

SEQUENCE LISTING

<110> Seattle Children's Hospital
 dba Seattle Children's Research Institute
 Crane, Courtney
 Jensen, Michael C.
 Moyes, Kara White
 Perry, Nicole

<120> GENETIC ENGINEERING OF MACROPHAGES FOR IMMUNOTHERAPY

<130> SCRI.101WO

<150> 62/361348
 <151> 2016-07-12

<150> 62/216224
 <151> 2015-09-09

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 <223> IL21-T2A forward primer

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<223> PDL1.1-Reverse primer

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<223> PD-L1 small guide RNA sequence

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<210> 28
 <211> 2197
 <212> DNA
 <213> Artificial Sequence

<220>
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<210> 29

<211> 1641

<212> DNA

<213> Artificial Sequence

<220>

<223> EGreen Fluorescent Protein

<400> 29

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 <211> 1038
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> T2A-CD19t

<400> 30

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 <213> Artificial Sequence

<220>
 <223> CD19t epitope tag

<400> 31

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972

<210> 32

<211> 567

<212> DNA

<213> Artificial Sequence

<220>

<223> T2A-sTGFBRII

<400> 32

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<210> 33

<211> 1539

<212> DNA

<213> Artificial Sequence

<220>

<223> CD19t-T2A-sTGFBRII

<400> 33

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<210> 34
 <211> 1990
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> EGFP:ffluc-T2A-CD19t

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<210> 35
 <211> 972
 <212> DNA
 <213> Artificial Sequence

<220>
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<210> 36

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Forward cloning primer

<400> 36

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<210> 37

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse cloning primer

<400> 37

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<210> 38

<211> 1162

<212> DNA

<213> Artificial Sequence

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<400> 38

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aataatcaaag gacttacgca ggccagtagg aatgcgaatg atggaataag catcgcacag 480
actacggaag gagcgctgaa cgaaatcaac aataacctcc agcgcgttcg cgaactctct 540
gtccaggcga caacgggcac gaattctgat agcgatctta aatcaatata agacgagata 600
cagcagcgct tggaagagat tgatagggtg agcggacaaa cgcaattcaa tggcgttaaa 660
gtgctttccc aggacaatca gatgaagata caagtcggcg caaacgacgg ggagacgatt 720
acaatcgact tgcaaaaaat agatgtgaaa agcctcgggt tggatgggtt taacgtcaat 780
agtcttgga ttagtggtgg cggcggtggg attttggaac gcatgggtac tcttattaat 840

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gaagatgccg cagcagctaa aaaaagtact gctaaccgcg tggcatccat cgattcagcg 900
ttgagtaaag ttgacgcagt cgcgacgagt ctgggcgcga tacaaaacag atttgattcc 960
gccattacca acctcggcaa taccgtcacc aatttgaatt cagcgagatc tcggattgag 1020
gacgccgatt acgcaacaga agtgtctaac atgtctaaag ctcaaattctt gcaacaggcc 1080
ggcacctccg tactggctca ggccaatcaa gttccacaga atgtgctgag tctgcttcga 1140
taagctctag acccgggctg ca 1162

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<210> 39
<211> 440
<212> PRT
<213> Artificial Sequence

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```

<220>
<223> scFV PD-1 inhibitor

```

```

<400> 39
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1          5          10          15
Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
20          25          30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
100          105          110
Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
115          120          125
Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
130          135          140
Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145          150          155          160
Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
165          170          175
Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys
180          185          190
Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
195          200          205
Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala
210          215          220
Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
225          230          235          240
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
245          250          255
Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
260          265          270
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
275          280          285
Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
290          295          300
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
305          310          315          320
Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
325          330          335

```

Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Gln	Glu	Glu	Met	Thr	
			340					345					350			
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	
		355					360					365				
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	
	370					375					380					
Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	
385					390					395					400	
Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Glu	Gly	Asn	Val	Phe	
				405				410						415		
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	
			420					425					430			
Ser	Leu	Ser	Leu	Ser	Leu	Gly	Lys									
		435					440									

<210> 40
 <211> 214
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Light chain sequence

<400> 40

Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Leu	Ser	Pro	Gly	
1				5					10					15		
Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	Val	Ser	Ser	Tyr	
			20					25					30			
Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	
		35				40						45				
Tyr	Asp	Ala	Ser	Asn	Arg	Ala	Thr	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	
	50				55					60						
Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	
65				70					75					80		
Glu	Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Ser	Ser	Asn	Trp	Pro	Arg	
				85				90					95			
Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	
			100					105					110			
Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	
		115				120						125				
Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	
	130				135						140					
Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	
145				150						155					160	
Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	
			165					170						175		
Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	
		180				185							190			
Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	
		195				200						205				
Phe	Asn	Arg	Gly	Glu	Cys											
		210														

<210> 41
 <211> 128
 <212> PRT

<213> Artificial Sequence

<220>

<223> Variable heavy

<400> 41

Gln	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Val	Val	Gln	Pro	Gly	Arg	
1				5				10						15		
Ser	Leu	Arg	Leu	Asp	Cys	Lys	Ala	Ser	Gly	Ile	Thr	Phe	Ser	Asn	Ser	
			20					25					30			
Gly	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	
		35					40					45				
Ala	Val	Ile	Trp	Tyr	Asp	Gly	Ser	Lys	Arg	Tyr	Tyr	Ala	Asp	Ser	Val	
	50					55					60					
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Leu	Phe	
65					70					75				80		
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	
			85					90					95			
Ala	Thr	Asn	Asp	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	
		100					105					110				
Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	
		115					120					125				

<210> 42

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> Variable light

<400> 42

Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Leu	Ser	Pro	Gly	
1				5				10						15		
Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	Val	Ser	Ser	Tyr	
			20					25					30			
Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	
		35					40					45				
Tyr	Asp	Ala	Ser	Asn	Arg	Ala	Thr	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	
	50					55					60					
Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	
65					70					75				80		
Glu	Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Ser	Ser	Asn	Trp	Pro	Arg	
			85					90					95			
Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys						
		100						105								

<210> 43

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Optimized heavy chain

<400> 43

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Phe Arg Gly
 1 5 10 15
 Val Gln Cys

<210> 44
 <211> 57
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Optimized heavy chain

<400> 44
 Ala Thr Gly Gly Ala Gly Thr Thr Thr Gly Gly Gly Cys Thr Gly Ala
 1 5 10 15
 Gly Cys Thr Gly Gly Gly Thr Thr Thr Thr Cys Cys Thr Cys Gly Thr
 20 25 30
 Thr Gly Cys Thr Cys Thr Thr Thr Thr Thr Ala Gly Ala Gly Gly Thr
 35 40 45
 Gly Thr Cys Cys Ala Gly Thr Gly Thr
 50 55

<210> 45
 <211> 22
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Optimized light chain

<400> 45
 Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
 1 5 10 15
 Leu Ser Gly Ala Arg Cys
 20

<210> 46
 <211> 66
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Optimized light chain

<400> 46
 Ala Thr Gly Gly Ala Cys Ala Thr Gly Ala Gly Gly Gly Thr Cys Cys
 1 5 10 15
 Cys Thr Gly Cys Thr Cys Ala Gly Cys Thr Cys Cys Thr Gly Gly Gly
 20 25 30
 Gly Cys Thr Cys Cys Thr Gly Cys Thr Gly Cys Thr Cys Thr Gly Gly
 35 40 45
 Cys Thr Cys Thr Cys Ala Gly Gly Thr Gly Cys Cys Ala Gly Ala Thr
 50 55 60
 Gly Thr
 65