

Supplementary Table 1. Antibodies used for flow cytometry staining of single cell suspensions

Antigen	Antibody clone	Supplier	Catalog number	Fluorophore
CD19	FMC63	Novus Biologicals	NBP2-52716	Unconjugated
CD19	HI19 α	Biolegend	302254	PE
CD8	B9.11	Beckman Coulter	IM0451U	FITC
CD4	SK3	BD Biosciences	341654	PerCP/Cy5.5
CCR7	150503	BD Biosciences	560765	PE
CD3	UCHT1	BD Biosciences	558117	Pacific Blue
CD45RA	L48	BD Biosciences	337167	PE/Cy7
CD25	B1.49.9	Beckman Coulter	A07774	PE
CD127	A019D5	Biolegend	351316	APC
Anti-mouse IgG (H+L), F(ab') ₂ Fragment		Cell Signaling Technology	4410s	Alexa Fluor 647

Supplementary Table 2. Computational alanine scanning on the complex of CD19 ECD and HI19 α scFv

Mutation	Mutation Energy	Effect of Mutation
C:LEU112>ALA	1.88	DESTABILIZING
C:GLY110>ALA	1.69	DESTABILIZING
C:PRO134>ALA	1.41	DESTABILIZING
C:GLU96>ALA	1.01	DESTABILIZING
C:GLN162>ALA	0.95	DESTABILIZING
C:LEU164>ALA	0.86	DESTABILIZING
C:GLY111>ALA	0.74	DESTABILIZING
C:PRO172>ALA	0.59	DESTABILIZING

Supplementary Table 3. Computational alanine scanning on the complex of CD19 ECD and FMC63 scFv

Mutation	Mutation Energy	Effect of Mutation
C:TRP177>ALA	3.29	DESTABILIZING
C:TRP61>ALA	2.37	DESTABILIZING
C:ARG56>ALA	2.09	DESTABILIZING
C:LEU58>ALA	1.63	DESTABILIZING
C:TRP91>ALA	1.39	DESTABILIZING
C:LEU164>ALA	1.32	DESTABILIZING
C:ASP218>ALA	1.23	DESTABILIZING
C:ARG230>ALA	1.15	DESTABILIZING
C:TRP220>ALA	0.94	DESTABILIZING

C:PHE63>ALA	0.84	DESTABILIZING
C:LEU168>ALA	0.84	DESTABILIZING
C:ASP158>ALA	0.8	DESTABILIZING
C:PRO183>ALA	0.71	DESTABILIZING
C:TRP261>ALA	0.67	DESTABILIZING
C:HIS54>ALA	0.65	DESTABILIZING
C:LEU160>ALA	0.52	DESTABILIZING
C:MET170>ALA	0.52	DESTABILIZING

Supplementary Table 4. Non-bond interaction between HI19 α and hCD19 ECD

Name	Distance	Category	Types
C:LYS130:HZ1-H:ASP197:OD2	2.99968	Hydrogen Bond; Electrostatic	Salt Bridge;Attractive Charge
C:LYS117:NZ-H:ASP197:OD2	4.74657	Electrostatic	Attractive Charge
C:LYS130:NZ - H:ASP195:OD2	4.75474	Electrostatic	Attractive Charge
C:GLN162:HE21 - H:TYR60:O	2.87565	Hydrogen Bond	Conventional Hydrogen Bond
C:GLN162:HE22 - H:SER61:O	2.06008	Hydrogen Bond	Conventional Hydrogen Bond
C:THR169:HG1 - H:TYR60:OH	1.86865	Hydrogen Bond	Conventional Hydrogen Bond
H:TYR64:HH - C:SER163:O	2.42486	Hydrogen Bond	Conventional Hydrogen Bond
H:TYR64:HH - C:ASP167:O	2.32071	Hydrogen Bond	Conventional Hydrogen Bond
H:SER67:HG - C:PRO172:O	2.00899	Hydrogen Bond	Conventional Hydrogen Bond
H:SER78:HG - C:ASN161:O	2.76553	Hydrogen Bond	Conventional Hydrogen Bond
H:TYR172:HH - C:GLY97:O	2.46944	Hydrogen Bond	Conventional Hydrogen Bond
H:ARG238:HH21 - C:GLU96:OE1	2.56241	Hydrogen Bond	Conventional Hydrogen Bond
H:ARG238:HH22 - C:GLU96:OE1	2.46999	Hydrogen Bond	Conventional Hydrogen Bond
H:SER242:HG - C:VAL95:O	2.8557	Hydrogen Bond	Conventional Hydrogen Bond
H:SER242:HG - C:GLU96:O	3.02546	Hydrogen Bond	Conventional Hydrogen Bond
H:TYR248:HH - C:GLU96:O	2.22702	Hydrogen Bond	Conventional Hydrogen Bond
H:TYR251:HH - C:GLU96:OE2	2.18266	Hydrogen Bond	Conventional Hydrogen Bond
C:LYS130:HE2 - H:ASP195:OD2	2.74364	Hydrogen Bond	Carbon Hydrogen Bond
C:PRO134:HD1 - H:ILE241:O	3.0158	Hydrogen Bond	Carbon Hydrogen Bond
C:LEU164:HA - H:TYR64:OH	2.12798	Hydrogen Bond	Carbon Hydrogen Bond
C:MET170:HA - H:ARG65:O	2.29093	Hydrogen Bond	Carbon Hydrogen Bond
H:THR42:HB - C:ASN161:O	2.84799	Hydrogen Bond	Carbon Hydrogen Bond
H:SER67:HB2 - C:ALA171:O	2.02977	Hydrogen Bond	Carbon Hydrogen Bond
H:GLY194:HA2 - C:GLY129:O	3.08142	Hydrogen Bond	Carbon Hydrogen Bond
H:THR240:HB - C:GLU96:O	3.01836	Hydrogen Bond	Carbon Hydrogen Bond
H:SER242:HB2 - C:GLU96:O	1.96151	Hydrogen Bond	Carbon Hydrogen Bond
C:THR169:HN - H:TYR64	2.94094	Hydrogen Bond	Pi-Donor Hydrogen Bond
C:PRO134 - H:ILE241	4.84992	Hydrophobic	Alkyl
C:PRO134 - H:VAL244	4.05062	Hydrophobic	Alkyl
H:VAL244 - C:LEU101	5.48013	Hydrophobic	Alkyl
H:VAL244 - C:LEU116	3.88092	Hydrophobic	Alkyl

H:VAL245 - C:LEU112	5.05273	Hydrophobic	Alkyl
H:TYR64 - C:LEU168	5.37667	Hydrophobic	Pi-Alkyl
H:TYR192 - C:LYS130	5.49221	Hydrophobic	Pi-Alkyl

Supplementary Table 5. Non-bond interaction between FMC63 and hCD19 ECD

Name	Distance	Category	Types
F:ARG53:NE - C:ASP218:OD2	4.97651	Electrostatic	Attractive Charge
C:ARG56:HH11 - F:GLY247:O	2.06903	Hydrogen Bond	Conventional Hydrogen Bond
C:ARG56:HH12 - F:TYR246:O	2.05558	Hydrogen Bond	Conventional Hydrogen Bond
C:ARG56:HH12 - F:GLY248:O	2.07919	Hydrogen Bond	Conventional Hydrogen Bond
C:ARG56:HH22 - F:GLY248:O	2.54991	Hydrogen Bond	Conventional Hydrogen Bond
C:THR169:HN - F:SER198:O	2.17681	Hydrogen Bond	Conventional Hydrogen Bond
C:ARG230:HH12 - F:LEU54:O	2.05587	Hydrogen Bond	Conventional Hydrogen Bond
F:TYR32:HH - C:PRO57:O	1.97098	Hydrogen Bond	Conventional Hydrogen Bond
F:TYR204:HH - C:SER159:O	2.09663	Hydrogen Bond	Conventional Hydrogen Bond
F:SER210:HG - C:ASP158:OD1	2.59296	Hydrogen Bond	Conventional Hydrogen Bond
F:THR213:HG1 - C:ASP158:O	1.99273	Hydrogen Bond	Conventional Hydrogen Bond
C:HIS54:HD2 - F:GLY247:O	1.96267	Hydrogen Bond	Carbon Hydrogen Bond
C:ARG56:HD2 - F:GLY247:O	3.00059	Hydrogen Bond	Carbon Hydrogen Bond
C:LEU164:HA - F:GLU199:O	2.0872	Hydrogen Bond	Carbon Hydrogen Bond
C:LEU168:HA - F:SER198:O	1.98845	Hydrogen Bond	Carbon Hydrogen Bond
F:SER198:HA - C:THR169:O	2.15073	Hydrogen Bond	Carbon Hydrogen Bond
F:THR201:HA - C:LEU164:O	2.24241	Hydrogen Bond	Carbon Hydrogen Bond
F:SER210:HA - C:ASP158:OD1	2.14936	Hydrogen Bond	Carbon Hydrogen Bond
F:THR213:HA - C:ASP158:O	2.71305	Hydrogen Bond	Carbon Hydrogen Bond
F:ILE215:HA - C:ASN161:OD1	2.47184	Hydrogen Bond	Carbon Hydrogen Bond
C:ARG56:NE - F:TYR251	4.30386	Electrostatic	Pi-Cation
C:ARG56:HH21 - F:TYR251	2.16168	Hydrogen Bond	Pi-Donor Hydrogen Bond
C:TRP177 - F:TRP195	5.42153	Hydrophobic	Pi-Pi Stacked
C:TRP177 - F:TRP195	4.34988	Hydrophobic	Pi-Pi Stacked
C:TRP177 - F:TRP195	4.01551	Hydrophobic	Pi-Pi Stacked
F:TRP195 - C:TRP177	4.5345	Hydrophobic	Pi-Pi Stacked
C:PRO183 - F:LEU96	4.28572	Hydrophobic	Alkyl
C:PRO184 - F:LEU96	4.52884	Hydrophobic	Alkyl
F:TYR32 - C:PRO57	4.90288	Hydrophobic	Pi-Alkyl
F:HIS50 - C:PRO57	5.19082	Hydrophobic	Pi-Alkyl
F:TYR203 - C:VAL182	5.28128	Hydrophobic	Pi-Alkyl
F:TYR203 - C:PRO183	4.73926	Hydrophobic	Pi-Alkyl

Supplementary Table 6. Univariate analysis for relapse-free survival

	B	SE	Wald	HR	P	95% CI
CD8 ⁺ T _N (Q2-4 /Q1) *	-1.940	0.781	6.169	0.144	0.013	0.031-0.664

CD4 ⁺ T _N (Q2-4 /Q1) *	-0.892	0.676	1.743	0.410	0.187	0.109-1.541
Age (≥18/<18 years old)	1.301	0.684	3.623	3.674	0.057	0.962-14.03
High-risk gene mutation or deletion *(yes/no)	0.141	0.628	0.050	1.151	0.823	0.336-3.938
Glucocorticoid therapy (yes/no)	-0.496	0.694	0.510	0.609	0.475	0.156-2.375
Transplantation (yes/no)	-1.919	0.688	7.789	0.147	0.005	0.038-0.565

T_N, naïve T cells; Q2-4 and Q1, CD8⁺ T_N cells percentage in 20 min after CD19 CAR T infusion were ranked and divided the into four quartiles, with the lowest CD8⁺ T_N cells frequency in quartile one (Q1) and the greatest in quartile four (Q4); High-risk gene mutation or deletion include T315I mutation, TP53 mutation, IKZF1 deletion

Supplementary Table 7. Multivariate analysis for relapse-free survival

	B	SE	Wald	HR	P	95% CI
CD8 ⁺ T _N (Q2-4 /Q1) *	-2.279	0.903	6.373	0.102	0.012	0.017-0.601
Transplantation (yes/no)	-1.213	0.928	1.710	0.297	0.191	0.048-1.832
Age (≥18/<18 years old)	0.814	0.876	0.864	2.257	0.353	0.406-12.56

T_N, naïve T cells; Q2-4 and Q1, CD8⁺ T_N cells percentage in 20 min after CD19 CAR T infusion were ranked and divided the into four quartiles, with the lowest CD8⁺ T_N cells frequency in quartile one (Q1) and the greatest in quartile four (Q4)