

Supplemental

Exposure-Efficacy Analysis of Antibody-Drug Conjugates Delivering an Excessive Level of Payload to Tissues

Donglu Zhang, Peter S Dragovich, Shang-Fan Yu, Yong Ma, Thomas H Pillow, Jack D Sadowsky, Dian Su, Wei Wang, Andrew Polson, S Cyrus Khojasteh, Cornelis ECA Hop

Affiliation: Drug Metabolism & Pharmacokinetics (DZ, YM, SCK, DS, WW, CECAH), Discovery Chemistry (THP, PSD), Translational Oncology (SY, AP), Protein Chemistry (JDS), Genentech, South San Francisco, CA 94080, USA.

Supplemental contains:

Table S1. Quantitation of MMAE, and DMx (DM1, DM3, and DM4) and associated methylated metabolites (methylated DMx, DM-Me) in tumors of xenograft mice following intravenously administration of corresponding ADC conjugates

Table S2. Quantitation of total antibody (Tab) in plasma of xenograft mice following intravenously administration of corresponding ADC conjugates

Figure S1. Correlation (X-Y plots) of tumor growth inhibition with intratumor DMx (DM+DM-Me) in mice bearing human CD22-expressing BJAB.Luc xenografts

Figure S2. Body weights from *In vivo* efficacy studies of PBD-, MMAE-, and DMx-ADCs in mice bearing human HER2-expressing Fo5 or human CD22-expressing BJAB.Luc xenografts (n = 8) after intravenous administration of corresponding ADCs A1-A4 (I), B1-B4 (II), C1-C8 (III), and D1-3 (IV, V, VI)

Figure S3. Correlation analysis of tumor growth inhibition with intratumor MMAE concentrations from Conjugates B1-B4 in mice bearing human CD22-expressing BJAB.Luc xenografts

Figure S4. Correlation analysis of tumor growth inhibition with intratumor DMx-Me concentrations from Conjugates C1-C8 in mice bearing human CD22-expressing BJAB.Luc xenografts

Table S1. Quantitation of MMAE and DMx (DM1, DM3, and DM4) and associated methylated metabolites (methylated DMx) in tumors of xenograft mice following intravenously administration of corresponding ADC conjugates

A

Conjugate	ADC name	MMAE Concentrations (nM) at Day 4
		Day 4
B1_1	CD22-Me-SS-MMAE	3.30±0.9
B1	CD22-Me-SS-MMAE	19.1±0.1
B2	CD22-Me-SS-PAB-MMAE	87.1±8.3
B3	CD22-DiMe-SS-MMAE	42.1±1.2
B4	CD22-Val-Cit-PAB-MMAE	55.6±13.3

B

Conjugate	ADC name	DMx Concentrations (nM) at Day 21	
		Methylated DMx	DMx thiol
C1	CD22-SS-DM1	2.5±0.6	68.4±11.2
C2	CD22-SS-DM3	43.4±14.6	24.8±3.1
C3	CD22-MPEO-DM1	<LLOQ	7.3±1.9
C4	CD22-SPDB-DM4	5.4±2.9	<LLOQ
C5	CD22-MBT-DM4	3.4±0.6	<LLOQ
C6	CD22-SS-DM4	53.6±16.0	15.0±0.0
C7	CD22-Fc-SS-DM4	13.5±2.9	7.8±1.8
C8	CD22-Fc-SS-DM3	4.3±1.9	<LLOQ

Table S2. Quantitation of total antibody (Tab) in plasma of xenograft mice following intravenously administration of corresponding ADC conjugates in mice bearing human human CD22-expressing BJAB.Luc xenografts

A

Conjugate	ADC name	Tab (µg/mL)			AUC _{0-7 day} µg/mL•day
		Day 1	Day 4	Day 7	
B1	CD22-Me-SS-MMAE	229±11.3	312±10.2	232±7.59	1746±404
B2	CD22-Me-SS-PAB-MMAE	193±10.2	140±56.8	125±107	1072±226
B3	CD22-DiMe-SS-MMAE	134±17.8	122±2.57	115±21.6	809±176
B4	CD22-Val-Cit-PAB-MMAE	10.8±2.57	5.87±1.20	6.30±2.35	49.0±10.0

B

Conjugate	ADC name	Tab (µg/mL)			AUC _{0-7 day} µg/mL•day
		Day 1	Day 3	Day 7	
C1	CD22-SS-DM1	13.9±3.02	15.4±0.47	11.2±0.06	103±167
C2	CD22-SS-DM3	11.7±0.28	14.6±1.56	11.7±1.65	95.7±17.9
C3	CD22-MPEO-DM1	15.5±3.30	14.0±2.50	12.1±1.00	107±15.9
C4	CD22-SPDB-DM4	7.91±0.31	6.63±0.59	4.63±0.45	48.9±5.54
C5	CD22-MBT-DM4	13.1±0.21	10.9±0.07	6.80±0.34	79.1±8.20
C6	CD22-SS-DM4	13.9±0.32	13.5±0.56	8.86±0.78	93.1±12.3
C7	CD22-Fc-SS-DM4	15.6±0.30	11.7±1.13	13.1±0.54	100±14.1
C8	CD22-Fc-SS-DM3	13.8±0.28	10.7±0.60	10.1±0.21	86.4±11.0

Figure S1. Correlation (X-Y plots) of tumor growth inhibition with intratumor concentrations of DMx and methylated DMx in mice bearing human CD22-expressing BJAB.Luc xenografts

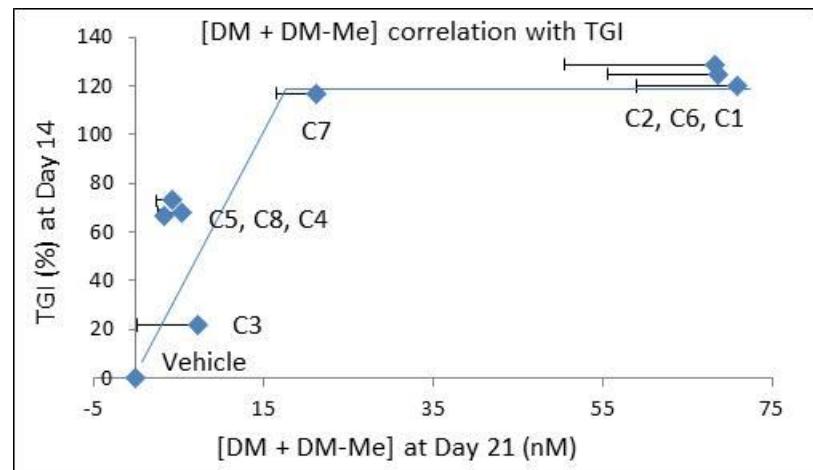


Figure S2. Body weights from *In vivo* efficacy studies of PBD-, MMAE-, and DMx-ADCs in mice bearing human HER2-expressing Fo5 or human CD22-expressing BJAB.Luc xenografts ($n = 8$) after intravenous administration of corresponding ADCs A1-A4 (I), B1-B4 (II), C1-C8 (III), and D1-3 (IV, V, VI).

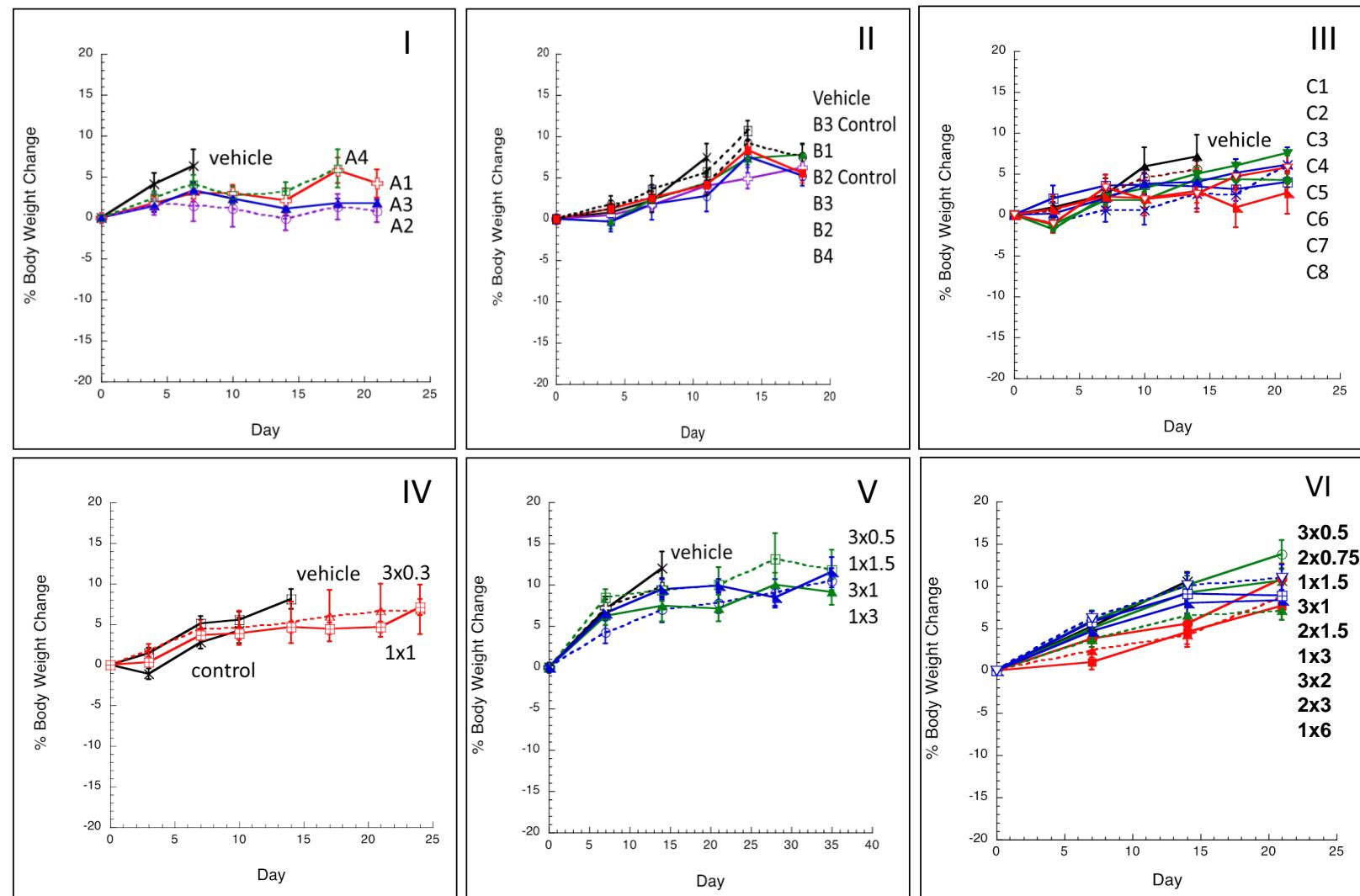
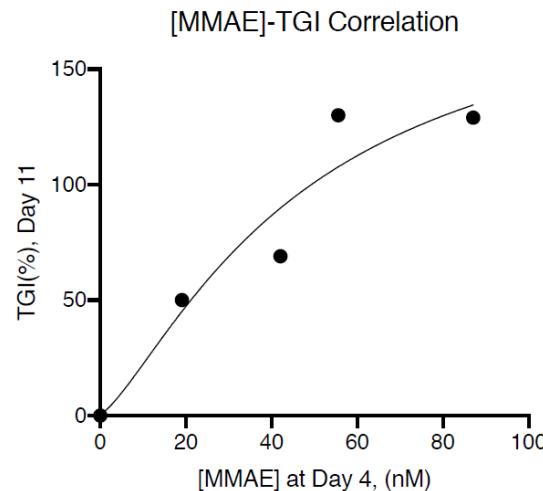
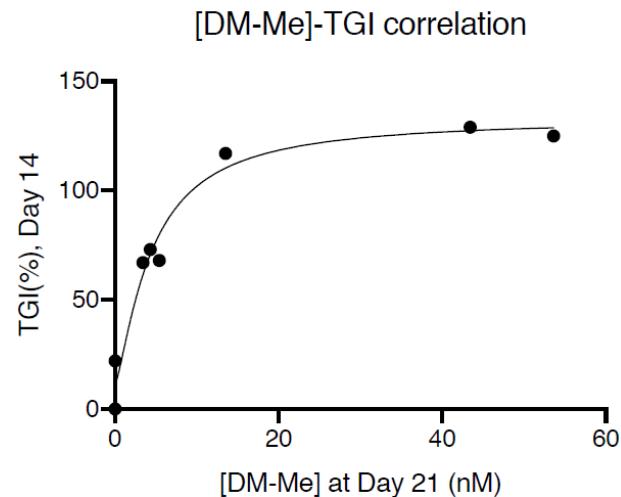


Figure S3. Correlation analysis of tumor growth inhibition with intratumor MMAE concentrations from Conjugates B1-B4 in mice bearing human CD22-expressing BJAB.Luc xenografts



[Agonist] vs. response -- Variable slope (four parameters)	
Best-fit values	
Bottom	0.7400
Hillslope	1.339
Top	192.8
EC50	46.87
logEC50	1.671
Span	192.1
95% CI (profile likelihood)	
Bottom	-399.2 to 247.7
Hillslope	???
Top	-102.5 to 746.3
EC50	???
logEC50	
Goodness of Fit	
Degrees of Freedom	1
R squared	0.9192
Sum of Squares	987.4
Sy.x	31.42
Constraints	
EC50	EC50 > 0
Number of points	
# of X values	5
# Y values analyzed	5

Figure S4. Correlation analysis of tumor growth inhibition with intratumor DMx-Me concentrations from Conjugates C1-C8 in mice bearing human CD22-expressing BJAB.Luc xenografts



[Agonist] vs. response -- Variable slope (four parameters)	
Best-fit values	
Bottom	11.28
Hillslope	1.326
Top	133.2
EC50	4.469
logEC50	0.6502
Span	121.9
95% CI (profile likelihood)	
Bottom	-10.33 to 32.95
Hillslope	0.3842 to 4.068
Top	108.3 to ???
EC50	1.867 to ???
logEC50	0.2712 to ???
Goodness of Fit	
Degrees of Freedom	4
R squared	0.9694
Sum of Squares	481.7
Sy.x	10.97
Constraints	
EC50	EC50 > 0
Number of points	
# of X values	8
# Y values analyzed	8

DMD/2019/087023