



BRAF and MEK Inhibitors Influence the Function of Reprogrammed T Cells: Consequences for Adoptive T-Cell Therapy

Jan Dörrie, Lek Babalija, Stefanie Hoyer, Kerstin F. Gerer, Gerold Schuler, Lucie Heinzerling and Niels Schaft

Table S1. Original data used for Figure 1A ¹.

Donor	Percentage positive cells	
	mock electroporated	CAR-RNA electroporated
HD 1	8.79	96.59
HD 2	2.9	86.75
HD 3	4.59	97.53
HD 4	11.33	97.91
HD 5	6.76	96.84
HD 6	2.16	93.85
HD 7	15.91	98.29
HD 8	1.08	93.79
Average (n=8)	6.69	95.19375
SEM (n=8)	1.80138835	1.35063204

¹ Percentage positive cells was determined by flow cytometry. See legend to figure 1 and materials and methods section for details.

Table S2. Original data used for Figure 2 ¹.

	HD 1					
	CD25 (MFI)					
	Mock T only	CAR-T only	Mock T + T2	Mock T + A375M	CAR T + T2	CAR T + A375M
no inhibitor	0.56	1.93	0.91	0.73	2.32	18.66
DMSO	0.55	2.49	1.44	0.75	3.81	17.53
Vem	0.4	1.8	0.56	0	2.26	3.95
Dabra	0.64	2.57	1.75	1.08	4.1	14.23
Cobi	0.66	2.05	1.23	0.57	3.77	16.33
Tram	0.55	2.2	1.31	0.75	3.53	11.42
V+C	0.92	2.49	1.52	0.84	3.55	20.87
D+T	1.18	2.73	1.84	0.86	4.42	17.02
	CD69 (MFI)					
	Mock T only	CAR-T only	Mock T + T2	Mock T + A375M	CAR T + T2	CAR T + A375M
no inhibitor	0	2.15	2.72	0.11	4.48	17.33
DMSO	0	2.83	2.38	0.11	5.96	12.83
Vem	0	1.95	0	0	1.8	6.1
Dabra	0	2.63	2.01	0.08	4.67	9.64
Cobi	0	1.48	0	0	1.84	9.37
Tram	0	1.63	0.14	0	2.33	5.54
V+C	0	1.64	0	0	1.23	6.08
D+T	0	1.59	0.05	0	1.62	4.95

	HD 2					
	CD25 (MFI)					
	Mock T only	CAR-T only	Mock T + T2	Mock T + A375M	CAR T + T2	CAR T + A375M
no inhibitor	1.62	2.66	1.38	1.93	2.24	11.26
DMSO	1.3	2.76	1.33	1.7	2.1	17.04
Vem	1.2	2.43	1.77	1.43	2.37	8.05
Dabra	1.96	3.82	1.89	1.96	2.68	18.36
Cobi	1.38	2.82	1.67	1.79	3.07	11.74
Tram	1.55	2.85	2.02	1.7	3.19	12.06
V+C	1.41	2.36	1.81	1.49	2.04	5.01
D+T	1.52	3.35	2.41	2.02	3.79	15.64
	CD69 (MFI)					
	Mock T only	CAR-T only	Mock T + T2	Mock T + A375M	CAR T + T2	CAR T + A375M
no inhibitor	0	2.65	1.74	0.44	2.68	11.22
DMSO	0	2.75	1.65	0.27	2.22	16.36
Vem	0	1.84	0.19	0	1.71	8.8
Dabra	0.13	2.95	1.4	0.34	2.27	14
Cobi	0	2.46	0.03	0.06	1.61	6.44
Tram	0	2.27	0.25	0	1.83	7.79
V+C	0	1.61	0	0	1.27	3.68
D+T	0	2.17	0	0.07	1.97	5.49

	HD 3					
	CD25 (MFI)					
	Mock T only	CAR-T only	Mock T + T2	Mock T + A375M	CAR T + T2	CAR T + A375M
no inhibitor	0.01	3.13	0.69	0.32	1.41	27.14
DMSO	0.58	2.25	0.51	0.56	2.74	31.55
Vem	0.04	1.04	0.26	0.16	1.16	2.08
Dabra	0.38	1.7	0.77	0.48	1.78	18.7
Cobi	0.68	1.67	0.84	0.36	1.92	9.74
Tram	0.16	0.91	0.33	0.85	1.82	10.34
V+C	0.79	2.05	0.54	0.75	2.44	13.76
D+T	0.7	2.1	0.47	0.47	2.44	19.25
	CD69 (MFI)					
	Mock T only	CAR-T only	Mock T + T2	Mock T + A375M	CAR T + T2	CAR T + A375M
no inhibitor	0	2.47	2.84	0.15	2.98	53.1
DMSO	0	2.03	2.51	0.68	5.52	100.14
Vem	0	2.53	0.36	0.09	2.53	13.71
Dabra	0	1.21	1.6	0.34	2.8	32.6
Cobi	0	1.03	0.5	-0.07	1.48	5.21
Tram	0	0.4	0.32	0.48	1.28	11.65
V+C	0	1.74	0.41	0.36	2.02	14.09
D+T	0	1.71	2.63	0.47	3.35	32.74

	HD 4					
	CD25 (MFI)					
	Mock T only	CAR-T only	Mock T + T2	Mock T + A375M	CAR T + T2	CAR T + A375M
no inhibitor	1.55	3.08	0.86	1.23	0	26.75
DMSO	1.45	3.08	1.66	1.49	3.18	33.58
Vem	1.23	1.98	1.48	1.15	2.31	6.57
Dabra	1.37	2.47	1.5	1.26	2.54	11.96
Cobi	1.33	2.97	1.4	1.02	2.17	9.9
Tram	1.54	2.42	1.02	1.41	1.92	10.01
V+C	1.77	2.54	1.6	1.45	2.64	7.28
D+T	1.69	3.03	1.83	1.85	2.52	11.72
	CD69 (MFI)					
	Mock T only	CAR-T only	Mock T + T2	Mock T + A375M	CAR T + T2	CAR T + A375M
no inhibitor	0	0.84	0.27	0.4	0	20.02
DMSO	0	1.19	0.66	0.69	3.08	25.37
Vem	0	0.9	0.14	0.37	0.4	9.62
Dabra	0	0.89	0.56	0.13	1.59	8.31
Cobi	0	0.7	0.01	0	0.08	4.42
Tram	0	0.55	0	0.15	0.72	5.71
V+C	0	1.06	0.08	0.1	0.95	5.36
D+T	0	0.98	0	0.48	0.13	4.32

	Average n=4					
	CD25 (MFI)					

	Mock T only	CAR-T only	Mock T + T2	Mock T + A375M	CAR T + T2	CAR T + A375M
no inhibitor	0.935	2.7	0.96	1.0525	1.4925	20.9525
DMSO	0.97	2.645	1.235	1.125	2.9575	24.925
Vem	0.7175	1.8125	1.0175	0.685	2.025	5.1625
Dabra	1.0875	2.64	1.4775	1.195	2.775	15.8125
Cobi	1.0125	2.3775	1.285	0.935	2.7325	11.9275
Tram	0.95	2.095	1.17	1.1775	2.615	10.9575
V+C	1.2225	2.36	1.3675	1.1325	2.6675	11.73
D+T	1.2725	2.8025	1.6375	1.3	3.2925	15.9075
CD69 (MFI)						
	Mock T only	CAR-T only	Mock T + T2	Mock T + A375M	CAR T + T2	CAR T + A375M
no inhibitor	0	2.0275	1.8925	0.275	2.535	25.4175
DMSO	0	2.2	1.8	0.4375	4.195	38.675
Vem	0	1.805	0.1725	0.115	1.61	9.5575
Dabra	0.0325	1.92	1.3925	0.2225	2.8325	16.1375
Cobi	0	1.4175	0.135	-0.0025	1.2525	6.36
Tram	0	1.2125	0.1775	0.1575	1.54	7.6725
V+C	0	1.5125	0.1225	0.115	1.3675	7.3025
D+T	0	1.6125	0.67	0.255	1.7675	11.875

SEM n=4						
CD25 (MFI)						
	Mock T only	CAR-T only	Mock T + T2	Mock T + A375M	CAR T + T2	CAR T + A375M
no inhibitor	0.39	0.28	0.15	0.35	0.54	3.78
DMSO	0.24	0.18	0.25	0.28	0.36	4.43
Vem	0.30	0.29	0.36	0.36	0.29	1.33
Dabra	0.36	0.44	0.25	0.30	0.48	1.64
Cobi	0.20	0.31	0.17	0.32	0.42	1.54
Tram	0.35	0.42	0.35	0.23	0.44	0.48
V+C	0.23	0.11	0.28	0.20	0.32	3.57
D+T	0.22	0.27	0.41	0.38	0.49	1.58
CD69 (MFI)						
	Mock T only	CAR-T only	Mock T + T2	Mock T + A375M	CAR T + T2	CAR T + A375M
no inhibitor	0.00	0.41	0.59	0.08	0.93	9.41
DMSO	0.00	0.38	0.42	0.15	0.91	20.66
Vem	0.00	0.34	0.07	0.09	0.44	1.58
Dabra	0.03	0.51	0.31	0.07	0.66	5.62
Cobi	0.00	0.38	0.12	0.03	0.40	1.09
Tram	0.00	0.45	0.07	0.11	0.35	1.42
V+C	0.00	0.15	0.10	0.09	0.23	2.32
D+T	0.00	0.25	0.65	0.13	0.66	6.96

¹MFI was determined by flow cytometry. See legend to figure 2 and materials and methods section for details.

Table S3. *p*-values of CD25 upregulation (paired students *t*-test) ¹.

Inhibitor	CAR-T Cells on T2 vs. CAR-T Cells on A375M	CAR-T Cells on A375M DMSO vs. Inhibitor ²	CAR-T Cells on A375M ³
no inhibitor	*		ns
DMSO control	*		
Vem	0.07	*	*
Dabra	**	ns	
Cobi	**	ns	ns
Tram	***	0.06	
Vem & Cobi	0.07	ns	ns
Dabra & Tram	**	ns	

¹ The table depicts the results of statistical analyses related to Figure 2a. Relative expression of CD25 on CAR-transfected T cells after stimulation was compared. *p*-values were calculated using the paired Student's *t* test: (*p*>0.1: ns, *p*≤0.05: *, *p*≤0.01: **, *p*≤0.001: ***; *p*-values >0.05 but ≤0.1 are indicated as numbers). ² Comparison between the indicated inhibitor and the DMSO solvent control.

³ Comparison between each 2 corresponding inhibitors i.e. no inhibitor vs DMSO control, Vem vs Dabra, Cobi vs. Tram, and Vem & Cobi vs. Dabra & Tram.

Table S4. *p*-values of CD69 upregulation (paired students *t*-test) ¹.

Inhibitor	CAR-T Cells on T2 vs. CAR-T Cells on A375M	CAR-T Cells on A375M DMSO vs. Inhibitor ²	CAR-T Cells on A375M ³
no inhibitor	0.09		ns
DMSO control	ns		
Vem	*	ns	ns
Dabra	0.10	ns	
Cobi	**	ns	ns
Tram	*	ns	
Vem & Cobi	0.07	ns	ns
Dabra & Tram	ns	ns	

¹ The table depicts the results of statistical analyses related to Figure 2b. Relative expression of CD69 on CAR-transfected T cells after stimulation was compared. *p*-values were calculated using the paired Student's *t* test: (*p*>0.1: ns, *p*≤0.05: *, *p*≤0.01: **; *p*-values >0.05 but ≤0.1 are indicated as numbers).

² Comparison between the indicated inhibitor and the DMSO solvent control. ³ Comparison between each 2 corresponding inhibitors i.e. no inhibitor vs DMSO control, Vem vs Dabra, Cobi vs. Tram, and Vem & Cobi vs. Dabra & Tram.

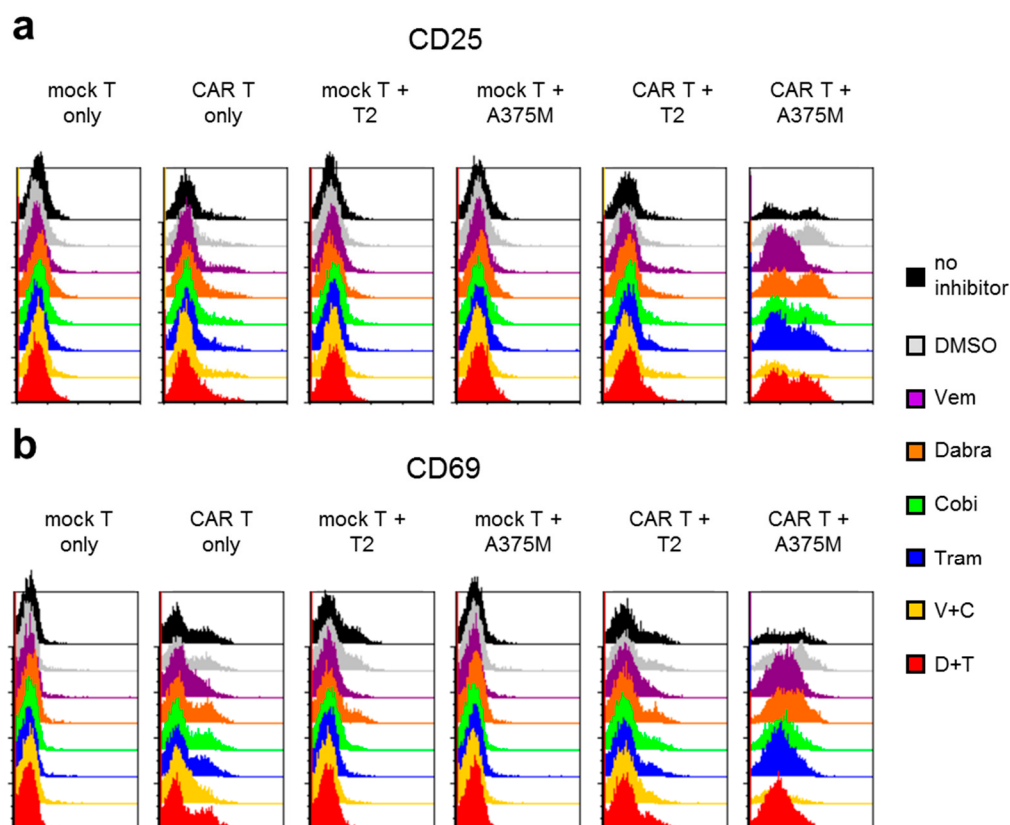


Figure S1. BRAF and MEK inhibitor treatment affects CAR-T-cell activation after antigen-specific stimulation. CAR-T cells were generated as described in figure 1. Four hours after electroporation, these cells were co-incubated over-night with CSPG4-negative T2 cells and the CSPG4⁺ melanoma cell line A375M at a 1:1 ratio. Mock-transfected T cells were used as control. Co-incubations were performed in the absence of inhibitors (no inhibitor), in the presence of DMSO only (solvent control), or in the presence of the different kinase inhibitors, either alone or in combination. The used kinase inhibitors vemurafenib (Vem, V), dabrafenib (Dabra, D), cobimetinib (Cobi, C), and trametinib (Tram, T) were used in final concentrations listed in Table 1. Mock-transfected T cells stimulated with T2 or A375M, and mock-transfected T cells and CAR-T cells incubated without target cells served as negative controls. After 16 hours of co-incubation, the cells were harvested and stained for the activation markers CD25 (a) and CD69 (b) and measured by flow cytometry. Histograms of a representative donor are depicted.

Table S5. Original data used for Figure 3 ¹.

HD1				
IL-2	TNF	IFN γ		
pg/ml	pg/ml	pg/ml		
0	8	3	no inhibitor	T2 only
0	1	4		A375M only
33	192	166		Mock T + T2
1	14	20		Mock T + A375M
28	353	454		CAR T + T2
654	2303	7594		CAR T + A375M
1	9	12	DMSO	T2 only
0	2	8		A375M only
57	232	278		Mock T + T2
1	9	23		Mock T + A375M
18	421	397		CAR T + T2
837	3234	9679		CAR T + A375M
1	7	5	Vem	T2 only
1	2	7		A375M only
1	58	32		Mock T + T2
1	4	13		Mock T + A375M
7	224	407		CAR T + T2
213	1257	2865		CAR T + A375M
1	4	5	Dabra	T2 only
1	2	10		A375M only
16	149	332		Mock T + T2
1	4	21		Mock T + A375M
13	297	649		CAR T + T2
674	2632	9460		CAR T + A375M
1	1	6	Cobi	T2 only
0	0	9		A375M only
10	18	65		Mock T + T2
1	3	6		Mock T + A375M
3	34	117		CAR T + T2
125	562	3333		CAR T + A375M
1	2	7	Tram	T2 only
0	2	6		A375M only
15	27	65		Mock T + T2
1	3	8		Mock T + A375M
3	52	129		CAR T + T2
238	850	4026		CAR T + A375M
1	4	8	V+C	T2 only
1	1	3		A375M only
0	3	11		Mock T + T2

0	0	5		Mock T + A375M
2	26	179		CAR T + T2
27	194	601		CAR T + A375M
1	2	6		T2 only
1	1	5		A375M only
1	13	72		Mock T + T2
1	2	9		Mock T + A375M
2	33	128		CAR T + T2
114	544	3217	D+T	CAR T + A375M
HD2				
IL-2	TNF	IFN γ		
pg/ml	pg/ml	pg/ml		
0	3	-1		T2 only
0	0	0		A375M only
29	76	324		Mock T + T2
2	6	28		Mock T + A375M
24	346	2614		CAR T + T2
2238	5148	14468	no inhibitor	CAR T + A375M
0	6	-2		T2 only
0	-1	0		A375M only
14	95	306		Mock T + T2
2	5	23		Mock T + A375M
13	373	2133		CAR T + T2
1975	5042	13321	DMSO	CAR T + A375M
0	4	-1		T2 only
0	-1	0		A375M only
0	9	4		Mock T + T2
0	0	3		Mock T + A375M
2	102	286		CAR T + T2
154	1321	1416	Vem	CAR T + A375M
0	3	1		T2 only
0	0	1		A375M only
4	40	530		Mock T + T2
0	1	9		Mock T + A375M
5	127	2344		CAR T + T2
1662	3986	14834	Dabra	CAR T + A375M
0	0	0		T2 only
-1	0	0		A375M only
3	6	118		Mock T + T2
0	0	6		Mock T + A375M
2	21	396		CAR T + T2
466	942	5575	Cobi	CAR T + A375M
0	0	2	Tram	T2 only

0	-1	2		A375M only
4	10	108		Mock T + T2
0	-1	5		Mock T + A375M
1	31	407		CAR T + T2
801	1241	6632		CAR T + A375M
0	-1	-2		T2 only
0	-1	1		A375M only
0	2	10		Mock T + T2
0	1	1		Mock T + A375M
0	14	132		CAR T + T2
485	649	3549	V+C	CAR T + A375M
0	0	1		T2 only
0	-1	0		A375M only
0	3	106		Mock T + T2
0	0	-1		Mock T + A375M
0	13	426		CAR T + T2
690	1088	6763	D+T	CAR T + A375M
HD3				
IL-2	TNF	IFNgamma		
pg/ml	pg/ml	pg/ml		
0	1	-1		T2 only
0	1	0		A375M only
69	16	95		Mock T + T2
4	100	29		Mock T + A375M
151	174	1432		CAR T + T2
2100	2328	9225	no inhibitor	CAR T + A375M
0	11	0		T2 only
0	1	-1		A375M only
53	59	195		Mock T + T2
4	101	31		Mock T + A375M
100	530	2580		CAR T + T2
1848	2160	9508	DMSO	CAR T + A375M
0	4	-1		T2 only
0	-1	-1		A375M only
1	13	3		Mock T + T2
0	40	8		Mock T + A375M
6	84	405		CAR T + T2
384	841	2156	Vem	CAR T + A375M

0	1	-1		T2 only
0	1	0		A375M only
33	11	178		Mock T + T2
1	40	15		Mock T + A375M
38	49	1473		CAR T + T2
1473	1446	9365	Dabra	CAR T + A375M
0	2	-1		T2 only
0	0	1		A375M only
20	4	48		Mock T + T2
0	4	1		Mock T + A375M
18	14	266		CAR T + T2
417	183	3021	Cobi	CAR T + A375M
0	0	-2		T2 only
0	1	0		A375M only
39	6	57		Mock T + T2
2	22	15		Mock T + A375M
51	35	498		CAR T + T2
998	745	5199	Tram	CAR T + A375M
1	1	-1		T2 only
1	0	1		A375M only
1	1	-1		Mock T + T2
0	3	2		Mock T + A375M
1	11	81		CAR T + T2
120	110	695	V+C	CAR T + A375M
0	1	0		T2 only
0	0	0		A375M only
5	3	60		Mock T + T2
1	6	4		Mock T + A375M
10	11	381		CAR T + T2
680	358	3883	D+T	CAR T + A375M
HD4				
IL-2	TNF	IFN γ		
pg/ml	pg/ml	pg/ml		
			no inhibitor	

0	3	-4		T2 only
0	0	-6		A375M only
35	43	142		Mock T + T2
1	8	21		Mock T + A375M
30	124	1226		CAR T + T2
2138	3159	9489		CAR T + A375M
0	3	-4	DMSO	T2 only
0	0	-1		A375M only
21	35	125		Mock T + T2
1	8	23		Mock T + A375M
21	113	1038		CAR T + T2
2370	3405	9788		CAR T + A375M
0	0	-3	Vem	T2 only
0	2	0		A375M only
0	2	-2		Mock T + T2
0	1	-1		Mock T + A375M
3	31	280		CAR T + T2
108	421	690		CAR T + A375M
0	0	1	Dabra	T2 only
0	-1	-3		A375M only
13	18	329		Mock T + T2
0	1	8		Mock T + A375M
18	50	1653		CAR T + T2
2038	2267	11235		CAR T + A375M
0	0	-4	Cobi	T2 only
0	1	-1		A375M only
6	3	97		Mock T + T2
0	0	7		Mock T + A375M
2	12	240		CAR T + T2
660	283	3934		CAR T + A375M
0	1	-2	Tram	T2 only
0	0	0		A375M only
10	10	169		Mock T + T2
0	2	5		Mock T +

				A375M
2	20	386		CAR T + T2
1126	720	5828		CAR T + A375M
0	1	-4		T2 only
0	0	-3		A375M only
2	1	51		Mock T + T2
0	0	8		Mock T + A375M
1	10	182		CAR T + T2
794	266	3442	V+C	CAR T + A375M
0	-1	-4		
0	58	85		
0	1	-6		T2 only
0	-1	-2		A375M only
27	17	625		Mock T + T2
0	3	25		Mock T + A375M
4	36	1157		CAR T + T2
2012	2304	10720	D+T	CAR T + A375M
n=4				
IL-2	TNF	IFNgamma		
pg/ml	pg/ml	pg/ml		
0	4	-1		T2 only
0	0	0		A375M only
42	82	182		Mock T + T2
2	32	24		Mock T + A375M
58	249	1432		CAR T + T2
1782	3234	10194	no inhibitor	CAR T + A375M
0	7	1		T2 only
0	0	1		A375M only
36	105	226		Mock T + T2
2	31	25		Mock T + A375M
38	359	1537		CAR T + T2
1758	3460	10574	DMSO	CAR T + A375M
0	4	0		T2 only
0	0	1		A375M only
1	20	9		Mock T + T2
0	11	6	Vem	Mock T +

				A375M
4	110	344		CAR T + T2
214	960	1782		CAR T + A375M
0	2	2		T2 only
0	1	2		A375M only
16	55	342		Mock T + T2
1	11	13		Mock T + A375M
18	131	1530		CAR T + T2
1462	2583	11224	Dabra	CAR T + A375M
0	1	0		T2 only
0	0	2		A375M only
10	8	82		Mock T + T2
0	2	5		Mock T + A375M
6	20	255		CAR T + T2
417	493	3966	Cobi	CAR T + A375M
0	1	1		T2 only
0	1	2		A375M only
17	13	100		Mock T + T2
1	6	8		Mock T + A375M
14	35	355		CAR T + T2
791	889	5421	Tram	CAR T + A375M
0	1	0		T2 only
0	0	0		A375M only
1	2	18		Mock T + T2
0	1	4		Mock T + A375M
1	15	144		CAR T + T2
357	305	2072	V+C	CAR T + A375M
0	1	0		T2 only
0	0	1		A375M only
8	9	215		Mock T + T2
0	3	9		Mock T + A375M
4	23	523		CAR T + T2
874	1073	6146	D+T	CAR T + A375M

¹ Cytokine concentrations were determined by Cytometric Bead Arrays. See legend to figure 3 and materials and methods section for details.

Table S6. *p*-values of IL-2 secretion (paired students *t*-test) ¹.

inhibitor	CAR-T cells on T2 vs CAR-T cells on A375M	CAR-T cells on A375M DMSO vs inhibitor ²	CAR-T cells on A375M ³
no inhibitor	*		ns
DMSO control	*		
Vem	*	*	*
Dabra	*	**	
Cobi	*	**	*
Tram	*	**	
Vem & Cobi	ns	**	ns
Dabra & Tram	ns	*	

¹ The table depicts the results of statistical analyses related to Figure 3a. Secretion of IL-2 by CAR-transfected T cells after stimulation was compared. *p*-values were calculated using the paired Student's *t* test: (*p*>0.1: ns, *p*≤0.05: *, *p*≤0.01: **).² Comparison between the indicated inhibitor and the DMSO solvent control. ³ Comparison between each 2 corresponding inhibitors i.e. no inhibitor vs DMSO control, Vem vs Dabra, Cobi vs. Tram, and Vem & Cobi vs. Dabra & Tram.

Table S7. *p*-values of TNF secretion (paired students *t*-test) ¹.

inhibitor	CAR-T cells on T2 vs CAR-T cells on A375M	CAR-T cells on A375M DMSO vs inhibitor ²	CAR-T cells on A375M ³
no inhibitor	*		ns
DMSO control	*		
Vem	*	*	*
Dabra	*	**	
Cobi	0.07	**	**
Tram	**	*	
Vem & Cobi	0.09	**	ns
Dabra & Tram	0.09	*	

¹ The table depicts the results of statistical analyses related to Figure 3b. Secretion of TNF by CAR-transfected T cells after stimulation was compared. *p*-values were calculated using the paired Student's *t* test: (*p*>0.1: ns, *p*≤0.05: *, *p*≤0.01: **; *p*-values >0.05 but ≤0.1 are indicated as numbers). ² Comparison between the indicated inhibitor and the DMSO solvent control. ³ Comparison between each 2 corresponding inhibitors i.e. no inhibitor vs DMSO control, Vem vs Dabra, Cobi vs. Tram, and Vem & Cobi vs. Dabra & Tram.

Table S8. *p*-values of IFN γ secretion (paired students *t*-test) ¹.

inhibitor	CAR-T cells on T2 vs CAR-T cells on A375M	CAR-T cells on A375M DMSO vs inhibitor ²	CAR-T cells on A375M ³
no inhibitor	**		ns
DMSO control	**		
Vem	*	**	**
Dabra	**	ns	
Cobi	**	***	*
Tram	**	**	
Vem & Cobi	0.10	**	*
Dabra & Tram	*	0.09	

¹ The table depicts the results of statistical analyses related to Figure 3c. Secretion of IFN γ by CAR-transfected T cells after stimulation was compared. *p*-values were calculated using the paired Student's *t* test: (*p*>0.1: ns, *p*≤0.05: *****, *p*≤0.01: ******, *p*≤0.001: *******; *p*-values >0.05 but ≤0.1 are indicated as numbers). ² Comparison between the indicated inhibitor and the DMSO solvent control. ³ Comparison between each 2 corresponding inhibitors i.e. no inhibitor vs DMSO control, Vem vs Dabra, Cobi vs. Tram, and Vem & Cobi vs. Dabra & Tram.

Table S9. Original data used for Figure 4 ¹.

HD1			
IL-6	IL-10		
pg/ml	pg/ml		
1	13	no inhibitor	T2 only
1	3		A375M only
1	11		Mock T + T2
3	3		Mock T + A375M
2	13		CAR T + T2
13	7		CAR T + A375M
1	12	DMSO	T2 only
0	3		A375M only
2	9		Mock T + T2
1	2		Mock T + A375M
1	10		CAR T + T2
16	7		CAR T + A375M
1	5	Vem	T2 only
1	0		A375M only
1	6		Mock T + T2
1	0		Mock T + A375M
1	6		CAR T + T2
4	1		CAR T + A375M
		Dabra	

1	11		T2 only
1	1		A375M only
7	11		Mock T + T2
1	1		Mock T + A375M
6	11		CAR T + T2
538	4		CAR T + A375M
0	4		T2 only
0	0		A375M only
1	4		Mock T + T2
1	1		Mock T + A375M
1	4		CAR T + T2
63	1	Cobi	CAR T + A375M
1	4		T2 only
1	1		A375M only
0	5		Mock T + T2
1	1		Mock T + A375M
1	4		CAR T + T2
72	1	Tram	CAR T + A375M
1	3		T2 only
0	1		A375M only
0	3		Mock T + T2
0	0		Mock T + A375M
0	3		CAR T + T2
2	1	V+C	CAR T + A375M
1	4		T2 only
1	0		A375M only
1	4		Mock T + T2
1	1		Mock T + A375M
1	4		CAR T + T2
231	2	D+T	CAR T + A375M
HD2			
IL-6	IL-10		
pg/ml	pg/ml		
0	18		T2 only
0	3		A375M only
1	21		Mock T + T2
0	3		Mock T + A375M
4	20		CAR T + T2
30	40	no inhibitor	CAR T + A375M
1	23	DMSO	T2 only

0	2		A375M only
1	17		Mock T + T2
0	2		Mock T + A375M
4	16		CAR T + T2
28	29		CAR T + A375M
		Vem	
0	5		T2 only
-1	0		A375M only
0	6		Mock T + T2
0	1		Mock T + A375M
1	8		CAR T + T2
3	1		CAR T + A375M
		Dabra	
3	20		T2 only
0	1		A375M only
5	19		Mock T + T2
1	0		Mock T + A375M
9	16		CAR T + T2
903	14		CAR T + A375M
		Cobi	
1	7		T2 only
0	0		A375M only
0	5		Mock T + T2
0	0		Mock T + A375M
2	6		CAR T + T2
204	4		CAR T + A375M
		Tram	
0	10		T2 only
0	0		A375M only
0	7		Mock T + T2
0	0		Mock T + A375M
1	8		CAR T + T2
141	5		CAR T + A375M
		V+C	
0	4		T2 only
-1	0		A375M only
0	3		Mock T + T2
-1	0		Mock T + A375M
1	4		CAR T + T2
9	1		CAR T + A375M
		D+T	
0	6		T2 only
0	1		A375M only
1	4		Mock T + T2
0	1		Mock T + A375M
3	6		CAR T + T2

618	3		CAR T + A375M
HD3			
IL-6	IL-10		
pg/ml	pg/ml		
0	23	no inhibitor	T2 only
1	3		A375M only
1	19		Mock T + T2
2	3		Mock T + A375M
2	22		CAR T + T2
19	27		CAR T + A375M
1	21	DMSO	T2 only
0	2		A375M only
1	20		Mock T + T2
1	2		Mock T + A375M
1	19		CAR T + T2
16	21		CAR T + A375M
0	5	Vem	T2 only
1	0		A375M only
0	6		Mock T + T2
0	1		Mock T + A375M
1	7		CAR T + T2
3	2		CAR T + A375M
1	20	Dabra	T2 only
1	0		A375M only
4	17		Mock T + T2
16	1		Mock T + A375M
3	17		CAR T + T2
1052	8		CAR T + A375M
0	5	Cobi	T2 only
1	1		A375M only
0	5		Mock T + T2
1	1		Mock T + A375M
1	5		CAR T + T2
79	2		CAR T + A375M
1	10	Tram	T2 only
1	2		A375M only
1	9		Mock T + T2
0	1		Mock T + A375M
1	10		CAR T + T2
90	7		CAR T + A375M

1	4		T2 only
1	1		A375M only
0	3		Mock T + T2
1	0		Mock T + A375M
1	3		CAR T + T2
2	0		CAR T + A375M
0	5		T2 only
1	1		A375M only
1	5		Mock T + T2
2	1		Mock T + A375M
2	5		CAR T + T2
547	2	D+T	CAR T + A375M
HD4			
IL-6	IL-10		
pg/ml	pg/ml		
0	2		T2 only
0	1		A375M only
1	1		Mock T + T2
0	0		Mock T + A375M
1	3		CAR T + T2
58	70	no inhibitor	CAR T + A375M
0	2		T2 only
1	0		A375M only
1	1		Mock T + T2
0	0		Mock T + A375M
0	3		CAR T + T2
62	79	DMSO	CAR T + A375M
0	1		T2 only
1	1		A375M only
1	1		Mock T + T2
0	0		Mock T + A375M
0	1		CAR T + T2
1	0	Vem	CAR T + A375M
0	2		T2 only
1	0		A375M only
6	1		Mock T + T2
1	0		Mock T + A375M
1	2		CAR T + T2
2895	39	Dabra	CAR T + A375M
0	1		T2 only
1	0	Cobi	A375M only

0	1		Mock T + T2
0	0		Mock T + A375M
1	1		CAR T + T2
469	1		CAR T + A375M
		Tram	
0	1		T2 only
0	1		A375M only
1	1		Mock T + T2
0	0		Mock T + A375M
0	1		CAR T + T2
497	5		CAR T + A375M
		V+C	
0	1		T2 only
-1	0		A375M only
0	1		Mock T + T2
1	0		Mock T + A375M
1	1		CAR T + T2
21	0		CAR T + A375M
0	0		D+T
0	0		
0	1	T2 only	
1	0	A375M only	
5	1	Mock T + T2	
2	0	Mock T + A375M	
1	1	CAR T + T2	
2128	30	CAR T + A375M	
n=4			
IL-6	IL-10		
pg/ml	pg/ml		
		no inhibitor	
0	14		T2 only
1	2		A375M only
1	13		Mock T + T2
1	2		Mock T + A375M
2	14		CAR T + T2
30	36		CAR T + A375M
		DMSO	
1	15		T2 only
0	2		A375M only
1	12		Mock T + T2
1	2		Mock T + A375M
2	12		CAR T + T2
30	34		CAR T + A375M
		Vem	
0	4		T2 only
1	1		A375M only

0	4		Mock T + T2
0	0		Mock T + A375M
1	6		CAR T + T2
3	1		CAR T + A375M
		Dabra	
1	13		T2 only
1	1		A375M only
5	12		Mock T + T2
5	0		Mock T + A375M
5	12		CAR T + T2
1347	16		CAR T + A375M
		Cobi	
0	4		T2 only
1	1		A375M only
0	4		Mock T + T2
1	1		Mock T + A375M
1	4		CAR T + T2
204	2		CAR T + A375M
		Tram	
0	6		T2 only
0	1		A375M only
1	5		Mock T + T2
0	1		Mock T + A375M
1	6		CAR T + T2
200	5		CAR T + A375M
		V+C	
0	3		T2 only
0	0		A375M only
0	3		Mock T + T2
0	0		Mock T + A375M
0	3		CAR T + T2
8	1		CAR T + A375M
		D+T	
0	4		T2 only
1	0		A375M only
2	4		Mock T + T2
1	1		Mock T + A375M
2	4		CAR T + T2
881	10		CAR T + A375M

¹Cytokine concentrations were determined by Cytometric Bead Arrays. See legend to figure 3 and materials and methods section for details.

Table S10. *p*-values of IL-6 secretion (paired students *t*-test) ¹.

inhibitor	CAR-T cells on T2 vs CAR-T cells on A375M	CAR-T cells on A375M DMSO vs inhibitor ²	CAR-T cells on A375M ³
no inhibitor	0.073		ns
DMSO control	0.081		
Vem	*	0.09	0.08
Dabra	0.08	0.08	
Cobi	ns	ns	ns
Tram	ns	ns	
Vem & Cobi	ns	*	ns
Dabra & Tram	ns	ns	

¹ The table depicts the results of statistical analyses related to Figure 4a. Secretion of IL-6 by CAR-transfected T cells after stimulation was compared. *p*-values were calculated using the paired Student's *t* test: (*p*>0.1: ns, *p*≤0.05: *; *p*-values >0.05 but ≤0.1 are indicated as numbers). ² Comparison between the indicated inhibitor and the DMSO solvent control. ³ Comparison between each 2 corresponding inhibitors i.e. no inhibitor vs DMSO control, Vem vs Dabra, Cobi vs. Tram, and Vem & Cobi vs. Dabra & Tram.

Table S11. *p*-values of IL-10 secretion (paired students *t*-test) ¹.

inhibitor	CAR-T cells on T2 vs CAR-T cells on A375M	CAR-T cells on A375M DMSO vs inhibitor ²	CAR-T cells on A375M ³
no inhibitor	ns		ns
DMSO control	ns		
Vem	*	ns	ns
Dabra	ns	ns	
Cobi	0.07	ns	ns
Tram	ns	ns	
Vem & Cobi	*	ns	ns
Dabra & Tram	ns	0.07	

¹ The table depicts the results of statistical analyses related to Figure 4b. Secretion of IL-10 by CAR-transfected T cells after stimulation was compared. *p*-values were calculated using the paired Student's *t* test: (*p*>0.1: ns, *p*≤0.05: *, *p*≤0.01; *p*-values >0.05 but ≤0.1 are indicated as numbers). ² Comparison between the indicated inhibitor and the DMSO solvent control. ³ Comparison between each 2 corresponding inhibitors i.e. no inhibitor vs DMSO control, Vem vs Dabra, Cobi vs. Tram, and Vem & Cobi vs. Dabra & Tram.

Table S12. Original data used for figure 5 ¹.

HD 1				
relative lysis				
	T2	54:1	18:1	6:1
no inhibitor	Mock T	1.45	-1.04	0.21

DMSO		0.93	-2.59	-0.10
Vem		-0.21	0.21	-0.62
Dabra		-1.14	0.31	-1.14
Cobi		-0.10	-0.72	-0.47
Tram		0.10	0.31	0.10
V+C		-1.76	0.47	0.10
D+T		-1.14	-0.72	1.35
	A375M	54:1	18:1	6:1
no inhibitor	Mock T	-2.56	-2.31	-2.25
DMSO		-1.85	-2.31	-3.17
Vem		-2.34	-2.50	-2.50
Dabra		-1.73	-1.85	-2.37
Cobi		-1.60	-2.07	-2.31
Tram		-2.40	-1.51	-3.27
V+C		-2.82	-2.96	-2.34
D+T		-2.64	-2.22	-2.43
	T2	54:1	18:1	6:1
no inhibitor	CAR T	-0.88	-1.81	-0.62
DMSO		-0.31	-2.54	-1.24
Vem		-2.33	-2.33	-1.45
Dabra		0.62	-1.60	-2.74
Cobi		-1.92	-2.12	-1.14
Tram		-1.19	-2.28	-2.17
V+C		-0.78	-1.24	-2.80
D+T		-1.92	-2.69	-2.07
	A375M	54:1	18:1	6:1
no inhibitor	CAR T	7.55	4.07	2.16
DMSO		8.60	4.96	3.76
Vem		9.12	6.50	2.87
Dabra		9.12	7.34	3.21
Cobi		3.61	1.08	-0.34
Tram		9.40	6.47	2.28
V+C		4.28	1.48	-0.34
D+T		7.40	4.35	1.11
HD 2				
relative lysis				
	T2	54:1	18:1	6:1
no inhibitor	Mock T	0.26	-1.39	0.69
DMSO		-1.65	-2.82	3.03
Vem		-2.69	-2.69	-0.74
Dabra		-1.56	0.17	0.56
Cobi		-0.35	-1.91	-2.56
Tram		-2.08	-1.78	3.94
V+C		-0.35	-2.95	2.64

D+T		-1.52	-2.69	1.73
	A375M	54:1	18:1	6:1
no inhibitor	Mock T	-0.76	-0.35	-1.06
DMSO		0.40	0.76	0.45
Vem		0.30	0.10	-0.86
Dabra		0.71	0.20	-0.76
Cobi		-0.45	-0.05	-0.91
Tram		-1.06	-0.81	-0.50
V+C		-0.86	0.00	-1.01
D+T			0.61	-1.51
	T2	54:1	18:1	6:1
no inhibitor	CAR T	0.26	-1.56	0.56
DMSO		-1.30	-1.91	-0.87
Vem		0.09	-1.21	-1.00
Dabra		-0.78	-0.61	-2.17
Cobi		-2.08	-1.65	-2.17
Tram		-0.43	-0.69	0.17
V+C		-2.77	-1.99	-2.69
D+T			-1.47	-1.91
	A375M	54:1	18:1	6:1
no inhibitor	CAR T	7.82	3.18	0.71
DMSO		7.52	2.88	0.81
Vem		7.02	3.43	-1.31
Dabra		8.58	3.84	-0.15
Cobi		2.78	0.86	-1.21
Tram		8.13	2.68	-0.86
V+C		2.37	-0.71	-1.72
D+T			6.51	1.16
HD 3				
relative lysis				
	T2	54:1	18:1	6:1
no inhibitor	Mock T	-0.90	-1.33	-1.06
DMSO		1.39	-1.59	-2.06
Vem		-1.06	-2.06	-2.72
Dabra		-0.73	-1.79	-1.79
Cobi		-2.92	-2.46	-3.32
Tram		-1.59	-1.73	-1.39
V+C		-1.79	-2.19	-2.72
D+T			-1.79	-1.59
	A375M	54:1	18:1	6:1
no inhibitor	Mock T	5.59	5.93	4.30
DMSO		2.59	4.22	0.11

Vem		1.31	-1.18	1.82
Dabra		2.08	1.31	0.19
Cobi		-0.32	-2.12	-0.15
Tram		0.45	0.36	1.13
V+C		-2.29	-2.80	-0.92
D+T		3.45	1.13	0.02
	T2	54:1	18:1	6:1
no inhibitor	CAR T	6.47	-1.69	1.10
DMSO		1.79	-0.40	3.78
Vem		0.00	-2.26	-1.59
Dabra		-0.33	-1.53	-0.80
Cobi		-2.39	-2.06	-2.12
Tram		-1.92	-2.12	-2.32
V+C		-2.06	-2.26	-2.46
D+T		-0.93	-1.59	-2.46
	A375M	54:1	18:1	6:1
no inhibitor	CAR T	25.11	12.18	6.27
DMSO		23.56	12.52	5.59
Vem		15.26	5.24	2.68
Dabra		26.30	13.89	7.47
Cobi		14.92	4.39	2.68
Tram		18.00	7.73	4.39
V+C		8.00	0.28	-2.38
D+T		24.10	7.13	3.02
n=3				
relative lysis				
	T2	54:1	18:1	6:1
no inhibitor	Mock T	0.27	-1.25	-0.05
DMSO		0.23	-2.33	0.29
Vem		-1.32	-1.51	-1.36
Dabra		-1.14	-0.44	-0.79
Cobi		-1.12	-1.70	-2.11
Tram		-1.19	-1.06	0.88
V+C		-1.30	-1.56	0.01
D+T		-1.48	-1.67	0.38
	A375M	54:1	18:1	6:1
no inhibitor	Mock T	0.76	1.09	0.33
DMSO		0.38	0.89	-0.87
Vem		-0.24	-1.19	-0.51
Dabra		0.35	-0.11	-0.98
Cobi		-0.79	-1.41	-1.12
Tram		-1.00	-0.65	-0.88
V+C		-1.99	-1.92	-1.42
D+T		0.47	-0.87	-1.12

	T2	54:1	18:1	6:1
no inhibitor	CAR T	1.95	-1.69	0.35
DMSO		0.06	-1.61	0.56
Vem		-0.75	-1.93	-1.35
Dabra		-0.16	-1.25	-1.90
Cobi		-2.13	-1.94	-1.81
Tram		-1.18	-1.70	-1.44
V+C		-1.87	-1.83	-2.65
D+T		-1.44	-2.06	-2.23
	A375M	54:1	18:1	6:1
no inhibitor	CAR T	13.49	6.48	3.05
DMSO		13.23	6.79	3.38
Vem		10.47	5.06	1.41
Dabra		14.67	8.35	3.51
Cobi		7.10	2.11	0.37
Tram		11.84	5.62	1.94
V+C		4.89	0.35	-1.48
D+T		12.67	4.21	1.06

¹Cytotoxicity was determined in a Cr⁵¹-release assay. See legend to figure 4 and materials and methods section for details.

Table S13. *p*-values of cytotoxicity (paired students *t*-test) ¹.

	CAR-T cells on T2 vs CAR-T cells on A375M		CAR-T cells on A375M DMSO vs inhibitor ²		CAR-T cells on A375M ³	
inhibitor	54 : 1 ⁴	18 : 1 ⁴	54 : 1 ⁴	18 : 1 ⁴	54 : 1 ⁴	18 : 1 ⁴
no inhibitor	*	*			ns	ns
DMSO control	*	*				
Vem	**	**	ns	ns	ns	ns
Dabra	*	*	0.06	*		
Cobi	*	*	**	*	**	*
Tram	**	*	ns	ns		
Vem & Cobi	*	**	*	0.05	0.08	*
Dabra & Tram	*	*	ns	0.09		

¹ The table depicts the results of statistical analyses related to Figure 5. Lytic activity of CAR-transfected T cells against T2 and A375M was compared. *p*-values were calculated using the paired Student's *t* test: (*p*>0.1: ns, *p*≤0.05: *, *p*≤0.01: **; *p*-values >0.05 but ≤0.1 are indicated as numbers). ² Comparison between the indicated inhibitor and the DMSO solvent control. ³ Comparison between each 2 corresponding inhibitors i.e. no inhibitor vs DMSO control, Vem vs Dabra, Cobi vs. Tram, and Vem & Cobi vs. Dabra & Tram. ⁴ Effector to target ratio.