

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.101W0

<150> 62/361348

<151> 2016-07-12

<150> 62/216224

<151> 2015-09-09

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<210> 28

<211> 2197

<212> DNA

<213> Artificial Sequence

<220>

<223> EGFRt-P2A-soluble VEGF Receptor

<400> 28

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<210> 29

<211> 1641

<212> DNA

<213> Artificial Sequence

<220>

<223> EGreen Fluorescent Protein

<400> 29

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<210> 30

<211> 1038

<212> DNA

<213> Artificial Sequence

<220>

<223> T2A-CD19t

<400> 30

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aaacccttct taaaactcag cctggggctg ccaggcctgg gaatccacat gaggcccctg 300
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<210> 31

<211> 972

<212> DNA

<213> Artificial Sequence

<220>

<223> CD19t epitope tag

<400> 31

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<210> 32

<211> 567

<212> DNA

<213> Artificial Sequence

<220>

<223> T2A-sTGFBR1I

<400> 32

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<210> 33

<211> 1539

<212> DNA

<213> Artificial Sequence

<220>

<223> CD19t-T2A-sTGFBR1I

<400> 33

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<210> 34

<211> 1990

<212> DNA

<213> Artificial Sequence

<220>

<223> EGFP:ffluc-T2A-CD19t

<400> 34

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<210> 35

<211> 972

<212> DNA

<213> Artificial Sequence

<220>

<223> IL-15

<400> 35

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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
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<220>
 <223> Reverse cloning primer

<400> 37	
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<210> 38
 <211> 1162
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Entolomid (TLR5 agonist)

<400> 38						
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cagcagcgct	tggaagagat	tgatagggtta	agcggacaaa	cgcaattcaa	tggcggttaa	660

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gtgctttccc aggacaatca gatgaagata caagtcggcg caaacgacgg ggagacgatt 720
acaatcgact tgcaaaaaat agatgtgaaa agcctcgggt tggatgggtt taacgtcaat 780
agtcttgcca ttagtggtgg cggcgggtggg attttggaca gcatgggtac tcttattaat 840
gaagatgccg cagcagctaa aaaaagtact gctaaccgcg tggcatccat cgattcagcg 900
ttgagtaaag ttgacgcagt cgcagcagct 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

<220>

<223> scFV PD-1 inhibitor

<400> 39

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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

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

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	

<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