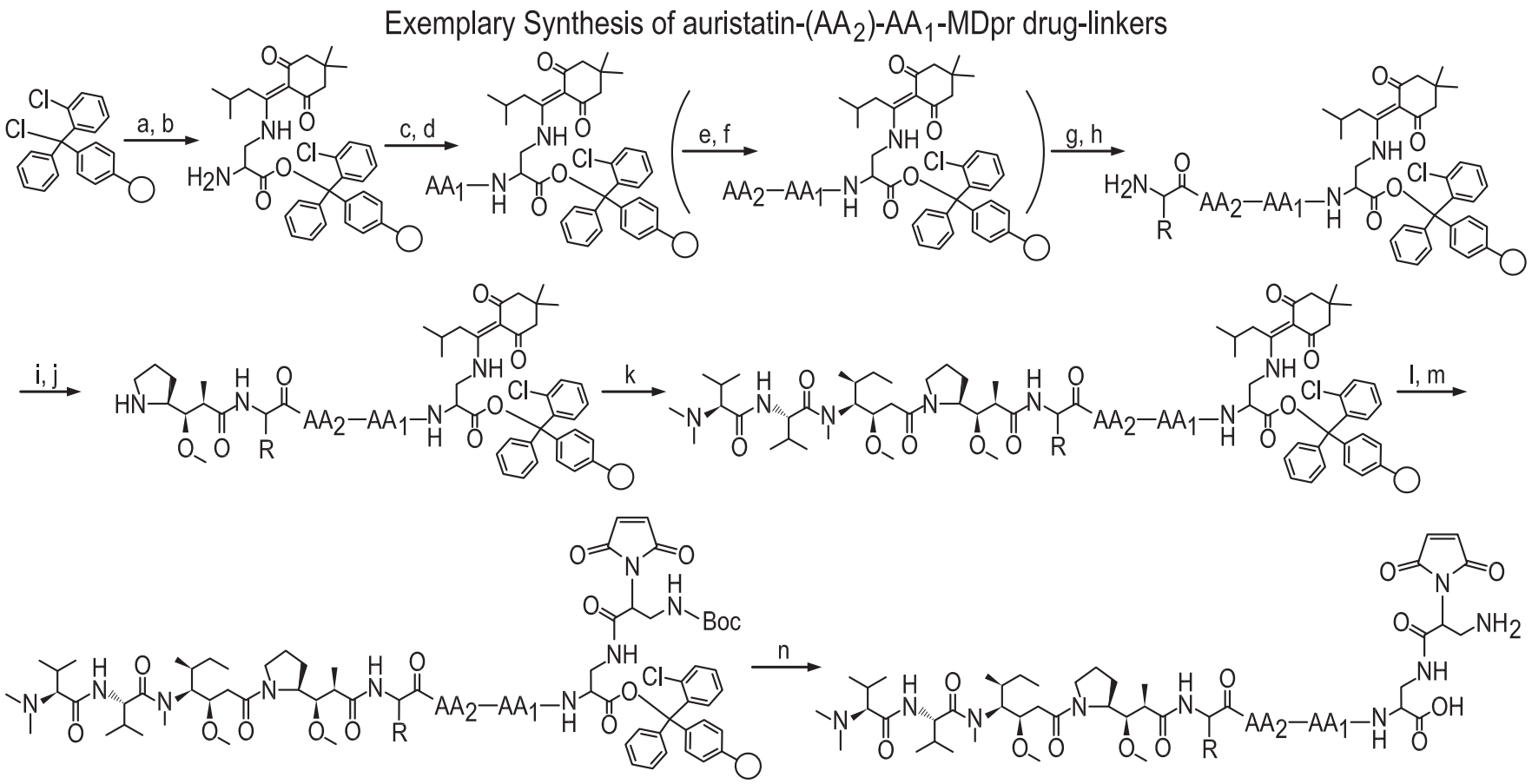


Reaction conditions: (a) Fmoc-Dpr(ivDde)-OH (1.5 equiv), DIEA (2.5 equiv), CH_2Cl_2 /DMF, 2 h; (b) 20% piperidine/DMF, 2h; (c) Fmoc-AA_1-OH (2 equiv), HATU (2 equiv), and DIEA (4 equiv), DMF, 3h; (d) 20% piperidine/DMF, 2h; (e, optional) Fmoc- AA_2-OH (2 equiv), HATU (2 equiv), and DIEA (4 equiv), DMF, 3h; (f, optional) 20% piperidine/DMF, 2h; (g) Fmoc-Phe-OH, or Fmoc-Met-OH, or Fmoc-Trp(Boc)-OH (2 equiv), HATU (2 equiv), and DIEA (4 equiv), DMF, 3h; (h) 20% piperidine/DMF, 2h; (i) Fmoc-Dap-OH (2 equiv), HATU (2 equiv), and DIEA (4 equiv), DMF, 3h; (j) 20% piperidine/DMF, 2h; (k) Dov-Val-Dil-OH (2 equiv), HATU (2 equiv), and DIEA (4 equiv), DMF, 2h; (l) 2% hydrazine/DMF, 2h; (m) MDpr(Boc)-OH (2 equiv), DIEA (4 equiv), CH_2Cl_2, 2h; (n) 20% TFA/CH_2Cl_2, 2h.

Fig. 1

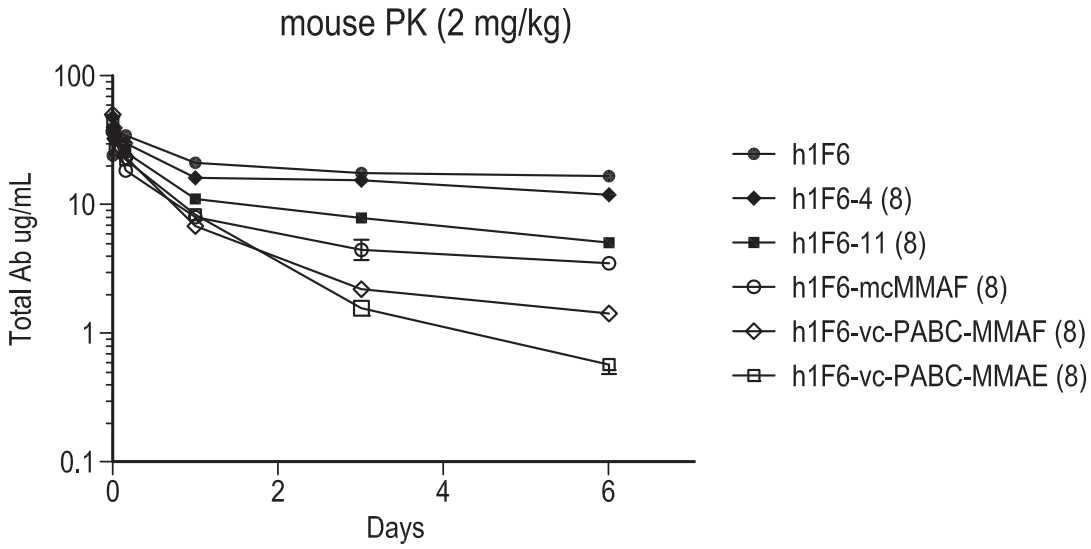

mouse PK (2 mg/kg)
Total Ab ug/mL
100
10
1
0.1
0
2
4
6
Days
h1F6
h1F6-4 (8)
h1F6-11 (8)
h1F6-mcMMAF (8)
h1F6-vc-PABC-MMAF (8)
h1F6-vc-PABC-MMAE (8)

Fig. 2

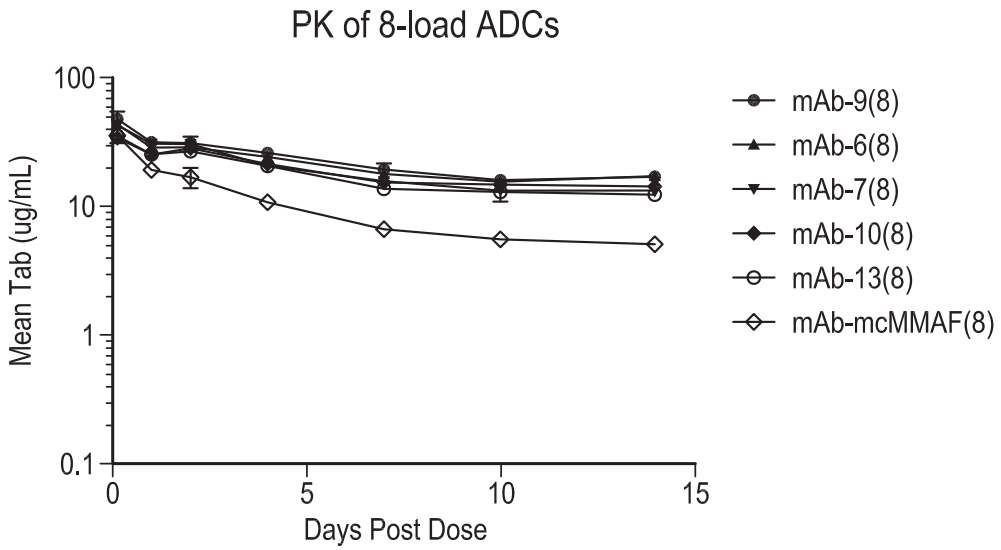

PK of 8-load ADCs
Mean Tab (ug/mL)
100
10
1
0.1
0
5
10
15
Days Post Dose
mAb-9(8)
mAb-6(8)
mAb-7(8)
mAb-10(8)
mAb-13(8)
mAb-mcMMAF(8)

Fig. 3

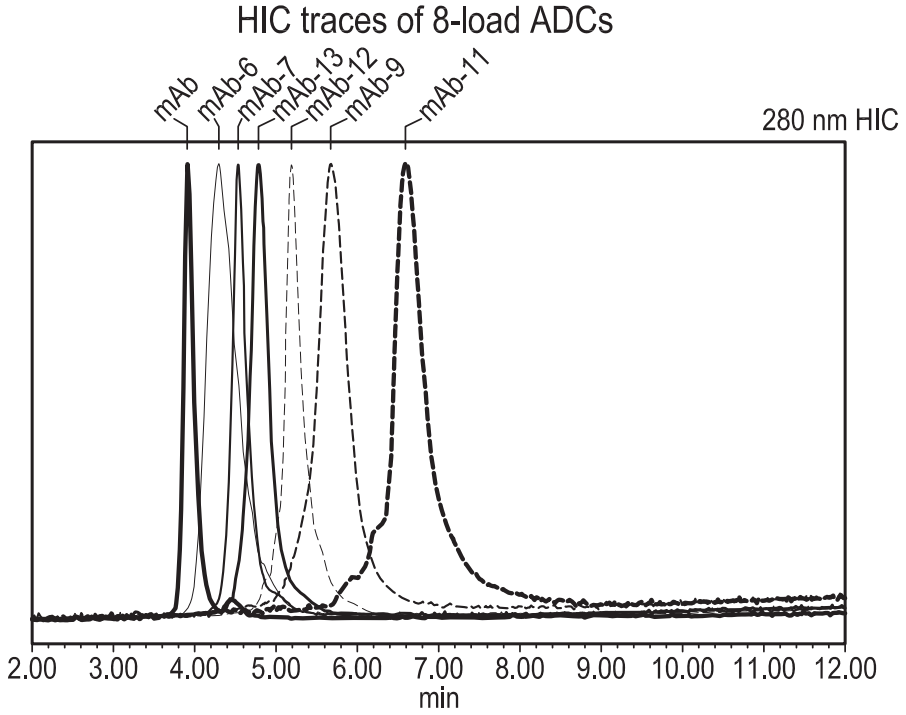

HIC traces of 8-load ADCs
mAb
mAb-6
mAb-7
mAb-13
mAb-12
mAb-9
mAb-11
280 nm HIC
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
min

Fig. 4

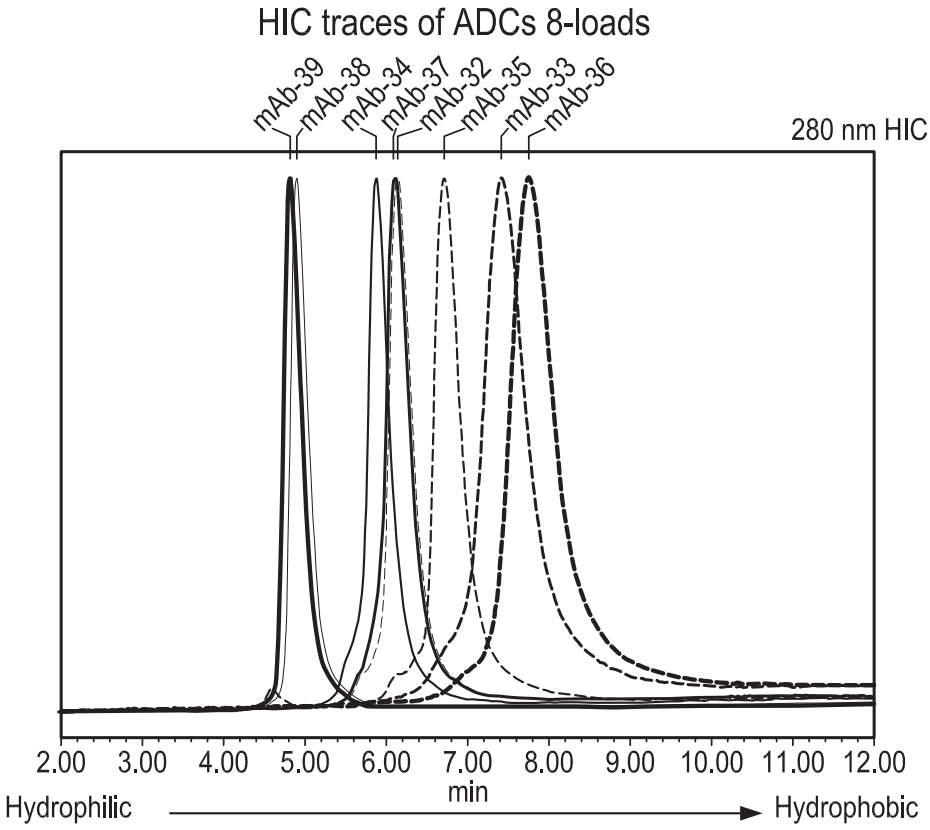

HIC traces of ADCs 8-loads
mAb-39
mAb-38
mAb-34
mAb-37
mAb-32
mAb-35
mAb-33
mAb-36
280 nm HIC
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
min
Hydrophilic
Hydrophobic

Fig. 5

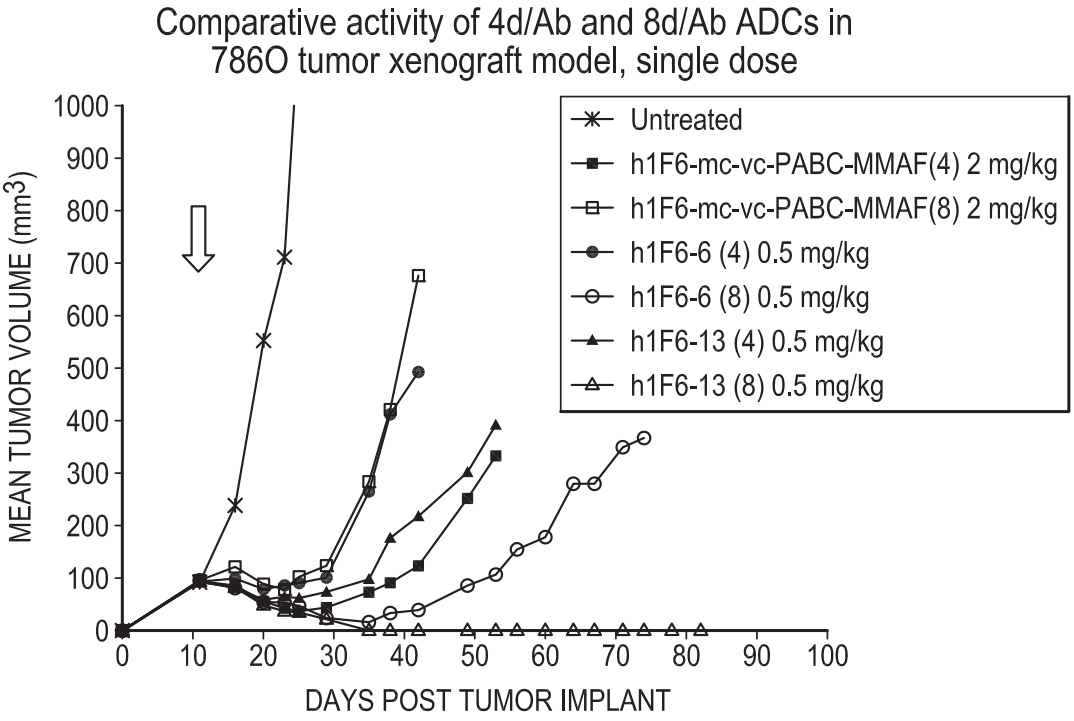

Comparative activity of 4d/Ab and 8d/Ab ADCs in
786O tumor xenograft model, single dose
MEAN TUMOR VOLUME (mm3)
DAYS POST TUMOR IMPLANT
Untreated
h1F6-mc-vc-PABC-MMAF(4) 2 mg/kg
h1F6-mc-vc-PABC-MMAF(8) 2 mg/kg
h1F6-6 (4) 0.5 mg/kg
h1F6-6 (8) 0.5 mg/kg
h1F6-13 (4) 0.5 mg/kg
h1F6-13 (8) 0.5 mg/kg

Fig. 6

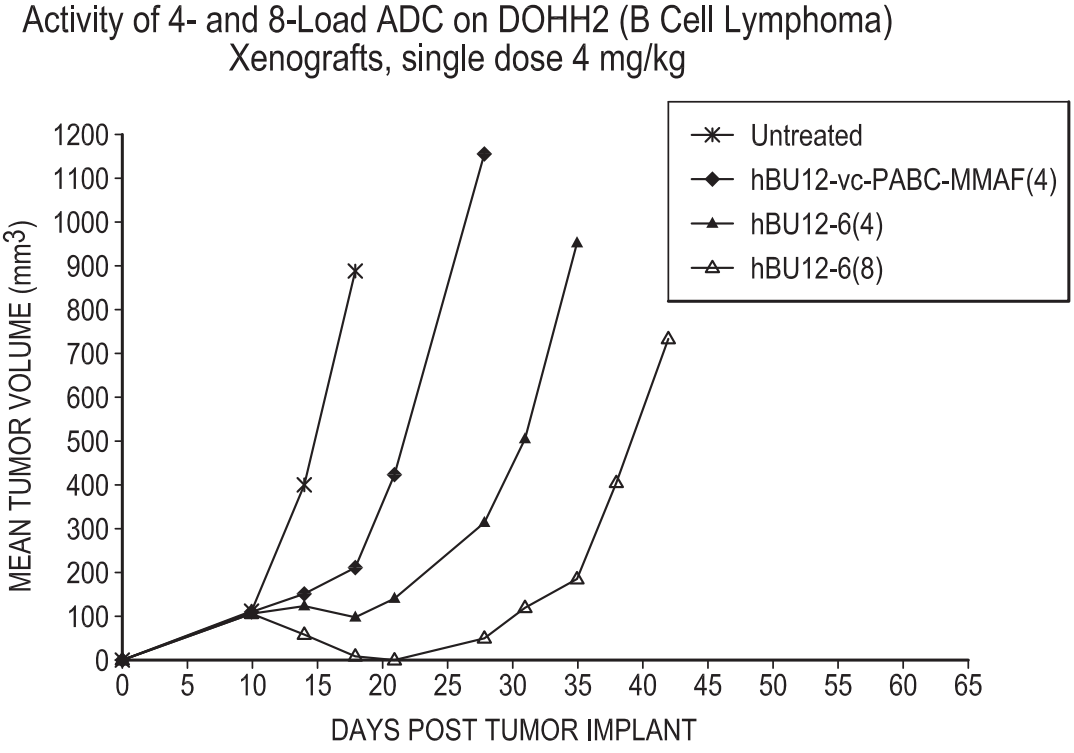

Activity of 4- and 8-Load ADC on DOHH2 (B Cell Lymphoma)
Xenografts, single dose 4 mg/kg
MEAN TUMOR VOLUME (mm3)
DAYS POST TUMOR IMPLANT
Untreated
hBU12-vc-PABC-MMAF(4)
hBU12-6(4)
hBU12-6(8)

Fig. 7

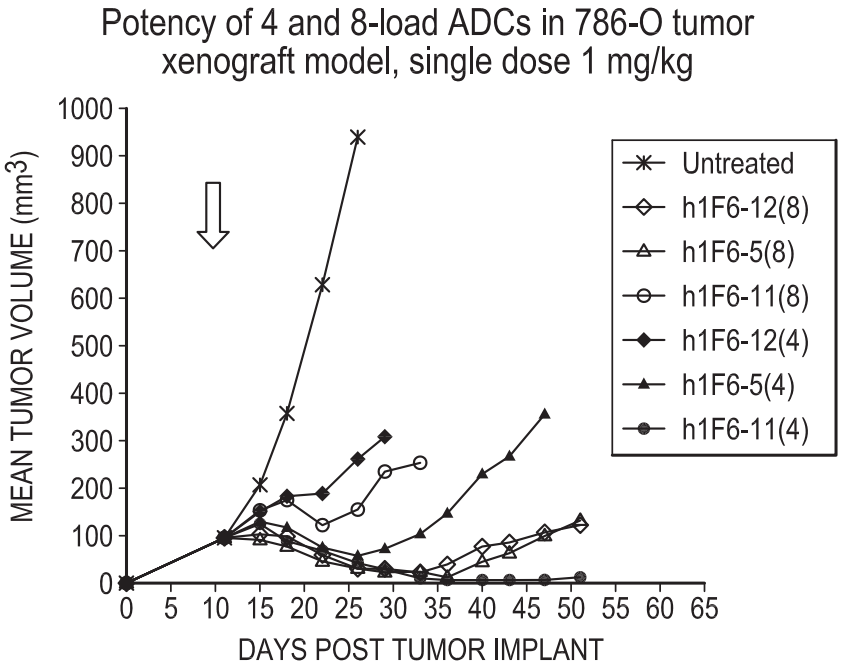

Potency of 4 and 8-load ADCs in 786-O tumor xenograft model, single dose 1 mg/kg
MEAN TUMOR VOLUME (mm3)
DAYS POST TUMOR IMPLANT
Untreated
h1F6-12(8)
h1F6-5(8)
h1F6-11(8)
h1F6-12(4)
h1F6-5(4)
h1F6-11(4)

Fig. 8

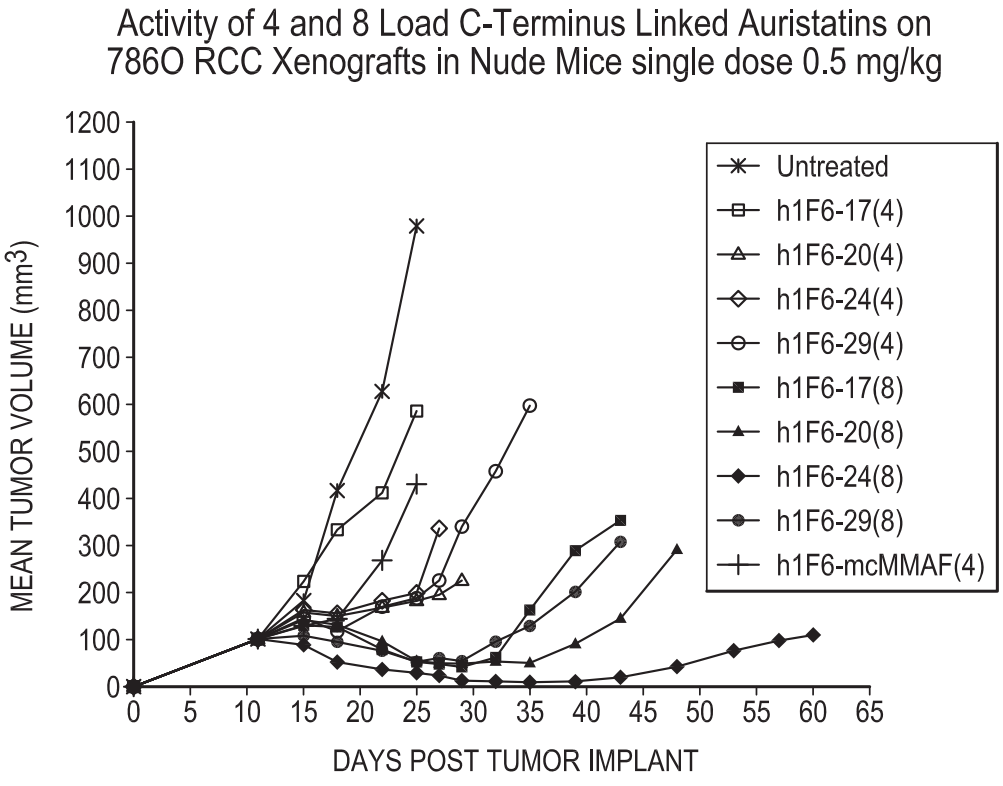

Activity of 4 and 8 Load C-Terminus Linked Auristatins on 786O RCC Xenografts in Nude Mice single dose 0.5 mg/kg
MEAN TUMOR VOLUME (mm3)
DAYS POST TUMOR IMPLANT
Untreated
h1F6-17(4)
h1F6-20(4)
h1F6-24(4)
h1F6-29(4)
h1F6-17(8)
h1F6-20(8)
h1F6-24(8)
h1F6-29(8)
h1F6-mcMMAF(4)

Fig. 9