Innovative bioconjugation technology for antibody-drug conjugates: proof of concept in a CD30-positive lymphoma mouse model

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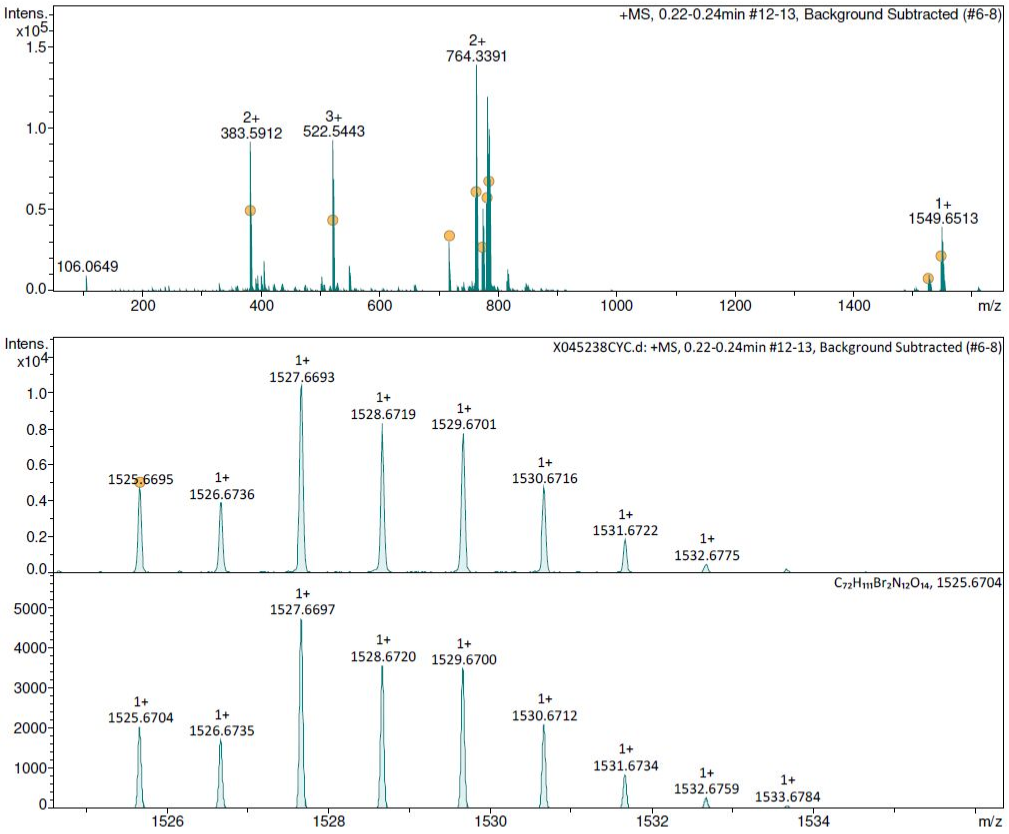
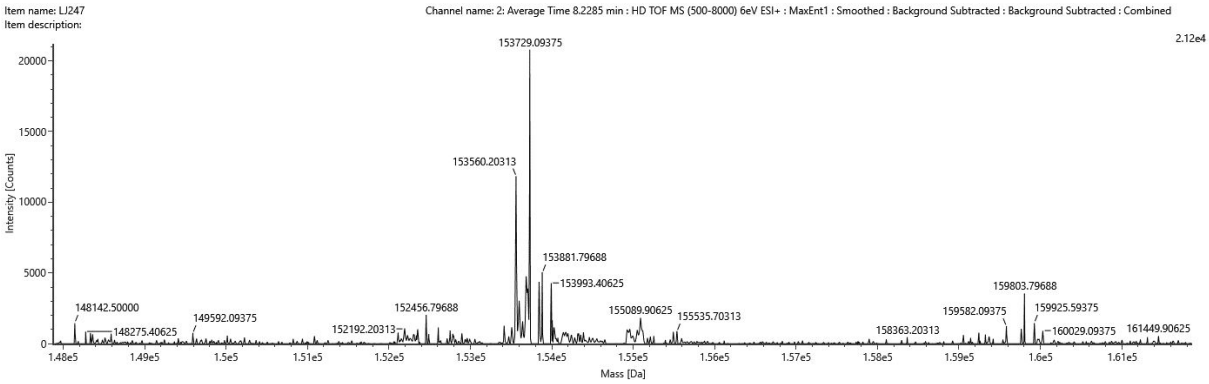


Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
106.064925	1+	1	C7H8N	106.065126	1.9	11.0	5.0	even
382.592270	2+	1	C32H45Br2N7O5	382.591897	-1.0	8.3	14.0	even
	2+	2	C33H41Br2N11O	382.592566	0.8	16.4	19.0	even
521.877882	3+	1	C72H111Br2CaN12O14	521.877297	-1.1	15.4	24.0	even
	3+	2	C76H115Br2CaN6O16	521.878192	0.6	21.5	23.0	even
	3+	3	C77H111Br2CaN10O12	521.878638	1.4	26.5	28.0	even
718.510986	1+	1	C39H68N5O7	718.511326	0.5	10.7	9.0	even
	1+	2	C38H72NO11	718.509989	-1.4	17.3	4.0	even

Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
763.339622	2+	1	C72H112Br2N12O14	763.338837	-1.0	17.2	23.0	even
	2+	2	C76H116Br2N6O16	763.340179	0.7	25.8	22.0	even
	2+	3	C77H112Br2N10O12	763.340848	1.6	32.1	27.0	even
774.329851	2+	1	C76H115Br2N6NaO16	774.331151	1.7	19.0	22.0	even
	2+	2	C72H111Br2N12NaO14	774.329809	-0.1	24.1	23.0	even
782.312829	2+	1	C77H110Br2CaN10O12	782.314319	1.9	286.7	28.0	even
	2+	2	C72H110Br2CaN12O14	782.312307	-0.7	289.0	24.0	even
	2+	3	C76H114Br2CaN6O16	782.313650	1.0	346.2	23.0	even
785.318499	2+	1	C67H110Br2N14Na2O16	785.318770	0.3	77.7	19.0	even
	2+	2	C72H110Br2N12Na2O14	785.320781	2.9	79.3	23.0	even
1525.669472	1+	1	C72H111Br2N12O14	1525.670397	0.6	19.1	23.0	even
	1+	2	C67H111Br2N14O16	1525.666374	-2.0	34.0	19.0	even
1547.652272	1+	1	C72H110Br2N12NaO14	1547.652341	0.0	18.4	23.0	even
	1+	2	C76H114Br2N6NaO16	1547.655026	1.8	22.6	22.0	even

Figure S1. Linker **7** mass spectrometry analysis.



MF-BTX-MMAE	DAR 0	DAR 1	DAR 2	DAR 3	DAR 4	DAR 5
Expected MW (Da)[1]	148083	149451	150819	152187	153555	154923
Measured MW (Da)[1]	n.o.[2]	n.o.[2]	n.o.[2]	152194	153561	154932
Measurement error (ppm)	-	-	-	-48.17	-38.8	-58.35
Area	-	-	-	77042	560913	59337
Area (%)	-	-	-	11.1	80.4	8.5
Average DAR	4.0					

[1]Only the first glycosylation peak (G0F) was considered.

[2]Not observed.

The mass increment of 1368 Da expected for the conjugated linker is confirmed.

Figure S2. MF-BTX-MMAE native mass spectrometry analysis.

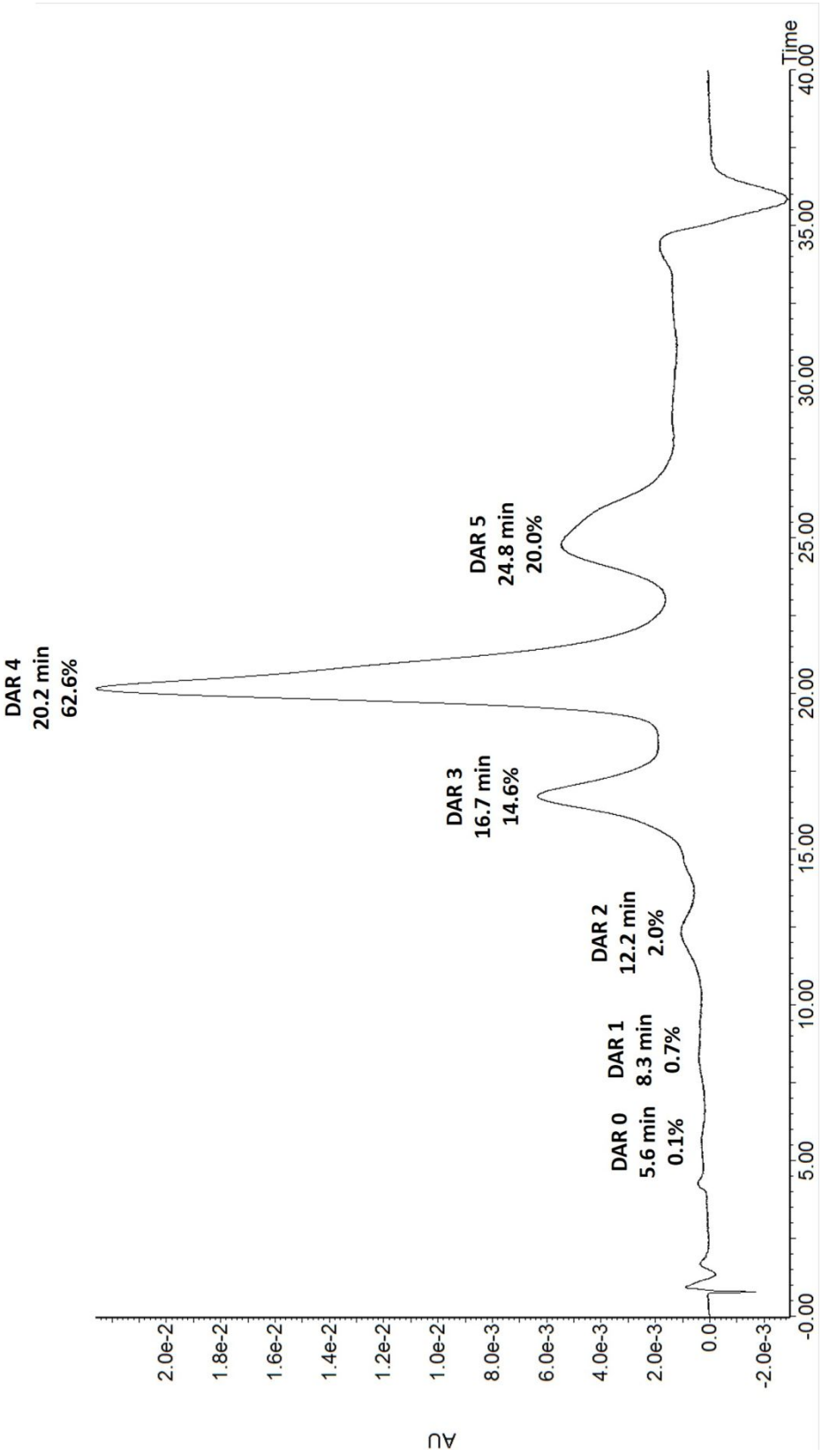
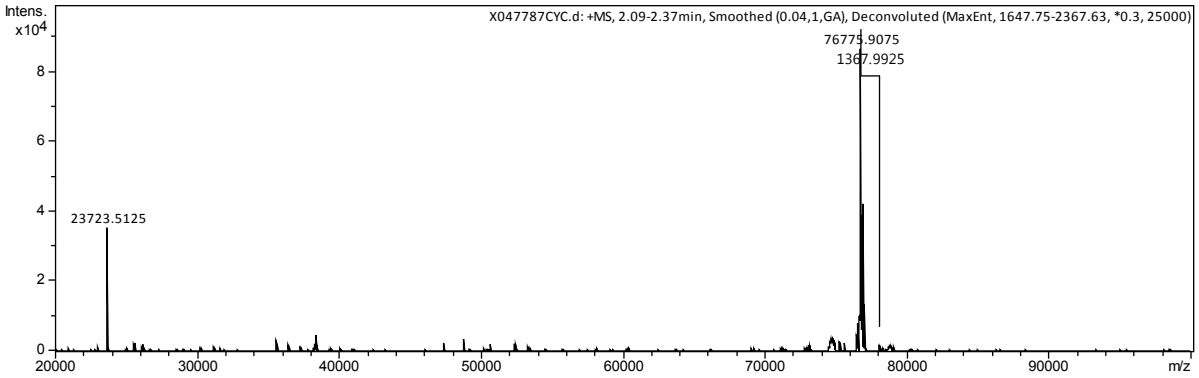
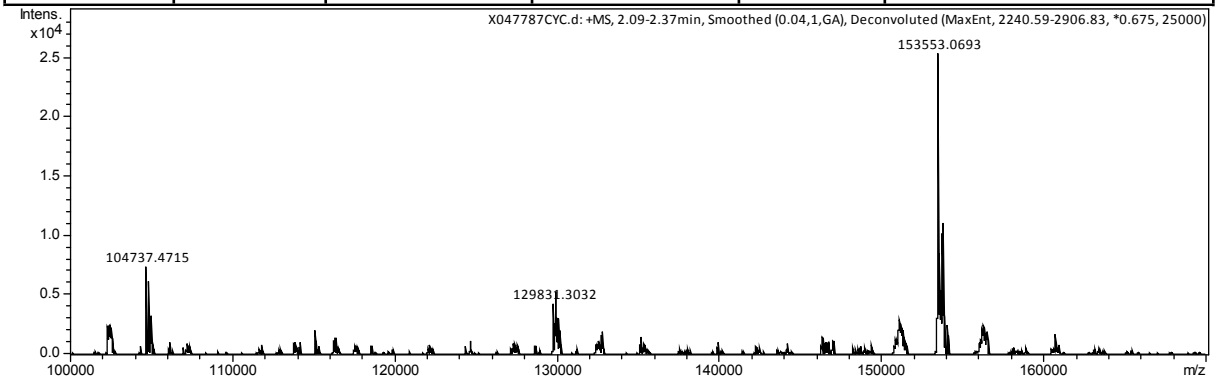


Figure S3. MF-BTX-MMAE HIC analysis.



Fragments observed	DAR observed	Expected MW (Da)[1]	Measured MW (Da)[1]	Intensity	Relative intensity (%)
L	0	23723	23723	35296	41.0
LH	2	76779	76776	86140	100.0
LH	3	78147	78144	1614	1.9



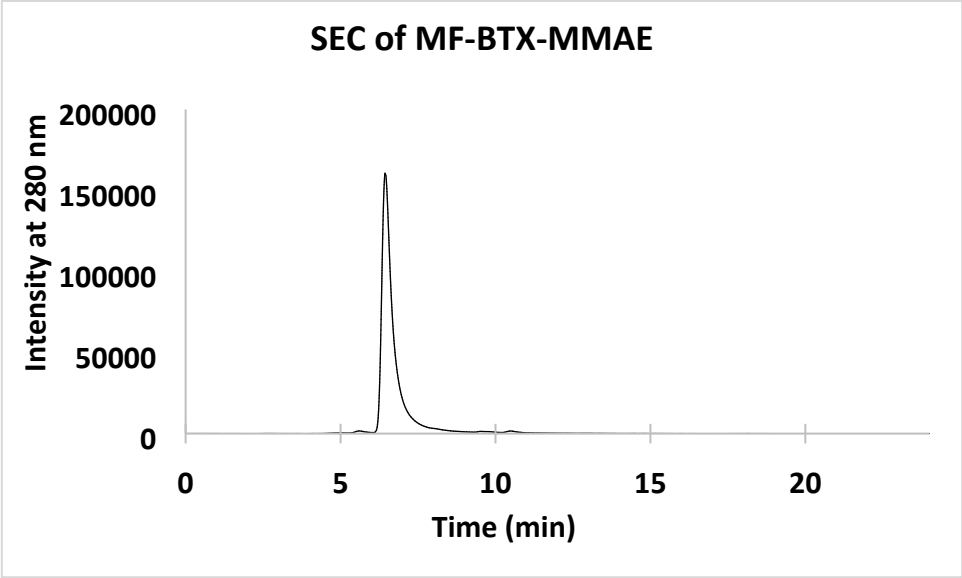
Fragments observed	DAR observed	Expected MW (Da)[1]	Measured MW (Da)[1]	Intensity	Relative intensity (%)
HH	3	104744	104737	7408	29.3
LHH	4	129832	129831	4272	16.9
LHHL	4	153555	153553	622804	100.00

Fragments	LHHL	LHH	HH	LH	H	L
Average DAR	4	4	3	2.02	-	0

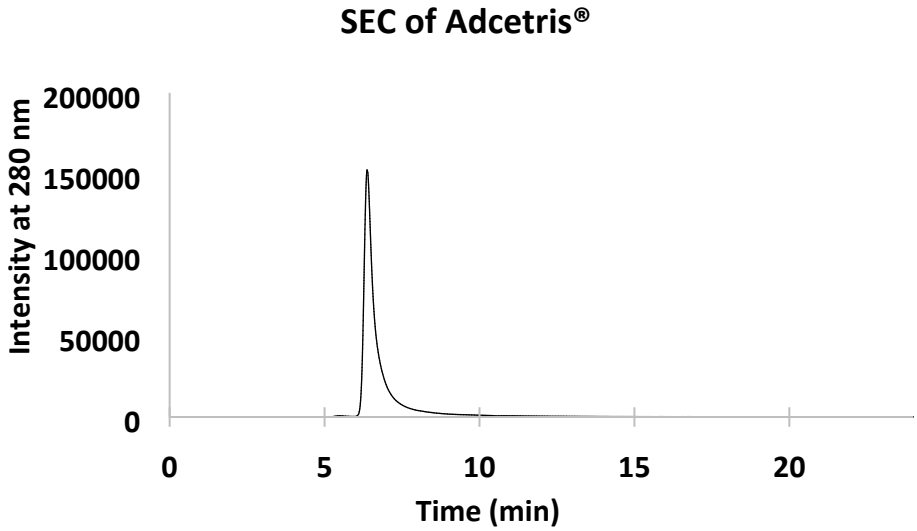
[1]Only the first glycosylation peak (G0F) was considered.

The mass increment of 1368 Da expected for the conjugated linker is confirmed.

Figure S4. MF-BTX-MMAE denaturing mass spectrometry analysis.



MF-BTX-MMAE	Aggregates	Oligomers	Monomer
Retention time(min)	2.71	5.59	6.44
Area (%)	0.05	1.4	98.55



Adcetris®	Aggregates	Oligomers	Monomer
Retention time(min)	2.77	5.49	6.38
Area (%)	0.04	0.45	99.51

Figure S5. HPLC SEC-UV analysis of **MF-BTX-MMAE** and Adcetris®.

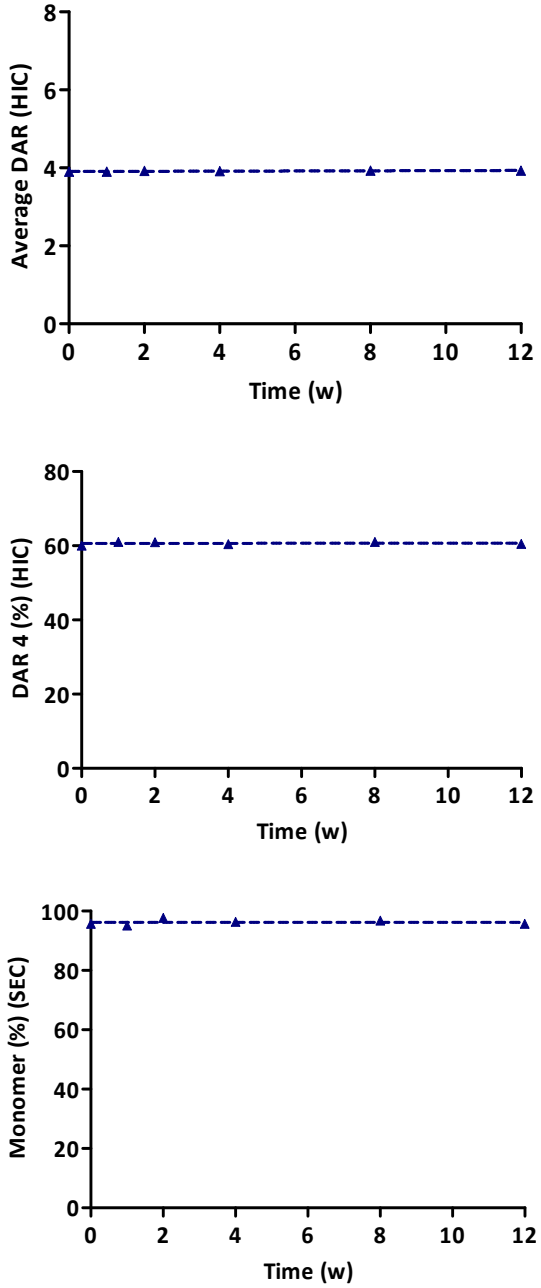


Figure S6. Stability of **MF-BTX-MMAE** upon storage at 4 °C in PBS pH 7.4 for 12 weeks (*N* = 1).

Name	Events	% of Vis	Mean	GeoMean	Median	CV
II^{ary} Ab only PE anti-human	8650	100.00	3.96	3.36	3.19	115.50
Isotype control ADC PE anti-human	8815	100.00	5.09	4.08	3.85	284.87
Adcetris® PE anti-human	8809	100.00	2829.78	2512.67	2617.99	45.10
MF-BTX-MMAE PE anti-human	8595	100.00	2693.90	2396.49	2525.48	44.85

Figure S7. Antigen-binding analysis data by FACS on Karpas-299 (CD30-positive).

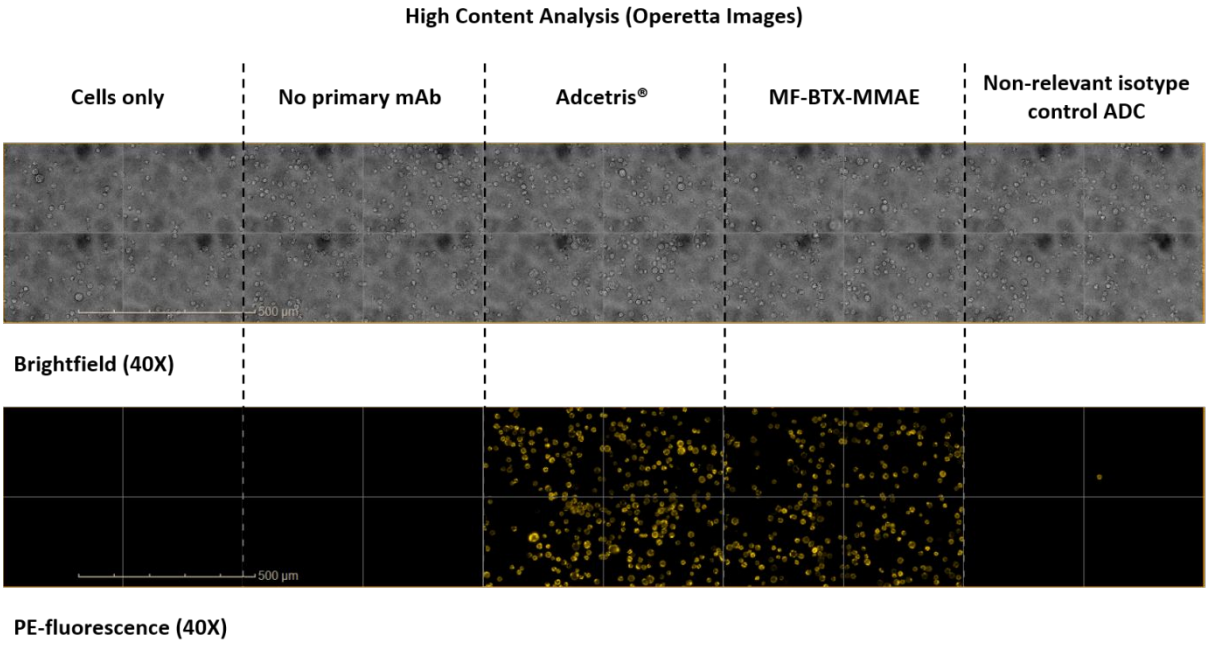


Figure S8. Antigen-binding analysis by high content analysis on Karpas-299 (CD30-positive).

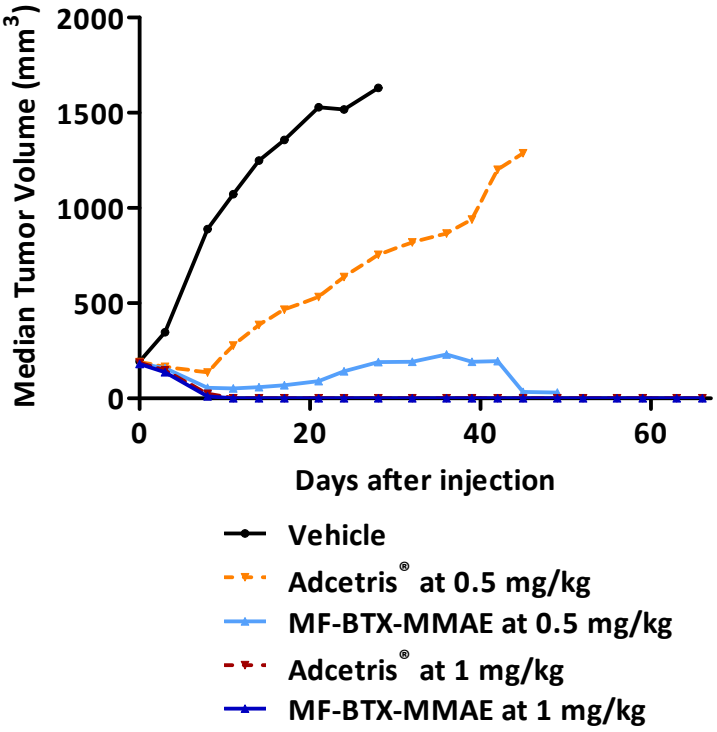


Figure S9. Median tumor volume curves of CB17-SCID mice bearing subcutaneous Karpas-299 human lymphoma tumors. Each point represents the median of the recorded tumor volume per group. Animals were randomized and treated on D0. Last mice were sacrificed on D66. Graphs are shown when at least 80% of the animals are alive.

Group	Data	D0	D3	D8	D11	D14	D17	D21	D24	D28	D32	D36
Vehicle	Median	194	347	889	1072	1250	1358	1529	1517	1631	1521	1401
	T/C%	100	100	100	100	100	100	100	100	100	100	100
Adcetris® 0.5 mg/kg	Median	191	166	135	277	385	465	532	637	755	819	865
	T/C%	98	48	15	26	31	34	35	42	46	54	62
Adcetris® 1 mg/kg	Median	187	147	24	0	0	0	0	0	0	0	0
	T/C%	96	42	3	0	0	0	0	0	0	0	0
MF-BTX-MMAE 0.5 mg/kg	Median	183	159	56	52	60	69	91	143	191	193	230
	T/C%	94	46	6	5	5	5	6	9	12	13	16
MF-BTX-MMAE 1 mg/kg	Median	181	137	10	0	0	0	0	0	0	0	0
	T/C%	93	39	1	0	0	0	0	0	0	0	0

Figure S10. Tumor growth inhibition (T/C%) results in treated CB17-SCID mice bearing subcutaneous Karpas 299 human lymphoma tumors. Animals were randomized and treated on D0. Last mice were sacrificed on D66. No further calculations were possible after D36 since there were less than four mice remaining in the vehicle group.

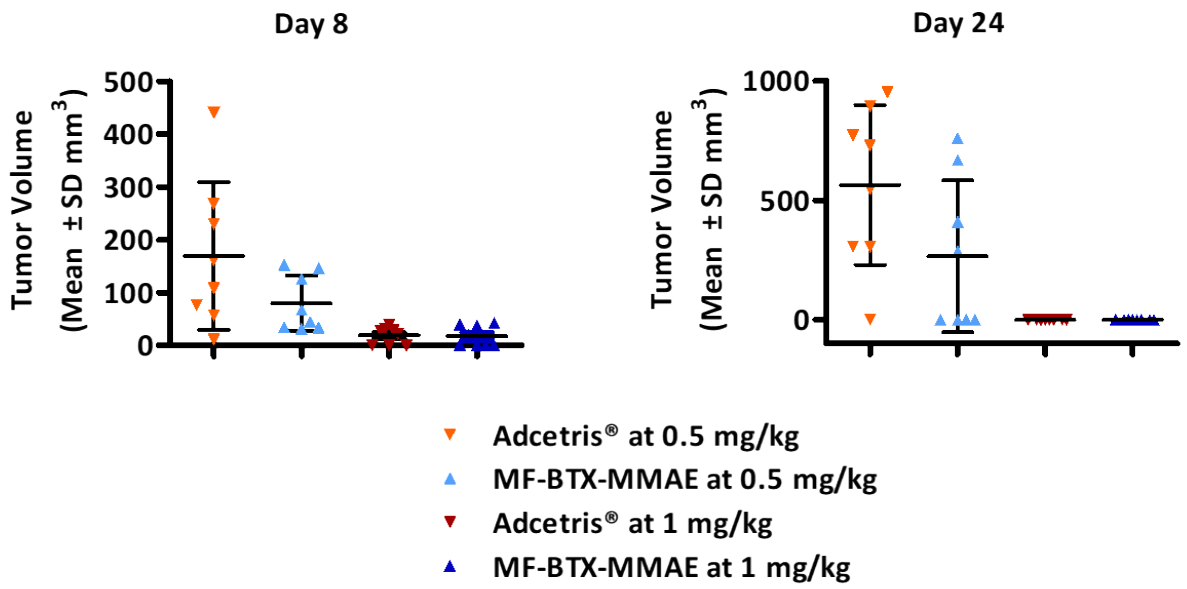


Figure S11. Individual tumor volumes of CB17-SCID mice bearing subcutaneous Karpas 299 human lymphoma tumors on D8 and D24. Animals were randomized and treated on D0. Last mice were sacrificed on D66.

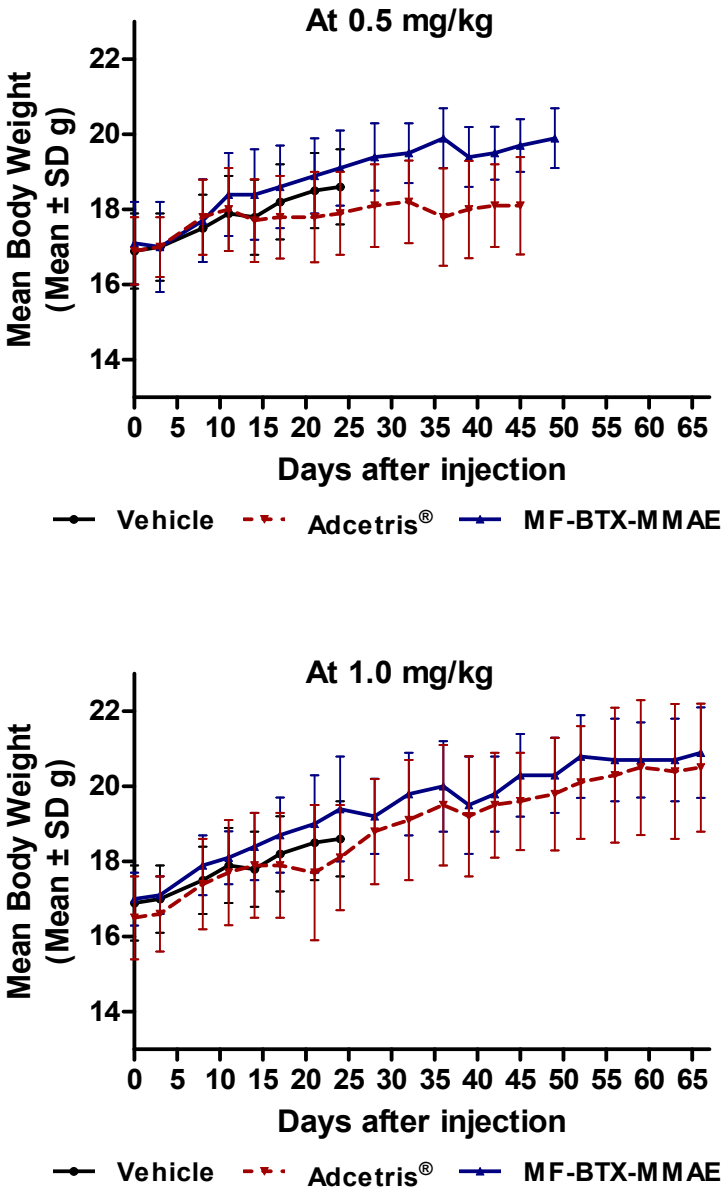
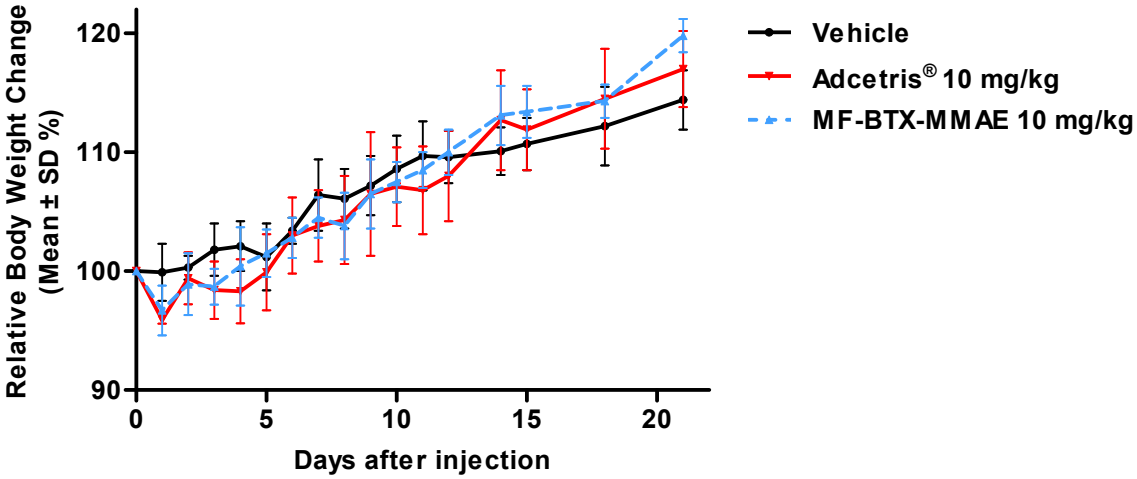
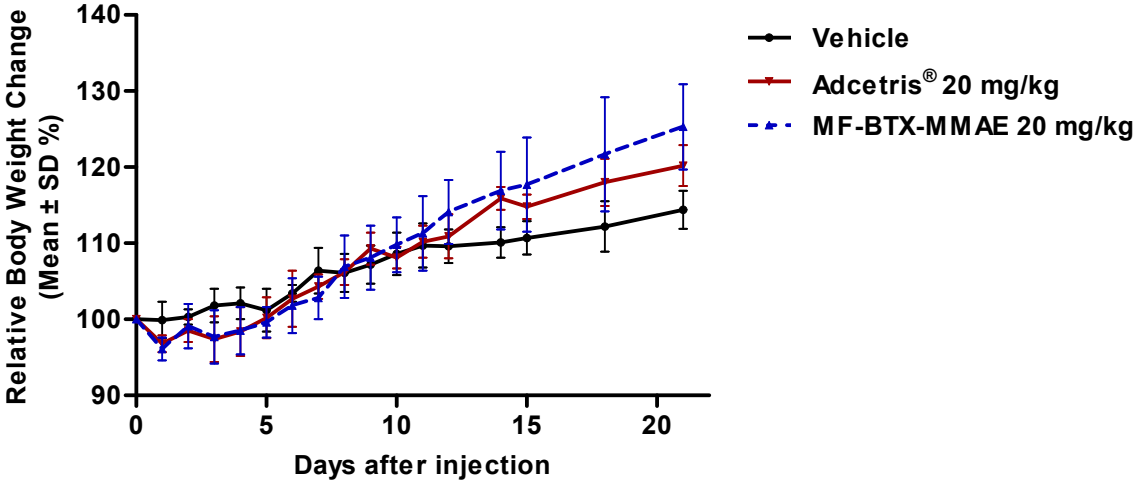
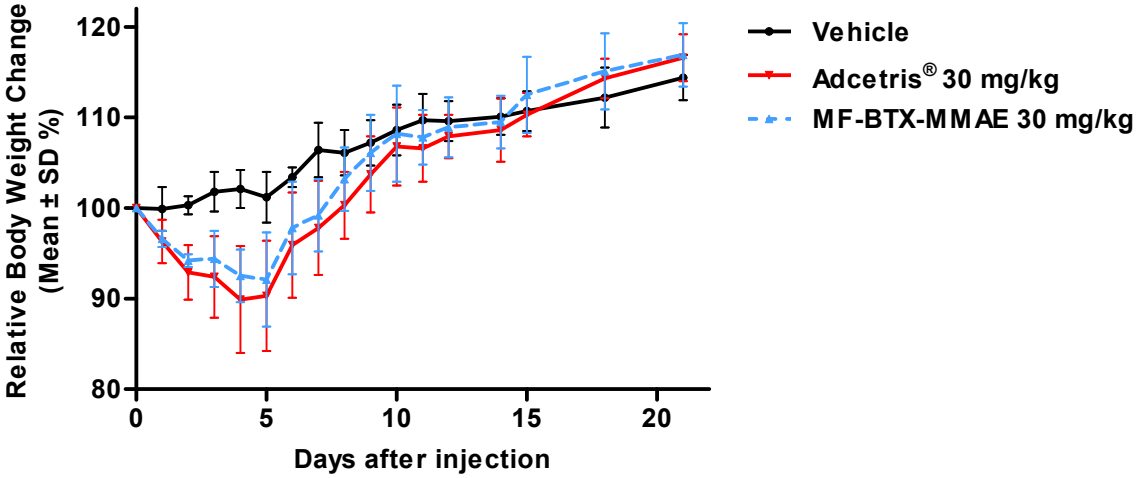


Figure S12. Mean body weight curves of CB17-SCID mice bearing subcutaneous Karpas 299 human lymphoma tumors treated with a single dose on day 0 either with vehicle, Adcetris® or **MF-BTX-MMAE** at 0,5 and 1 mg/kg. Each point represents the mean of the recorded body weight per group. Graphs are shown when more than 80% of the animals are alive. Data are mean ± SD, *N*=16 for vehicle and *N*=8 for other groups.


Relative Body Weight Change
(Mean ± SD %)
120
110
100
90
0
5
10
15
20
Days after injection
Vehicle
Adcetris® 10 mg/kg
MF-BTX-MMAE 10 mg/kg


Relative Body Weight Change
(Mean ± SD %)
140
130
120
110
100
90
0
5
10
15
20
Days after injection
Vehicle
Adcetris® 20 mg/kg
MF-BTX-MMAE 20 mg/kg


Relative Body Weight Change
(Mean ± SD %)
120
110
100
90
80
0
5
10
15
20
Days after injection
Vehicle
Adcetris® 30 mg/kg
MF-BTX-MMAE 30 mg/kg

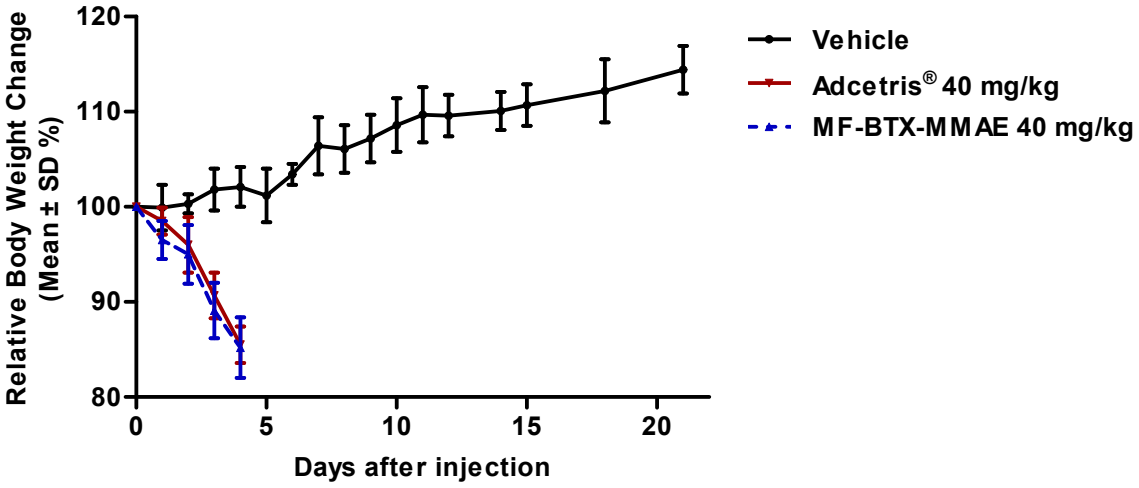


Figure S13. Relative body weight change curves of Sprague-Dawley rats treated with a single dose on day 0 either with vehicle, Adcetris® or **MF-BTX-MMAE** at 10, 20, 30 and 40 mg/kg. Each point represents the mean of the recorded relative body weight change per group. Data are mean ± SD, *N*=3 for vehicle, N=9 for **MF-BTX-MMAE** at 40 mg/kg and *N*=5 for other groups.