	Length
HFR1	QVQLQQSGAELVRPGTSVKVSCAS (SEQ ID NO: 102)	1 - 25	25
CDR-H1	GYAFNNY (SEQ ID NO: 103)	26 - 32	7
HFR2	LIEWVKQRPGQGLEWIGVI (SEQ ID NO: 104)	33 - 51	19
CDR-H2	NPGSGG (SEQ ID NO: 105)	52 - 57	6
HFR3	TNDNEKFRGKATLTADTSSSTAYMQLTSLTSDSAVYFCAR (SEQ ID NO: 106)	58 - 98	41
CDR-H3	GNYYGTRDWYFDV (SEQ ID NO: 107)	99 - 111	13
HFR4	WGAGTTVTVSS (SEQ ID NO: 108)	112 - 122	11

AVS-3705LC [574.2.1D12.1.6 light chain]

Region	Sequence Fragment	Residues	Length
LFR1	QIVLTQSPAISASLGARVTMT (SEQ ID NO: 109)	1 - 23	23
CDR-L1	TASSSVSSSLH (SEQ ID NO: 110)	24 - 35	12
LFR2	WYQQKPGSSPKLWIY (SEQ ID NO: 111)	36 - 50	15
CDR-L2	SISSLAS (SEQ ID NO: 112)	51 - 57	7
LFR3	GVPARFSGSGTSYSLTISLEAEDAATYYC (SEQ ID NO: 113)	58 - 89	32
CDR-L3	HQYHRSPPT (SEQ ID NO: 114)	90 - 98	9
LFR4	FGGGTKLEIK (SEQ ID NO: 44)	99 - 108	10

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3705	574.2.1D12.1.6	AVS-3705HCT.2	HC	GYAFNNY (SEQ ID NO: 103)	NPGSGG (SEQ ID NO: 105)	GNYYGTRDWYFDV (SEQ ID NO: 107)
Protein sequence			Nucleotide sequence				

QVQLQQSGAELVRPGTSVKVSKASG YAFNNYLIEWVKQRPGQGLEWIGVINP GSGGTNDNEKFRGKATLTADTSSSTA YMQLTSLTSDDSAVYFCARGNYYGTR DWYFDVWGAGTTVTVSS (SEQ ID NO: 115)	CAGGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGTA AGGCCTGGGACTTCAGTGAAGGTGTCCTGCAAGGCT TCTGGATACGCCTTCAATAATTACTTGATAGAGTGGG TAAAGCAGAGGCCTGGACAGGGCCTTGAGTGGATTG GAGTGATCAATCCTGGAAGTGGTGGTACTAACGACA ATGAGAAGTTCAGGGGCAAGGCTACCCTGACTGCAG ACACATCCTCCAGCACTGCCTACATGCAGCTCACCA GCCTGACATCTGATGACTCTGCGGTCTATTTCTGTGC AAGAGGGAATTACTACGGTACTCGCGACTGGTACTT CGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTC CTCA (SEQ ID NO: 116)
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SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3705	574.2.1D12.1.6	AVS-3705LCP.2	LC	TASSSV SSSLH (SEQ ID NO: 110)	SISSLA S (SEQ ID NO: 112)	HQYHRSPPT (SEQ ID NO: 114)
Protein sequence			Nucleotide sequence				
QIVLTQSPAIMASASLGARVTMTCTASSS VSSSLHWHYQQKPGSSPKLWIYSSSLA SGVPARFSGSGSGTSYSLTISSELEAEDAA TYYCHQYHRSPPTFGGGTKLEIK (SEQ ID NO: 117)			CAAATTGTTCTCACCCAGTCTCCAGCAATCATGTCT GCATCTCTAGGGGCACGGGTCACCATGACCTGCACT GCCAGCTCAAGTGTAAGTTCAGTTCCTTGCACTGG TACCAGCAGAAGCCAGGATCCTCCCCAAACTCTGG ATTTATAGTATATCCAGCCTGGCTTCTGGAGTCCCA GCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTAC TCTCTACAATCAGCAGCTTGGAGGCTGAAGATGCT GCCACTTATTACTGCCACCAGTATCATCGCTCCCCA CCGACGTTCCGTGGAGGCACCAAGCTGGAAATCAA A (SEQ ID NO: 118)				

8C2 Heavy Chain Variable (vH) and Light Chain Variable (vL) DNA and Amino Acid Sequences*

AVS-3706HC [574.2.8C2.12.3.10 heavy chain]

Region	Sequence Fragment	Residues	Length
HFR1	KVQLQQSGAELVRPGTSVKVSKAS (SEQ ID NO: 119)	1 - 25	25
CDR-H1	GYAFTNY (SEQ ID NO: 120)	26 - 32	7
HFR2	LIEWVKQRPGQGLEWIGVI (SEQ ID NO: 104)	33 - 51	19
CDR-H2	NPGSGG (SEQ ID NO: 105)	52 - 57	6
HFR3	TNDNEKFRDKATLTADKSSSTAYMQLSSLTSDDSAVYFCAR (SEQ ID NO: 121)	58 - 98	41
CDR-H3	GNYYGTTDWYFDV (SEQ ID NO: 122)	99 - 111	13
HFR4	WGAGTTVTVSS (SEQ ID NO: 108)	112 - 122	11

AVS-3706LC [574.2.8C2.12.3.10 light chain]

Region	Sequence Fragment	Residues	Length
LFR1	QIVLTQSPAIMASASLGARVTMTCT (SEQ ID NO: 123)	1 - 23	23
CDR-L1	TASASVSSSLH (SEQ ID NO: 124)	24 - 35	12
LFR2	WYQQKPGSPPKFWIY (SEQ ID NO: 125)	36 - 50	15
CDR-L2	STTNLAS (SEQ ID NO: 126)	51 - 57	7
LFR3	GVPTFRSGSGSGTSYSLTISNMEAEDAATYYC (SEQ ID NO: 127)	58 - 89	32

CDR-L3	HQYHRSPPT (SEQ ID NO: 114)	90 - 98	9
LFR4	FGGGTKLEIK (SEQ ID NO: 44)	99 - 108	10

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3706	574.2.8C2.12.3.10	AVS-3706HCT.1	HC	GYAFTN Y (SEQ ID NO: 120)	NPGSG G (SEQ ID NO: 105)	GNYYGTT DWYFDV (SEQ ID NO: 122)
Protein sequence			Nucleotide sequence				
KVQLQQSGAELVRPGTSVKVSK ASGYAFTNYLIEWVKQRPQGGL WIGVINPGSGGTNDNEKFRDKATL TADKSSSTAYMQLSSLTSDSAVY FCARGNYYGTTDWYFDVWGAGT TVTVSS (SEQ ID NO: 128)			AAGGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGTAAG GCCTGGGACTTCAGTGAAGGTGTCTGCAAGGCTTCTGG ATACGCCTTCACTAATTATTTGATAGAGTGGGTAAAGCA GAGGCCTGGACAGGGCCTTGAGTGGATTGGAGTGATTAA TCCTGGAAGTGGTGGTACTAATGACAATGAGAAGTTTCAG GGACAAGGCAACATTGACTGCAGACAAATCCTCCAGCA CTGCCTACATGCAGCTCAGCAGCCTGACATCTGATGACT CTGCGGTCTATTTCTGTGCAAGAGGGAATTACTACGGTA CTACCGACTGGTACTTCGATGTCTGGGGCGCAGGGACCA CGGTCACCGTCTCCTCA (SEQ ID NO: 129)				

SR linked	AVS Linked	Client Sample Name	LP Sample Name	Chain type	CDR-1	CDR-2	CDR-3
SR-14910	AVS-3706	574.2.8C2.12.3.10	AVS-3706LCP.1	LC	TASAS VSSSL H (SEQ ID NO: 124)	STTNLAS (SEQ ID NO: 126)	HQYHRSPPT (SEQ ID NO: 114)
Protein sequence			Nucleotide sequence				
QIVLTQSPAISASLGERVTMTCT ASASVSSSSLHWYQQKPGSPKFW IYSTTNLASGVPTFRSGSGSGTSYS LTISNMEAEDAATYYCHQYHRSPPT TFGGGTKLEIK (SEQ ID NO: 130)			CAAATTGTTCTCACCCAGTCTCCAGCAATCATGTCTGCAT CTCTAGGGGAACGGGTACCATGACCTGCACTGCCAGCG CAAGTGTAAGTTCCAGCTCCTTGCACTGGTACCAGCAGA AGCCAGGATCCCCCCCCAAATTCTGGATTATAGCACAA CCAACCTGGCTTCTGGAGTCCCAACTCGCTTCAGTGGCA GTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAACA TGGAGGCTGAAGATGCTGCCACTTATTACTGCCACCAGT ATCATCGTTCCCCACCGACGTTCTGGTGGAGGCACCAAGC TGGAAATCAAA (SEQ ID NO: 131)				

* CDR definitions and protein sequence numbering according to Kabat.

* Included in Table 2 are RNA nucleic acid molecules (e.g., thymines replaced with uridines), nucleic acid molecules encoding orthologs of the encoded proteins, as well as DNA, cDNA, or RNA nucleic acid sequences comprising a nucleic acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or more identity across their full length with the nucleic acid sequence of any SEQ ID NO listed in Table 2, or a portion thereof. Such nucleic acid molecules can have a function of the full-length nucleic acid as described further herein.

were generated by puromycin (0.25 µg/ml) and G418 (1000 µg/ml) double selection and limiting dilution. The chosen stable cell clone was maintained with complete cell culture medium supplemented with puromycin and G418. CHO-K1 cells were co-transfected sequentially with TCRa_pIRESHyg3 and HHLA2_pIRESneo3 by Lipofectamine 2000 (Invitrogen). Stable clones were generated by hygromycin and G418 double selection and limiting dilution. The chosen stable cell clone was maintained with complete cell culture medium supplemented with hygromycin and G418.

sgRNA targeting the human β 2-microglobulin gene (5'-GCTACTCTCTCTTTCTGGCC (SEQ ID NO: 132)) (World Wide Web at addgene.org/84381/) was ordered from Synthego with 2'-O-methyl 3' phosphorothionate modification in the first and last 3 nucleotides. 2 µl of 66.88 µM of Cas9 Nuclease (Aldevron, SpyFi Cas9 Nuclease 9214-0.25MG) was mixed with 3 µl of 100 µM sgRNA. Cas9 Nuclease and sgRNA were incubated at room temperature for 20 minutes. Raji cells were electroporated with Cas9 RNP using Lonza 4D Nucleofector following Lonza recommended setup for Raji cells. Raji cells were cultured for 48 hours, then sorted for β 2-microglobulin negativity using human β 2-microglobulin antibody (Biolegend 395711). β 2-microglobulin negative cells were cultured and re-sorted multiple times until a pure β 2-microglobulin negative cells population was obtained.

REPORTER ASSAYS

TMIGD2_NFAT_Jurkat reporter activity:

HHLA2-TCR-CHO and TCR-CHO cells were seeded at 2×10^4 cells/well density in CHOK1 growth medium in a white opaque bottom 96-well plate and incubated overnight at 37°C with 5% CO₂. The next day, medium was removed and cells were incubated with HHLA2 antibody in 50 µl Jurkat cell medium for one hour before the addition of TMIGD2_NFAT_Jurkat reporter cell line (clone # 62) at $4-5 \times 10^4$ cells/well in 50 µl Jurkat cell medium. The culture was incubated for 3-6 hours. Luciferase signal was produced by adding 100 µl ONE-Step™ Luciferase Assay System (BPS Bioscience 60690), according to manufacturer's protocol and luminescence measured in a luminometer.

KIR3DL3_IL2_Jurkat reporter activity

HHLA2-TCR-CHO (clone # 28) or TCR-CHO cells were seeded at 2×10^4 cells/well density in CHO-K1 growth medium in a white opaque bottom 96-well plate and incubated overnight at 37°C with 5% CO₂. The next day, the medium was removed and

K562 cells were incubated at 37°C with 5% CO₂ in RPMI1640 medium (Life Technologies A10491-01) supplemented with 10% fetal bovine serum (FBS, Life Technologies 26140-079), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

K562 cells were electroporated (300 volts, 1600 uFarads) with 50 ug of Mlu I linearized HHLA2 cDNA in the pEF-Puro vector, selected for puromycin resistance, stained with PE-conjugated HHLA2 mab 6F10, sorted and single cell cloned. K562 HHLA2 stable cell line was cultured in above indicated RPMI1640 media supplemented with 1 µg/mL of puromycin.

WESTERN BLOT ANALYSIS

Protein lysates were prepared with RIPA buffer with protease inhibitor cocktail per manufacturer's instructions (Thermo Scientific; complete Ultra tablets, mini, EDTA-free, Roche). Lysates were loaded into a single wide lane 4-15% gradient mini-Protean TGX gel (Biorad) and transferred by a semidry method. Membranes were blocked with 12% non-fat milk and 1% normal goat serum in Tris-buffered saline with Tween20 (TBST) for 1 hour at room temperature. The membrane was washed with TBST and incubated with anti-KIR3DL3 mAb, 574.1F12, at 5 ug/ml in TBST and 1% BSA at 4°C overnight in a multi-well-mini-blotter. Membranes were washed with TBST three times at room temperature and incubated with secondary antibody (1:4000, HRP-conjugated goat anti-mouse IgG, Southern Biotech) in TBST, 6% non-fat milk and 0.5% normal goat serum for 30 min. After 3 additional washes with TBST, a 1:1 ratio of ECL substrate: enhancer was added to the membrane (SuperSignal West Pico Stable Peroxide Solution, Supersignal West Pico Luminol/Enhancer Solution, ThermoScientific) and imaged on Hyblot CL autoradiography film (Denville Scientific).

RNA EXTRACTION AND QUANTITATIVE REAL-TIME PCR

Total RNA was extracted from cell pellets using Purelink RNA mini Kit (ThermoFisher). cDNA synthesis was performed with the High Capacity RNA-to-cDNA Kit (Applied Biosystems, ThermoFisher) according to the manufacturer's recommendations. RT-PCR was performed using Power SYBR™ Green PCR Master Mix according to the manufacturer's recommendations. Primers used included:

18S:	fw 5'-GTAACCCGTTGAACCCCAT-3' (SEQ ID NO: 133) ;
	5'-CCATCCAATCGGTAGTAGCG-3' (SEQ ID NO: 134)
KIR3DL3:	fw 5'- AGAAGACGGGATGCCTGTC-3' (SEQ ID NO: 135) ;

rv 5'-GTGAACTGCAACATCTGTAGGT-3' (SEQ ID NO: 136)
HHLA2: fw 5'-TACAAAGGCAGTGACCATTG-3' (SEQ ID NO: 137);
rv 5'-AGGTGTAAATTCCTTCGTCCAGA-3' (SEQ ID NO: 138)
PD-L1 (CD274): fw 5'-TGGCATTGCTGAACGCATTT-3' (SEQ ID NO: 139);
rv 5'-TGCAGCCAGGTCTAATTGTTTT-3' (SEQ ID NO: 140)

18S was used as internal control for each sample. Relative mRNA levels were determined by the $2^{-\Delta\Delta CT}$ formula, and experiments were repeated three times.

ANALYSIS OF mRNA EXPRESSION IN ccRCC PATIENTS

TCGA RSEM RNASeqV2 data for TCGA Kidney Clear Cell patients was downloaded from the TCGA GDAC at World Wide Web at gdac.broadinstitute.org/runs/stddata__2016_01_28/data/KIRC/20160128/gdac.broadinstitute.org_KIRC.Merge_rnaseqv2__illumina_hiseq_rnaseqv2__unc_edu__Level_3__RSEM_genes__data.Level_3.2016012800.0.0.tar.gz. To derive Transcripts per Million (TPM), the "scaled_estimate" output value was multiplied by 106. These TPM values were transformed to $\log_2(\text{TPM}+1)$ and tumor and normal expression was compared for each gene using a Wilcoxon rank sum test.

CYTOMETRY:

Cells were analyzed on a Gallios Flow Cytometer (Configuration: 488 nm, 561 nm, 405 nm, 355 nm, and 635 nm) or a BD Fortessa Flow Cytometer. Data were analyzed with FlowJo or with Kaluza Software. For each experiment, 10,000 to 20,000 cells were analyzed.

STATISTICS:

Data were analyzed using GraphPad Prism® Software 7, unless otherwise indicated. All data are represented as mean $\pm$ S.D. (error bars) except where stated otherwise (see Figure legends). Student's t test and two-way ANOVA were used as indicated in the legends (non-significant (ns), $P \geq 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$).

Example 2: Identification and Characterization of KIR3DL3 as second receptor for HHLA2

In order to identify an inhibitory receptor for HHLA2, a receptor screen was performed using soluble human HHLA2-mIgG2a fusion protein (HHLA2-Ig) on a library

Table 7: Representative exemplary amino acid sequences of PD-1 EH13 and KIR3DL3 2F11 mAb human IgG4 (SEQ ID NOS 141-144, respectively, in order of appearance)

Antibody: PD-1 EH13.2A11.17.1

Heavy Chain

Reference: MHC801HC.2

Isotype: Human IgG4 (S228P)

SignalPeptide EVKLVESGGGLVQPGGSLKLSAASGFTFSSFTLSWVRQTPEKRLEWVAYISYGGADTYYPDTVKGR
FTIFRDNAKNTLYLQMSLKSEDTARYYCARHGPGYKGDYYAMDYWGQGTSTVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPPC
PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLG*

Light Chain

Reference: MHC801LC.1

Isotype: Human Kappa (Km3 allotype)

SignalPeptide DIVLTQSPASLAVSLGQRATISCRASESVDDFGISFMNWFQQKPGQPPKLLIFAASKQSGVPARFS
SGSGGTDFTSLNIHPMEEDDTAMYFCQQSKEVPWTFGGVTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC*

Antibody: KIR3DL3 mAb 574.2.2F11.2.7.4

Heavy Chain

Reference: AVS-3703HCT.2

Isotype: Human IgG4 (S228P)

SignalPeptide DVOLVESGGGLVQPGGSRKLSCAASGFTFSYFGMHVRRRAPEKGLEWVAFISSDSTTIYYTDTVKGR
FTISRDNPKNLTLFQMTSLRSDTAIYYCARFYDGAMDYWGQGTSTVTVSTASTKGPSVFPLAPCSRSTSESTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPPCP
EFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLG*

Light Chain

Reference: AVS-3703LCT.2

Isotype: Human Kappa (Km3 allotype)

SignalPeptide DIVMTQSQKFMSTSVGDRVSITCKASQSVRTAVAWYQQKPGQSPKVLIIYASNHRHTGVPDRFTGSGS
GTDFTLTISNVQSEDLADYFCLQHWNYPLTFGAGTKLELKRVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW
KVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC*

Table 8: Representative exemplary amino acid sequences of PD-1 EH13 and KIR3DL3 2F11 mAb scFv's (SEQ ID NOS 145-146, respectively, in order of appearance)

Protein: PD-1 scFv

Reference: MHC801HC.2, MHC801LC.1

Isotype: Human IgG4 (S228P) Fc

SignalPeptide_ESKYGPPCP[CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSLT
CLVKGFPYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLK
GGGGAGGGGEVKLVESGGGLVQPGGSLKLSCAASGFTFSSTLSWVRQTPEKRLEWVAYISYGGADTYYPDTVKGRFTIFR
DNAKNTLYLQMSSSLKSEDTARYYCARHGPYKGDDYAMDYWGQGTSTVTVSSGGGGSGGGSGGGGSDIVLTQSPASLAVSL
GQRATISCRASESVDVDFGISFMNWFQKPGQPPKLLIFAAASKQSGVPAFSGSGSGTDFTSLNIHPMEEDDTAMYFCQQSK
EVPWTFGGVTKLEIK*

Protein: KIR3DL3 scFv

Reference: AVS-3703HCT.2, AVS-3703LCT.2

Isotype: Human IgG4 (S228P) Fc

SignalPeptide_ESKYGPPCP[CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSLT
CLVKGFPYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLK
GGGGAGGGGDVQLVESGGGLVQPGGSRKLSCAASGFTFSYFGMHWVRRAPKGLEWVAFISSDSTTIYYTDTVKGRFTISR
DNPKNLTLFQMTSLRSEDTAIYYCARFYDGAMDYWGQGTSTVTVSTGGGGSGGGSGGGGSDIVMTQSQKFMSTSVGDRVSI
TCKASQSVRTAVAWYQKPGQSPKVLIIYLASNRHTGVPDRFTGSGSGTDFTLTISNVQSEDLADYFCLQHWNYPITFGAGT
KLELK*

In some embodiments, the heavy chain/Fc construct is designed with the C-terminal lysine removed to create a more homogeneous product, as referenced by Cai *et al.* (2011) *Biotechnol Bioeng*, 108(2):404-12. The sequences presented in Table 8 are the sequences in which the terminal lysine has been removed.

In some embodiments, sequences are codon optimized for mammalian expression.

In some embodiments, the gene inserts with the listed elements and tags will be included in the final construct for expression. In some embodiments, the full insert will be cloned into a high expression mammalian vector.

Table 9: Representative amino acid sequences of an exemplary PD-1 x KIR3DL3 bispecific antibody - PD-1 EH13 IgG4 mAb and KIR3DL3 2F11 scFv Bispecific antibody (SEQ ID NOS 147 and 142, respectively, in order of appearance)

Antibody: PD-1-IgG4_KIR3DL3-ScFv

Heavy Chain

References:

- H12858, pLEV123-PD-1 EH13.2A11.17.1-hIgG4 (S228P, L235E)
- J2876, pLEV123-KIR3DL3 scFv

Isotype: Human IgG4 (S228P, L235E)

SignalPeptide EVKLVESGGGLVQPGGSLKLSAASGFTFSSFTLSWVRQTPEKRLEWVAYISYGGADTYYPDTVKGK
FTIFRDNAKNTLYLQMSLKSSEDARYYCARHGPYKGDDYAMDYWGQTSVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPPC
PPCPAPEFEGGGSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVSVLT
VLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSGGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNNHYTQKSLSLSLGSGGGGAGGGGDVQLVESGGGLVQPG
GGRKLSAASGFTFSYFGMHVRRRAPEKGLEWVAFISSDSTTIYYTDTVKGRFTISRDNPKNLTLFQMTSLRSEDTAIYY
CARFYDGAMDYWGQTSVTVSTGGGGSGGGSGGGGSDIVMTQSQKFMSTSVGDRVSIITCKASQSVRTAVAWYQQKPGQSP
KVLIIYLASNRHTGVPDRFTGSGSGTDFTLTISNVQSEDLADYFCLQHWNYPLTFGAGTKLEL*

Light Chain [SAME LC AS L12858]

Reference: L12858, pLEV123-PD-1 EH13.2A11.17.1-hKappa

Isotype: Human Kappa (Km3 allotype)

SignalPeptide DIVLTQSPASLAVSLGQRATISCRASESVDDFGISFMNWFQQKPGQPPKLLIFAASKQSGGVPARFS
GSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPWTFGGVTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC*

In some embodiments, the heavy chain/Fc construct is designed with the C-terminal lysine removed to create a more homogeneous product, as referenced by Cai *et al.* (2011) *Biotechnol Bioeng*, 108(2):404-12. The sequences presented in Table 9 are the sequences in which the terminal lysine has been removed.

In some embodiments, sequences are codon optimized for mammalian expression. The sequences in Table 9 have been previously codon optimized and constructed in SR-18230.

In some embodiments, the gene inserts with the listed elements and tags will be included in the final construct for expression. In some embodiments, the full insert will be cloned into a high expression mammalian vector.

What is claimed is CLAIMS:

1. A monoclonal antibody, or antigen-binding fragment thereof, comprising:
 - a) ~~a heavy chain sequence with at least about 95% identity to a heavy chain sequence selected from the group consisting of the sequences listed in Tables 2, 7, and 8; and/or~~
 - b) ~~a light chain sequence with at least about 95% identity to a light chain sequence selected from the group consisting of the sequences listed in Tables 2, 7, and 8.~~
2. ~~A monoclonal antibody, or antigen-binding fragment thereof, comprising:~~
 - a) one, two, or three heavy chain CDR sequences each with at least about 95% identity to a heavy chain CDR sequence ~~selected from the group consisting of the sequences listed in Tables 2(s) set forth in SEQ ID NOS: 67, 769, and 8/or 71; and/or~~
 - b) one, two, or three light chain CDR sequences each with at least about 95% identity to a light chain CDR sequence ~~selected from the group consisting of the sequences listed in Tables 2(s) set forth in SEQ ID NOS: 73, 775, and 8/or 77.~~
32. ~~A~~The monoclonal antibody, or antigen-binding fragment thereof of claim 1, comprising:
 - a) a heavy chain sequence ~~selected from the group consisting of the sequences listed in Tables 2, 7, and 8~~with at least about 95% identity to a heavy chain sequence set forth in SEQ ID NO: 79; and/or
 - b) a light chain sequence ~~selected from the group consisting of the sequences listed in Tables 2, 7, and 8~~with at least about 95% identity to a light chain sequence set forth in SEQ ID NO: 81.
4. ~~A monoclonal antibody, or antigen-binding fragment thereof, comprising:~~
 - a) ~~one, two, or three heavy chain CDR sequences each selected from the group consisting of the sequences listed in Tables 2, 7, and 8; and/or~~
 - b) ~~one, two, or three light chain CDR sequences each selected from the group consisting of the sequences listed in Tables 2, 7, and 8.~~
53. The monoclonal antibody, or antigen-binding fragment thereof, of ~~any one of claims 1-4~~claim 1 or 2, wherein the monoclonal antibody, or antigen-binding fragment thereof, is chimeric, humanized, composite, murine, or human.

~~64.~~ The monoclonal antibody, or antigen-binding fragment thereof, of any one of claims ~~1-51-3~~, wherein the monoclonal antibody, or antigen-binding fragment thereof, is (a) detectably labeled, (b) conjugated to a cytotoxic agent, optionally a chemotherapeutic agent, a biologic agent, a toxin, and/or a radioactive isotope, (c) comprises an effector domain, (d) comprises an Fc domain, and/or (e) is selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, and diabodies fragments.

~~7.~~ ~~The monoclonal antibody, or antigen-binding fragment thereof, of any one of claims 1-6, wherein said monoclonal antibody, or antigen-binding fragment thereof, is obtainable from hybridoma _____ deposited under deposit accession number _____.~~

~~85.~~ The monoclonal antibody, or antigen-binding fragment thereof, of any one of claims ~~1-71-4~~, wherein the monoclonal antibody, or antigen-binding fragment thereof, inhibits the binding of HHLA2 to KIR3DL3.

~~96.~~ The monoclonal antibody, or antigen-binding fragment thereof, of any one of claims ~~1-81-5~~, wherein the monoclonal antibody, or antigen-binding fragment thereof, specifically binds KIR3DL3.

~~107.~~ A bispecific antibody, or antigen-binding fragment thereof, comprising:

~~a) a heavy chain sequence with at least about 95% identity to a heavy chain sequence selected from the group consisting of the sequences listed in Tables 2 and 7-9; and/or~~

~~b) a light chain sequence with at least about 95% identity to a light chain sequence selected from the group consisting of the sequences listed in Tables 2 and 7-9.~~

~~11.~~ A bispecific antibody, or antigen-binding fragment thereof, comprising:

~~a) one, two, or three heavy chain CDR sequences each with at least about 95% identity to a heavy chain CDR sequence selected from the group consisting of the sequences listed in Tables 2(s) set forth in SEQ ID NOS: 67, 69, and 7-9/or 71; and/or~~

~~b) one, two, or three light chain CDR sequences each with at least about 95% identity to a light chain CDR sequence selected from the group consisting of the sequences listed in Tables 2(s) set forth in SEQ ID NOS: 73, 75, and 7-9/or 77.~~

~~128.~~ ~~A~~The bispecific antibody, or antigen-binding fragment thereof of claim 7, comprising:

a) a heavy chain sequence ~~selected from the group consisting of the sequences listed in Tables 2 and 7-9~~ with at least about 95% identity to a heavy chain sequence set forth in SEQ ID NO: 79; and/or

b) a light chain sequence ~~selected from the group consisting of the sequences listed in Tables 2 and 7-9~~ with at least about 95% identity to a light chain sequence set forth in SEQ ID NO: 81.

~~13. A bispecific antibody, or antigen-binding fragment thereof, comprising:~~

~~a) one, two, or three heavy chain CDR sequences each selected from the group consisting of the sequences listed in Tables 2 and 7-9; and/or~~

~~b) one, two, or three light chain CDR sequences each selected from the group consisting the sequences listed in Tables 2 and 7-9.~~

~~149.~~ The bispecific antibody, or antigen-binding fragment thereof, of ~~any one of claims 10-13~~ claim 7 or 8, wherein the bispecific antibody, or antigen-binding fragment thereof, is chimeric, humanized, composite, murine, or human.

~~1510.~~ The bispecific antibody, or antigen-binding fragment thereof, of any one of claims ~~10-147-9~~, wherein the bispecific antibody, or antigen-binding fragment thereof, is (a) detectably labeled, (b) conjugated to a cytotoxic agent, optionally a chemotherapeutic agent, a biologic agent, a toxin, and/or a radioactive isotope, (c) comprises an effector domain, (d) comprises an Fc domain, and/or (e) is selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, and diabodies fragments.

~~16. The bispecific antibody, or antigen-binding fragment thereof, of any one of claims 10-15, wherein said bispecific antibody, or antigen-binding fragment thereof, is obtainable from hybridoma _____ deposited under deposit accession number _____.~~

~~1711.~~ The bispecific antibody, or antigen-binding fragment thereof, of any one of claims ~~10-1467-10~~, wherein the bispecific antibody, or antigen-binding fragment thereof, inhibits the binding of (a) HHLA2 to KIR3DL3, and (b) PD-1 to PD-L1 and/or PD-L2.

~~18~~12. The bispecific antibody, or antigen-binding fragment thereof, of any one of claims ~~10-17~~7-11, wherein the bispecific antibody, or antigen-binding fragment thereof, specifically binds KIR3DL3 and PD-1.

~~19~~. ~~The bispecific antibody, or antigen-binding fragment thereof, of any one of claims 10-18, wherein the bispecific antibody, or antigen-binding fragment thereof, comprises~~

~~a) a heavy chain sequence listed in Table 9; and/or~~

~~b) a light chain sequence listed in Table 9.~~

~~20~~13. An immunoglobulin heavy and/or light chain selected from the group consisting of immunoglobulin heavy and light chain sequences ~~listed in Tables 2~~set forth in SEQ ID NOS: 79 and 7981.

~~21~~14. An isolated nucleic acid molecule that (a) encodes an immunoglobulin heavy chain, an immunoglobulin light chain, and/or an antibody, or antigen-binding fragment thereof, of any one of claims ~~1-20~~1-13; and/or (b) hybridizes, under stringent conditions, with the complement of a nucleic acid encoding a polypeptide ~~selected from the group consisting of polypeptide sequences listed in Tables 2~~sequence(s) set forth in SEQ ID NOS: 79 and 79or 81, or a sequence with at least about 95% homology to a nucleic acid encoding a polypeptide ~~selected from the group consisting of the polypeptide sequences listed in Tables 2~~sequence(s) set forth in SEQ ID NOS: 79 and 79or 81.

~~22~~15. A vector comprising the isolated nucleic acid of claim ~~21~~14.

~~23~~16. A host cell which comprises the isolated nucleic acid of claim ~~21~~14, comprises the vector of claim ~~22~~15, expresses the antibody, or antigen-binding fragment thereof, of any one of claims ~~1-19~~, or is accessible under deposit accession number ~~_____~~1-12.

~~24~~17. A device or kit comprising at least one antibody, or antigen-binding fragment thereof, according to any one of claims ~~1-19~~1-12, said device or kit optionally comprising a label to detect the at least one antibody, or antigen-binding fragment thereof, or a complex comprising the antibody, or antigen-binding fragment thereof.

~~25~~18. A method of producing at least one antibody, or antigen-binding fragment thereof, according to any one of claims ~~1-19~~1-12, which method comprises the steps of:

- (i) culturing a transformed host cell which has been transformed by a nucleic acid comprising a sequence encoding at least one antibody according to any one of claims ~~1-19~~1-12 under conditions suitable to allow expression of said antibody, or antigen-binding fragment thereof; and

- (ii) recovering the expressed antibody, or antigen-binding fragment thereof.

~~26~~19. A method of detecting the presence or level of an KIR3DL3 polypeptide comprising detecting said polypeptide in a sample by use of at least one antibody, or antigen-binding fragment thereof, according to any one of claims ~~1-19~~1-12,

~~27~~. ~~The method of claim 26, optionally~~ wherein the at least one antibody, or antigen-binding fragment thereof, forms a complex with an KIR3DL3 polypeptide and the complex is detected in the form of an enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), immunochemically, Western blot, or using an intracellular flow assay.

~~28~~20. A method of predicting the responsiveness to a therapy targeting KIR3DL3, the method comprising:

a) determining the level of KIR3DL3 and/or HHLA2 in a subject sample using at least one antibody, or antigen-binding fragment thereof, according to any one of claims ~~1-19~~1-12;

b) determining the level of KIR3DL3 and/or HHLA2 in a sample from at least one control subject having good responsiveness to a therapy targeting KIR3DL3, using the at least one antibody, or antigen-binding fragment thereof; and

c) comparing the level of KIR3DL3 and/or HHLA2 in the subject sample and in the sample from the control subject;

wherein

- the same or higher level of KIR3DL3 and/or HHLA2 in the subject sample as compared to the level in the sample from the at least one control subject is an indication that the subject will be responsive to the therapy; and

~~29~~. ~~The method of claim 28, wherein the therapy targets KIR3DL3 using at least one antibody, or antigen-binding fragment thereof, according to any one of claims 1-19~~1-12.

~~30~~21. A method of predicting the responsiveness to a therapy targeting KIR3DL3 using at least one antibody, or antigen-binding fragment thereof, according to any one of claims ~~1-19~~1-12, the method comprising:

- a) determining the level of KIR3DL3 and/or HHLA2 in a subject sample;
 - b) determining the level of KIR3DL3 and/or HHLA2 in a sample from at least one control subject having good responsiveness to a therapy targeting KIR3DL3; and
 - c) comparing the level of KIR3DL3 and/or HHLA2 in the subject sample and in the sample from the control subject;
- wherein
- the same or higher level of KIR3DL3 and/or HHLA2 in the subject sample as compared to the level in the sample from the at least one control subject is an indication that the subject will be responsive to the therapy-

; optionally 31. ~~The method of any one of claims 28-30,~~ wherein the sample is a portion of a single sample obtained from at least one subject or portions of pooled samples obtained from at least one subject-;

~~32. The method of any one of claims 28-31, wherein the therapy blocks the interaction and/or signaling between (a) HHLA2 and KIR3DL3; and/or (b) PD-1 and PD-L1 and/or PD-L2-;~~

~~33. The method of any one of claims 26-32,~~optionally wherein the sample comprises cells, serum, peritumoral tissue, and/or intratumoral tissue obtained from the subject-; and/or

~~34. The method of claim 33,~~optionally wherein the cells are T cells or natural killer (NK) cells.

~~35~~22. ~~A method of treating a subject afflicted with cancer~~composition comprising administering to the subject at least one antibody, or antigen-binding fragment thereof, according to any one of claims ~~1-19~~1-12 for use in therapy.

23. A composition comprising at least one anti-KIR3DL3 antibody or antigen-binding fragment thereof for use in therapy.

24. Use of at least one antibody or antigen-binding fragment thereof according to any one of claims 1-12 in the manufacture of a medicament for treating a subject afflicted with cancer.

25. The use of claim 24, wherein

~~36. The method of claim 35, wherein the at least one antibody, or antigen-binding fragment thereof, (a) reduces the number of proliferating cancer cells in the cancer; (b) reduces the volume or size of a tumor of the cancer; and/or (c) activates a T cell and/or an NK cell;~~

~~37. The method of claim 35 or 36, wherein the at least one antibody, or antigen-binding fragment thereof, is to be administered in a pharmaceutically acceptable formulation;~~

~~38. The method of any one of claims 35-37, further comprising administering to the subject is to be further administered a therapeutic agent or regimen for treating cancer;~~

~~39. The method of any one of claims 35-38, further comprising administering to the subject is to be further administered an additional therapy selected from the group consisting of immunotherapy, checkpoint blockade, cancer vaccines, chimeric antigen receptors, chemotherapy, radiation, target therapy, and surgery;~~

~~40. The method of claim 39, wherein the chimeric antigen receptor targets receptors target CD19;~~

~~41. The method of any one of claims 35-40, wherein cancer cells and/or tumor immune infiltrating cells in the subject express HHLA2;~~

~~42. The method of any one of claims 35-41, wherein the cancer is selected from the group consisting of adenocarcinoma, chronic myelogenous leukemia (CML), lung cancer, renal cancer, pancreatic cancer, colorectal cancer, acute myeloid leukemia, head and neck carcinoma, liver cancer, ovarian cancer, prostate cancer, uterine cancer, gliomas, glioblastoma, neuroblastoma, breast cancer, pancreatic ductal carcinoma, thymoma, B-CLL, leukemia, B cell lymphoma, and a cancer infiltrated with immune cells expressing a receptor to HHLA2;~~

~~43. The method of claim 42, wherein the cancer is selected from the group consisting of lung cancer, renal cancer, pancreatic cancer, colorectal cancer, acute myeloid leukemia (AML), head and neck carcinoma, liver cancer, ovarian cancer, prostate cancer, and uterine cancer;~~

~~44. The method of any one of claims 35-43, wherein the subject is an animal model of cancer;~~

~~45. The method of claim 44, wherein the animal model is optionally a mouse model, optionally wherein;~~

- the mouse model is optionally a humanized mouse model;:
the subject is optionally a mammal; and/or
46. ~~The method of any one of claims 35-45, wherein the subject is a mammal.~~
47. ~~The method of claim 46, wherein the mammal is optionally a humanized mouse or a human.~~
48. ~~The method of claim 47, wherein the mammal is a human.~~
4926. ~~A method of modulating an immune response using~~ Use of at least one anti-KIR3DL3 antibody, or antigen-binding fragment thereof in the manufacture of a medicament for modulating an immune response.
27. The use of claim 26, wherein
50. ~~The method of claim 49, wherein the at least one anti-KIR3DL3 antibody, or antigen-binding fragment thereof, inhibits or disrupts the interaction between HHLA2 and its binding inhibitor receptor, KIR3DL3;:~~
51. ~~The method of claim 49 or 50, wherein at least one anti-KIR3DL3 antibody, or antigen-binding fragment thereof, is conjugated to a cytotoxic agent;:~~
52. ~~The method of claim 51, wherein the cytotoxic agent is selected from the group consisting of a chemotherapeutic agent, a biologic agent, a toxin, and a radioactive isotope;:~~
53. ~~The method of any one of claims 49-52, wherein the immune response is downregulated or upregulated;:~~
54. ~~The method of any one of claims 49-53, wherein the at least one anti-KIR3DL3 antibody, or antigen-binding fragment thereof, is according to any one of claims 1-49;1-12;:~~
55. ~~The method of any one of claims 49-54, wherein the interaction between (a) HHLA2 and KIR3DL3; and/or (b) PD-1 and PD-L1 and/or PD-L2 is blocked;:~~
56. ~~The method of any one of claims 49-55, wherein the the at least one anti-KIR3DL3 antibody, or antigen-binding fragment thereof, is a checkpoint inhibitor of T cell activation for cancer immunotherapy;:~~
57. ~~The method of any one of claims 49-56, wherein the modulating an immune response comprises modulating a T cell function or NK cell function.~~

~~58. The method of claim 57, optionally wherein the T cell function or NK cell function comprises a cytotoxic activity;~~

~~59. The method of claim 58, optionally wherein the cytotoxic activity is against cancer cells;~~

~~60. The method of claim 59, wherein the cancer cells express HHLA2;~~

~~61. The method of any one of claims 56-60, wherein the cancer is selected from the group consisting of adenocarcinoma, chronic myelogenous leukemia (CML), lung cancer, renal cancer, pancreatic cancer, colorectal cancer, acute myeloid leukemia, head and neck carcinoma, liver cancer, ovarian cancer, prostate cancer, uterine cancer, gliomas, glioblastoma, neuroblastoma, breast cancer, pancreatic ductal carcinoma, thymoma, B-CLL, leukemia, B cell lymphoma, and a cancer infiltrated with immune cells expressing a receptor to HHLA2;~~

~~62. The method of claim 61, wherein the cancer is selected from the group consisting of lung cancer, renal cancer, pancreatic cancer, colorectal cancer, acute myeloid leukemia (AML), head and neck carcinoma, liver cancer, ovarian cancer, prostate cancer, and uterine cancer;~~

~~63. The method of any one of claims 49-62, further comprising an additional therapy selected from the group consisting of immunotherapy, checkpoint blockade, cancer vaccines, chimeric antigen receptors, chemotherapy, radiation, target therapy, and surgery;~~

~~64. The method of claim 63, wherein the chimeric antigen receptors target CD19;~~

~~65. The method of any one of claims 49-64, wherein the ~~immunue~~immune response is modulated in an animal model of cancer;~~

~~66. The method of claim 65, wherein the animal model is a mouse model, optionally wherein the mouse model is a humanized mouse model;~~

~~67. The method of any one of claims 49-66, wherein the immune response is modulated in a mammal; and/or~~

~~68. The method of claim 67, wherein the mammal is optionally a humanized mouse or a human.~~

28. A monoclonal antibody, or antigen-binding fragment thereof, comprising:

a) a heavy chain sequence with at least about 95% identity to a heavy chain sequence selected from the group consisting of the sequences listed in Tables 2, 7, and 8; and/or

b) a light chain sequence with at least about 95% identity to a light chain sequence selected from the group consisting of the sequences listed in Tables 2, 7, and 8.

29. A monoclonal antibody, or antigen-binding fragment thereof, comprising:

a) one, two, or three heavy chain CDR sequences each with at least about 95% identity to a heavy chain CDR sequence selected from the group consisting of the sequences listed in Tables 2, 7, and 8; and/or

b) one, two, or three light chain CDR sequences each with at least about 95% identity to a light chain CDR sequence selected from the group consisting of the sequences listed in Tables 2, 7, and 8.

30. A monoclonal antibody, or antigen-binding fragment thereof, comprising:

a) a heavy chain sequence selected from the group consisting of the sequences listed in Tables 2, 7, and 8; and/or

b) a light chain sequence selected from the group consisting of the sequences listed in Tables 2, 7, and 8.

31. A monoclonal antibody, or antigen-binding fragment thereof, comprising:

a) one, two, or three heavy chain CDR sequences each selected from the group consisting of the sequences listed in Tables 2, 7, and 8; and/or

b) one, two, or three light chain CDR sequences each selected from the group consisting of the sequences listed in Tables 2, 7, and 8.

32. A bispecific antibody, or antigen-binding fragment thereof, comprising:

a) a heavy chain sequence with at least about 95% identity to a heavy chain sequence selected from the group consisting of the sequences listed in Tables 2 and 7-9; and/or

b) a light chain sequence with at least about 95% identity to a light chain sequence selected from the group consisting of the sequences listed in Tables 2 and 7-9.

33. A bispecific antibody, or antigen-binding fragment thereof, comprising:

a) one, two, or three heavy chain CDR sequences each with at least about 95% identity to a heavy chain CDR sequence selected from the group consisting of the sequences listed in Tables 2 and 7-9; and/or

b) one, two, or three light chain CDR sequences each with at least about 95% identity to a light chain CDR sequence selected from the group consisting of the sequences listed in Tables 2 and 7-9.

34. A bispecific antibody, or antigen-binding fragment thereof, comprising:

a) a heavy chain sequence selected from the group consisting of the sequences listed in Tables 2 and 7-9; and/or

b) a light chain sequence selected from the group consisting of the sequences listed in Tables 2 and 7-9.

35. A bispecific antibody, or antigen-binding fragment thereof, comprising:

a) one, two, or three heavy chain CDR sequences each selected from the group consisting of the sequences listed in Tables 2 and 7-9; and/or

b) one, two, or three light chain CDR sequences each selected from the group consisting of the sequences listed in Tables 2 and 7-9.

36. An immunoglobulin heavy and/or light chain selected from the group consisting of immunoglobulin heavy and light chain sequences listed in Tables 2 and 7-9.

37. An isolated nucleic acid molecule that (a) encodes an immunoglobulin heavy chain, an immunoglobulin light chain, and/or an antibody, or antigen-binding fragment thereof, of any one of claims 28-36; and/or (b) hybridizes, under stringent conditions, with the complement of a nucleic acid encoding a polypeptide selected from the group consisting of polypeptide sequences listed in Tables 2 and 7-9, or a sequence with at least about 95% homology to a nucleic acid encoding a polypeptide selected from the group consisting of the polypeptide sequences listed in Tables 2 and 7-9.

38. A vector comprising the isolated nucleic acid of claim 37, wherein the mammal is a human.

39. A host cell which comprises the isolated nucleic acid of claim 37, comprises the vector of claim 38, expresses the antibody, or antigen-binding fragment thereof, of any one of claims 28-36.

40. A device or kit comprising at least one antibody, or antigen-binding fragment thereof, according to any one of claims 28-36, said device or kit optionally comprising a label to detect the at least one antibody, or antigen-binding fragment thereof, or a complex comprising the antibody, or antigen-binding fragment thereof.

41. A method of producing at least one antibody, or antigen-binding fragment thereof, according to any one of claims 28-36, which method comprises the steps of: (i) culturing a transformed host cell which has been transformed by a nucleic acid comprising a sequence encoding at least one antibody according to any one of claims 28-36 under conditions suitable to allow expression of said antibody, or antigen-binding fragment thereof; and (ii) recovering the expressed antibody, or antigen-binding fragment thereof.

42. A method of detecting the presence or level of an KIR3DL3 polypeptide comprising detecting said polypeptide in a sample by use of at least one antibody, or antigen-binding fragment thereof, according to any one of claims 28-36.

43. A method of predicting the responsiveness to a therapy targeting KIR3DL3, the method comprising:

a) determining the level of KIR3DL3 and/or HHLA2 in a subject sample using at least one antibody, or antigen-binding fragment thereof, according to any one of claims 28-36;

b) determining the level of KIR3DL3 and/or HHLA2 in a sample from at least one control subject having good responsiveness to a therapy targeting KIR3DL3, using the at least one antibody, or antigen-binding fragment thereof; and

c) comparing the level of KIR3DL3 and/or HHLA2 in the subject sample and in the sample from the control subject;

wherein the same or higher level of KIR3DL3 and/or HHLA2 in the subject sample as compared to the level in the sample from the at least one control subject is an indication that the subject will be responsive to the therapy.

44. A method of predicting the responsiveness to a therapy targeting KIR3DL3 using at least one antibody, or antigen-binding fragment thereof, according to any one of claims 28-36, the method comprising:

a) determining the level of KIR3DL3 and/or HHLA2 in a subject sample;

b) determining the level of KIR3DL3 and/or HHLA2 in a sample from at least one control subject having good responsiveness to a therapy targeting KIR3DL3; and

c) comparing the level of KIR3DL3 and/or HHLA2 in the subject sample and in the sample from the control subject;

wherein the same or higher level of KIR3DL3 and/or HHLA2 in the subject sample as compared to the level in the sample from the at least one control subject is an indication that the subject will be responsive to the therapy.

45. Use of at least one antibody or antigen-binding fragment thereof according to any one of claims 28-36 in the manufacture of a medicament for treating a subject afflicted with cancer.