

Engineered T Cells Secreting α B7-H3- α CD3 Bispecific Engagers for Enhanced Anti-Tumor Activity Against B7-H3 Positive Multiple Myeloma: A Novel Therapeutic Approach

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Supplementary Information

Table 1. Cytokine production profiles of untransduced T cells and α B7-H3- α CD3 ENG T cells following co-culture with the with SupT-1 (B7-H3^{Neg}) cell line. Cytokine levels were assessed using the LEGENDplex™ Human CD8/NK cell panel Cytokine Bead Array (CBA), measuring 13 cytokines and proteins after a 24 hours co-culture period.

Cytokines	UTD T cells	α B7-H3- α CD3 ENG T cells	
	Mean $\pm$ SEM (pg/ml)	Mean $\pm$ SEM (pg/ml)	P*
IL-2	9.1 $\pm$ 2.2	8.5 $\pm$ 1.7	0.8220
IL-4	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.7341
IL-6	4.7 $\pm$ 0.5	32.7 $\pm$ 31.2	0.4051
IL-10	1.9 $\pm$ 0.3	2.6 $\pm$ 0.5	0.2522
IL-17A	2.3 $\pm$ 0.9	2.3 $\pm$ 0.5	0.9760
TNF-α	7.5 $\pm$ 3.6	8.4 $\pm$ 5.8	0.8933
sFas	9.0 $\pm$ 1.2	10.8 $\pm$ 2.3	0.5123
sFasL	60.9 $\pm$ 16.3	33.6 $\pm$ 11.2	0.2156
IFN-γ	926.7 $\pm$ 331.5	1363.1 $\pm$ 457.2	0.4690
Granzyme A	1329.6 $\pm$ 140.3	1838.9 $\pm$ 356.9	0.2325
Granzyme B	18582.0 $\pm$ 6110.3	25290.0 $\pm$ 5452.8	0.4439
Perforin	349.6 $\pm$ 81.1	333.6 $\pm$ 82.6	0.8947
Granulysin	2513.1 $\pm$ 635.8	2466.7 $\pm$ 990.5	0.9699

* The data were derived from 4 independent experiments (N=4), and the results are presented as mean $\pm$ standard error of the mean (SEM). Statistical significance was determined using unpaired Student's t-tests (* p <0.05, ** p <0.01, *** p <0.001, and **** p <0.0001).

Table 2. Cytokine production profiles of untransduced T cells and α B7-H3- α CD3 ENG T cells following co-culture with the NCI-H929 (B7-H3^{Low}) cell line. Cytokine levels were assessed using the LEGENDplex™ Human CD8/NK cell panel Cytokine Bead Array (CBA), measuring 13 cytokines and proteins after a 24 hours co-culture period.

Cytokines	UTD T cells	α B7-H3- α CD3 ENG T cells	
	Mean $\pm$ SEM (pg/ml)	Mean $\pm$ SEM (pg/ml)	<i>P</i> *
IL-2	2.9 $\pm$ 1.5	12.8 $\pm$ 1.0	0.0013
IL-4	0.3 $\pm$ 0.1	0.3 $\pm$ 0.0	0.6250
IL-6	5.0 $\pm$ 2.0	5.9 $\pm$ 1.8	0.7530
IL-10	6.5 $\pm$ 1.2	4.8 $\pm$ 2.2	0.5109
IL-17A	2.1 $\pm$ 0.9	2.3 $\pm$ 0.5	0.8014
TNF-α	3.4 $\pm$ 0.8	23.4 $\pm$ 4.8	0.0063
sFas	6.3 $\pm$ 0.7	11.3 $\pm$ 0.7	0.0021
sFasL	56.3 $\pm$ 6.5	43.2 $\pm$ 1.5	0.0980
IFN-γ	308.5 $\pm$ 72.1	2158.6 $\pm$ 489.3	0.0096
Granzyme A	361.0 $\pm$ 94.6	4835.6 $\pm$ 1584.1	0.0304
Granzyme B	6021.4 $\pm$ 1539.5	18352.2 $\pm$ 4268.4	0.0348
Perforin	261.7 $\pm$ 34.8	437.1 $\pm$ 27.4	0.0074
Granulysin	1065.3 $\pm$ 211.1	2795.6 $\pm$ 587.2	0.0323

* The data were gathered from 4 independent experiments (N=4), and the results are presented as mean $\pm$ standard error of the mean (SEM). Statistical significance was assessed using unpaired Student's t-tests (* p <0.05, ** p <0.01, *** p <0.001, and **** p <0.0001).

Table 3. Cytokine production profiles of untransduced T cells and α B7-H3- α CD3 ENG T cells following co-culture with the L-363 (B7-H3^{Medium}) cell line. Cytokine levels were assessed using the LEGENDplex™ Human CD8/NK cell panel Cytokine Bead Array (CBA), measuring 13 cytokines and proteins after a 24 hour co-culture period.

Cytokines	UTD T cells	α B7-H3- α CD3 ENG T cells	
	Mean $\pm$ SEM (pg/ml)	Mean $\pm$ SEM (pg/ml)	<i>P</i> *
IL-2	5.0 $\pm$ 2.8	346.8 $\pm$ 130.4	0.0395
IL-4	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	0.6922
IL-6	7.5 $\pm$ 7.0	3.7 $\pm$ 2.8	0.6370
IL-10	1.2 $\pm$ 0.3	1.3 $\pm$ 0.6	0.9224
IL-17A	2.3 $\pm$ 0.5	3.1 $\pm$ 0.9	0.4799
TNF-α	6.3 $\pm$ 3.6	30.5 $\pm$ 6.9	0.0205
sFas	7.7 $\pm$ 0.2	11.6 $\pm$ 1.0	0.0106
sFasL	85.5 $\pm$ 12.2	75.0 $\pm$ 4.7	0.4487
IFN-γ	603.4 $\pm$ 227.3	7508.0 $\pm$ 1852.6	0.0101
Granzyme A	397.9 $\pm$ 109.8	4606.3 $\pm$ 1520.0	0.0328
Granzyme B	4038.1 $\pm$ 1286.9	21877.4 $\pm$ 7174.4	0.0500
Perforin	157.6 $\pm$ 80.4	470.8 $\pm$ 43.5	0.0140
Granulysin	257.5 $\pm$ 85.1	4221.2 $\pm$ 967.3	0.0065

* The data were derived from 4 independent experiments (N=4), and the results are presented as mean $\pm$ standard error of the mean (SEM). Statistical significance was assessed using unpaired Student's t-tests (* p <0.05, ** p <0.01, *** p <0.001, and **** p <0.0001).

Table 4. Cytokine production profiles of untransduced T cells and α B7-H3- α CD3 ENG T cells, in response following co-culture with the KMS-12-PE (B7-H3^{High}) cell line. Cytokine levels were quantified using the LEGENDplex™ Human CD8/NK cell panel Cytokine Bead Array (CBA), measuring 13 cytokines and proteins after a 24 hour co-culture period.

Cytokines	UTD T cells	α B7-H3- α CD3 ENG T cells	
	Mean $\pm$ SEM (pg/ml)	Mean $\pm$ SEM (pg/ml)	<i>P</i> *
IL-2	3.0 $\pm$ 1.2	316.8 $\pm$ 127.4	0.0489
IL-4	0.4 $\pm$ 0.1	0.7 $\pm$ 0.2	0.3697
IL-6	0.6 $\pm$ 0.3	2.7 $\pm$ 1.8	0.2727
IL-10	1.2 $\pm$ 0.1	1.9 $\pm$ 0.4	0.1564
IL-17A	1.2 $\pm$ 0.3	5.4 $\pm$ 1.4	0.0252
TNF-α	3.8 $\pm$ 3.4	68.0 $\pm$ 24.0	0.0381
sFas	9.5 $\pm$ 0.6	31.2 $\pm$ 7.3	0.0245
sFasL	76.7 $\pm$ 16.6	83.0 $\pm$ 3.4	0.7245
IFN-γ	1668.3 $\pm$ 349.3	7696.0 $\pm$ 1611.3	0.0106
Granzyme A	550.7 $\pm$ 192.4	7674.5 $\pm$ 1933.8	0.0105
Granzyme B	4225.0 $\pm$ 2655.5	20740.5 $\pm$ 3581.5	0.0100
Perforin	191.4 $\pm$ 39.6	746.7 $\pm$ 104.0	0.0025
Granulysin	389.7 $\pm$ 115.9	5122.7 $\pm$ 228.7	<0.0001

* The data were collected from 4 independent experiments (N=4), and the results are presented as mean $\pm$ standard error of the mean (SEM). Statistical significance was assessed using unpaired Student's t-tests (* p <0.05, ** p <0.01, *** p <0.001, and **** p <0.0001).