

Supplementary information

Supplementary table S1. Cell-based FcγRI signaling assay. Mean normalized luminescence ± SD (N=3).

[ANTIBODY] in nM	Siplizumab		DG siplizumab		FcS anti-CD2 IgG1		FcS anti-CD2 IgG2		FcS anti-CD2 IgG4	
6.67	100.0	0.0	125.6	2.7	5.7	0.4	3.8	0.7	3.2	0.4
1.11	103.1	6.0	123.0	11.0	5.8	0.9	3.5	0.5	3.0	0.4
0.19	106.0	11.9	127.3	8.5	6.0	0.5	3.2	0.3	2.9	0.2
0.031	101.7	13.6	107.8	6.9	3.4	0.4	2.9	0.7	2.8	0.2
0.0051	26.7	3.9	41.9	2.6	2.5	0.3	2.6	0.1	2.6	0.5
0.00086	5.1	0.4	8.5	1.2	2.5	0.3	2.5	0.5	2.9	0.4
0.000143	2.4	0.7	2.5	0.4	2.4	0.6	2.4	0.4	2.6	0.5
0.000024	2.4	0.5	2.6	0.6	2.7	0.4	2.4	0.3	2.8	0.5

Supplementary table S2. Cell-based FcγRIIA signaling assay. Mean normalized luminescence ± SD (N=3).

[ANTIBODY] in nM	Siplizumab		DG siplizumab		FcS anti-CD2 IgG1		FcS anti-CD2 IgG2		FcS anti-CD2 IgG4	
666.7	100.0	0.0								
111.1	107.8	17.5								
18.52	70.1	3.7								
3.09	45.3	1.2								
0.51	40.7	4.0								
0.086	11.0	1.4								
0.014	3.2	0.6								
0.002	3.0	0.3								
66.7			4.4	0.9	3.1	0.6	6.6	2.0	3.8	0.9
11.1			3.2	0.4	3.4	0.8	5.7	0.4	3.1	0.6
1.85			3.2	0.4	3.1	0.7	4.4	0.4	3.7	1.4
0.31			2.9	0.7	3.0	0.6	4.4	0.5	3.5	0.2
0.051			3.4	0.6	2.8	0.6	3.6	0.4	3.3	1.2
0.009			2.6	0.4	2.7	0.7	2.9	0.4	2.4	0.2
0.001			3.0	0.4	3.0	1.1	3.2	0.1	2.3	0.5
0.00024			2.6	0.4	2.5	0.5	3.0	0.5	3.4	0.4

Supplementary table S3. Cell-based Fc γ RIIIA signaling assay. Mean normalized luminescence \pm SD (N=3).

[ANTIBODY] in nM	Siplizumab		DG siplizumab		FcS anti-CD2 IgG1		FcS anti-CD2 IgG2		FcS anti-CD2 IgG4	
6.67	100.0	0.0	7.3	0.6	6.8	0.7	3.8	0.5	6.8	0.3
1.11	100.0	13.8	8.4	1.9	7.0	0.2	3.2	0.1	7.3	1.1
0.19	89.0	7.1	7.0	0.2	7.3	0.9	2.8	0.2	6.7	1.1
0.031	74.8	4.4	6.5	0.9	6.4	0.8	4.1	0.4	6.2	0.4
0.0051	44.5	3.9	6.5	0.7	6.3	0.5	5.4	0.2	6.4	0.9
0.00086	13.4	3.0	6.0	0.9	6.8	1.4	6.2	0.3	6.3	0.1
0.000143	6.6	0.9	7.5	2.0	6.7	0.5	6.5	0.6	6.1	1.4
0.000024	6.1	0.4	5.9	1.0	6.6	1.5	6.1	0.5	6.4	0.5

Supplementary table S4. CD2 expression. Average CD2 median fluorescent intensity \pm SEM (N=5).). Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01).

	AVERAGE MFI	SEM
T CELLS	651.9	56.3
CD4 TN	442.6	7.6
CD4 TCM	644.7	48.9
CD4 TEM	791.8	102.4
CD4 TMERA	468.9	36.6
CD8 TN	655.7	28.3
CD8 TCM	852.5	147.9
CD8 TEM	1165.1	92.7
CD8 TEMRA	740.4	117.7
RTREG	442.1	43.2
NSTREG	621.1	38.7
ATREG	777.5	97.0
B CELLS	2.7**	21.2
CD56^{BRIGHT}	713.3	120.6
CD56^{DIM}	150.5**	33.1
CD56^{NEG} CD16⁺	145.1*	46.0

Supplementary table S5. CD2 expression on resting and activated PBMC. Two-tailed paired t-test results (* p<0.05, ** p<0.01). Data shown as average median fluorescence intensity \pm SEM.

	T cells		B cells		CD56 ^{bright}		CD56 ^{dim}		CD56 ^{neg} CD16 ⁺	
RESTING	657.3	102.0	2.7	47.4	713.3	269.6	150.5	74.0	145.1	102.8
ACTVATED	11226.6**	3749.1	0.0	196.4	3465.0*	1674.6	990.8**	256.1	990.8**	256.1

Supplementary table S6. CD69+ NK cells in mixed lymphocyte reaction over time. Mean percentage of CD69+ NK cells \pm SD (N=12). Repeated-measure two-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

Time in days	No antibody		Siplizumab		DG siplizumab	
0	5.6	4.4				
1	14.2	7.9	43.2****	12.4	14.6	6.6
2	15.5	6.4	32.7***	10.8	8.1***	4.3
4	33.2	19.4	36.5	17.7	16.1**	12.3
7	37.8	20.0	36.5	14.6	20.4**	12.7

Supplementary table S7. CD69+ NK cells in autologous lymphocyte culture over time. Mean percentage of CD69+ NK cells \pm SD (N=12). Repeated-measure two-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

Time in days	No antibody		Siplizumab		DG siplizumab	
0	5.7	4.4				
1	12.0	7.2	43.5****	17.7	15.1*	8.6
2	6.3	3.8	34.5****	13.9	7.3	4.5
4	9.6	7.7	24.4***	10.7	6.5**	6.3
7	15.5	11.1	25.7**	8.2	13.1	8.2

Supplementary table S8. CD69+ NK cells after seven days of mixed lymphocyte reaction incubated with increasing doses of anti-CD2 antibody. Mean percentage of CD69+ NK cells \pm SD (N=9). Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001).

[Antibody] in μ g/ml	Siplizumab		DG siplizumab		FcS anti-CD2 IgG1		FcS anti-CD2 IgG2		FcS anti-CD2 IgG4	
0	28.6	15.1								
0.0001	29.6	15.8	31.3	14.3	34.9	16.3	33.1	14.8	31.3	15.9
0.001	28.3	14.9	28.6	13.2	34.2	15.5	29.9	13.7	31.3	14.3
0.01	41.0	23.1	23.2	10.9	27.2	14.3	29.4	13.7	28.4	14.6
0.1	49.7***	17.7	23.2	11.3	19.8*	10.9	11.9*	4.2	19.9*	10.7
1	42.9**	18.5	18.1**	12.0	18.7*	8.1	8.7**	4.3	18.6*	9.9
10	42.1*	19.7	14.4**	8.0	14.3***	8.7	8.1**	4.9	14.1**	9.5

Supplementary table S9. CD2 expression on NK cells after seven days of mixed lymphocyte reaction incubated with increasing doses of anti-CD2 antibody. Average median CD2 fluorescence intensity on NK cells \pm SD (N=9). Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

[Antibody] in μ g/ml	Siplizumab	DG siplizumab	FcS anti-CD2 IgG1	FcS anti-CD2 IgG2	FcS anti-CD2 IgG4
0	3091.0	1074.0			
0.0001	2912.0	1193.5	2910.0*	1115.2	3190.3
0.001	2783.6*	1043.3	3033.1	1242.4	3146.7
0.01	1968.2**	1110.7	2393.1**	919.6	2378.8**
0.1	159.8***	61.5	909.3***	405.0	1557.1***
1	124.8***	51.6	856.3***	439.6	1404.9***
10	156.2***	86.5	1073.4****	686.5	1466.1***

Supplementary table S10. NK cell lysis after incubation of purified NK cells with increasing doses of anti-CD2 antibody. Mean percentage of 7-AAD+ NK cells \pm SD (N=6). Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001).

[Antibody] in μ g/ml	Siplizumab	FcS anti-CD2 IgG1	FcS anti-CD2 IgG2	FcS anti-CD2 IgG4
0	6.0	2.4		
0.001	7.1	2.5	6.7	2.4
0.01	14.6**	3.5	6.6	2.4
0.1	28.8**	6.7	6.6	2.5
1	33.3***	6.4	6.7	2.6
10	35.0***	6.3	6.6	2.7

Supplementary table S11. Normalized NK cell count after incubation of purified NK cells with increasing doses of anti-CD2 antibody. Mean normalized NK cell count \pm SD (N=6). Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

[Antibody] in μ g/ml	Siplizumab	FcS anti-CD2 IgG1	FcS anti-CD2 IgG2	FcS anti-CD2 IgG4
0	100.0	0.0		
0.001	103.1	3.0	105.7	6.1
0.01	81.0*	11.3	105.3	7.3
0.1	47.8***	10.6	102.0	15.1
1	41.0****	8.4	105.5	10.9
10	41.1****	8.2	107.4	8.2

Supplementary table S12. Normalized CD56^{dim} NK cell count after incubation of purified NK cells with increasing doses of anti-CD2 antibody. Mean normalized CD56^{dim} NK cell count \pm SD (N=6). Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

[Antibody] in μ g/ml	Siplizumab	FcS anti-CD2 IgG1	FcS anti-CD2 IgG2	FcS anti-CD2 IgG4
0	100.0	0.0		
0.001	102.8	2.8	106.1	6.1
0.01	81.5*	9.8	105.7	6.9
0.1	50.7***	10.7	102.4	14.5
1	43.8****	8.6	106.3	10.3
10	44.0****	8.4	107.2	8.1

Supplementary table S13. Normalized CD56^{bright} NK cell count after incubation of purified NK cells with increasing doses of anti-CD2 antibody. Mean normalized CD56^{bright} NK cell count \pm SD (N=6). Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

[Antibody] in μ g/ml	Siplizumab	FcS anti-CD2 IgG1	FcS anti-CD2 IgG2	FcS anti-CD2 IgG4
0	100.0	0.0		
0.001	94.6	10.2	95.4	13.7
0.01	42.0**	25.8	98.5	22.3
0.1	10.2****	5.1	93.9	22.2
1	7.0****	2.3	96.1	27.8
10	7.3****	3.3	108.9	12.6

Supplementary table S14. CD107a expression on NK cells after incubation of purified NK cells with increasing doses of anti-CD2 antibody. Mean percentage of CD107a+ NK cells \pm SD (N=6). Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001).

[Antibody] in μ g/ml	Siplizumab	FcS anti-CD2 IgG1	FcS anti-CD2 IgG2	FcS anti-CD2 IgG4
0	1.6	1.9		
0.001	2.0*	1.9	1.9	2.4
0.01	4.3**	2.4	1.7	2.1
0.1	10.9**	4.8	1.7	2.0
1	13.0**	4.9	1.6	1.7
10	14.8***	4.4	1.5	1.7

Supplementary table S15. CD16 expression on NK cells after incubation of purified NK cells with increasing doses of anti-CD2 antibody. Average median CD16 fluorescence intensity on NK cells \pm SD (N=6). Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

[Antibody] in μ g/ml	Siplizumab	FcS anti-CD2 IgG1	FcS anti-CD2 IgG2	FcS anti-CD2 IgG4
0	1673.8	546.3		
0.001	1577.5	594.2	1645.5	612.3
0.01	553.7**	344.3	1671.2	636.6
0.1	118.9**	105.5	1624.7	589.7
1	80.3**	52.6	1599.2	557.4
10	69.3**	37.0	1677.5	594.5

Supplementary table S16. CD107a expression on NK cells after incubation of purified NK cells with increasing doses of anti-CD2 antibody and SPI-801 target cells. Mean percentage of CD107a+ NK cells \pm SD (N=6). Pre-incubation of NK cells with anti-CD2 mAbs for 30 minutes before target cell addition. Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001).

[Antibody] in μ g/ml	Siplizumab	FcS anti-CD2 IgG1	FcS anti-CD2 IgG2	FcS anti-CD2 IgG4	NK only
0	14.7***	3.8			0.4***
0.001	17.6	6.1	19.1	4.8	13.9
0.01	23.3	7.9	18.6	5.8	13.0
0.1	28.8*	8.0	16.0	5.5	13.1
1	37.9**	10.9	16.8	6.2	11.3
10	43.5**	12.6	16.6	5.3	12.6

Supplementary table S17. CD107a expression on NK cells after incubation of purified NK cells with increasing doses of anti-CD2 antibody and SPI-801 target cells. Mean percentage of CD107a+ NK cells \pm SD (N=6). Pre-incubation of NK cells with anti-CD2 mAbs for 48 hours before target cell addition. Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001).

[Antibody] in μ g/ml	Siplizumab	FcS anti-CD2 IgG1	FcS anti-CD2 IgG2	FcS anti-CD2 IgG4	NK only
0	31.6	9.3			1.5**
0.001	27.0	4.4	30.7	7.4	29.3
0.01	28.8	6.9	31.8	7.5	29.1
0.1	31.9	9.6	31.0	8.5	26.9
1	31.8	7.5	28.7	6.1	26.5
10	31.9	9.4	28.3	4.8	28.9

Supplementary table S18. CD107a expression on NK cells after incubation of purified NK cells with increasing doses of Rituximab and CD20⁺ target cells. Mean percentage of CD107a⁺ NK cells \pm SD (N=6). Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (*^(*) p<0.1, * p<0.05, ** p<0.01, *** p<0.001).

[Rituximab] in μ g/ml	No anti-CD2		Siplizumab	FcS anti-CD2 IgG1		FcS anti-CD2 IgG2		FcS anti-CD2 IgG4		
0	2.2	2.5								
0.0001	2.4	2.4	5.1 ^(*)	4.3	2.2	2.1	1.7	1.7	2.3	2.7
0.001	2.7	2.0	5.3*	4.3	2.6	2.2	1.9	1.8	2.6	2.2
0.01	5.8 ^(*)	2.8	5.4*	4.3	5.5*	3.0	4.7	3.0	5.3	3.4
0.1	13.7*	7.1	7.0*	4.2	13.4*	7.0	12.8 ^(*)	8.0	13.3 ^(*)	8.8
1	18.8 ^(*)	10.9	8.7**	3.7	17.9*	9.8	15.4*	8.5	15.7*	8.4

Supplementary table S19. Normalized target cell count after incubation of purified NK cells with increasing doses of Rituximab and CD20⁺ target cells. Mean normalized target cell count \pm SD (N=6). Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (*^(*) p<0.1, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

[Rituximab] in μ g/ml	No anti-CD2		Siplizumab	FcS anti-CD2 IgG1		FcS anti-CD2 IgG2		FcS anti-CD2 IgG4		
0	100	0								
0.0001	91.9	16.2	155.3	49.8	113.9	16.7	137.1	30.8	106.0	21.1
0.001	82.4	22.8	145.3	47.9	99.0	31.4	114.9	32.1	85.5	26.5
0.01	47.5**	15.2	132.9	35.9	53.2*	21.6	67.0 ^(*)	24.6	59.9*	24.0
0.1	29.6****	10.3	94.7	20.7	29.0***	13.3	28.2****	9.5	30.3***	13.1
1	21.0****	8.0	64.1*	22.1	22.1****	10.6	22.8****	8.4	26.0****	10.8

Supplementary table S20. CD2 expression on NK cells after incubation of purified NK cells with increasing doses of Rituximab and CD20⁺ target cells. Median CD2 fluorescence intensity on NK cells \pm SEM (N=6). Repeated-measure one-way ANOVA results followed by Dunnett's multiple comparison test (* p<0.05, ** p<0.01, *** p<0.001).

[Rituximab] in μ g/ml	No anti-CD2		Siplizumab	FcS anti-CD2 IgG1		FcS anti-CD2 IgG2		FcS anti-CD2 IgG4		
0	3299.7	1105.6								
0.0001	3346.0	1118.4	1050.0**	603.1	2469.3**	816.0	679.2**	175.8	1136.7**	369.9
0.001	3452.2	1118.3	1082.0**	602.4	2610.5*	819.7	706.5**	169.7	1249.7**	397.0
0.01	3780.0*	1204.6	1098.8**	614.6	3037.2	966.1	792.5**	206.8	1632.5**	618.7
0.1	4155.3*	1440.2	1206.5**	690.8	3647.2	1153.5	898.2**	258.2	2369.7*	776.7
1	4303.0*	1473.3	1287.3**	743.2	3898.5*	1212.8	889.3**	267.4	2660.2*	841.5