Herceptin-Acestatins Antibody Conjugates in the Treatment of Subcutaneous HCC1954 Human Her2 Positive Breast Cancer Xenograft Model

Shanghui Sean Hu[1,2,3], Lucy X. Zhao[1], Zunrong Wang[1], Xufang F. Tian[1], Xu Liu[3], Chao Li[2], Wei Hu[2], Xingyan Bao[2], Xiaopeng Yue [2], and Bonnie Y. Hu[1]

[1]ACES Pharma Inc, PO BOX 2049, Princeton NJ 08543, USA
[2]Huansheng Pharmtech (Shandong) Co. Ltd, Jinan, Shandong, China
[3]Beijing Deming Biotechnology Co. Ltd, Beijing, China

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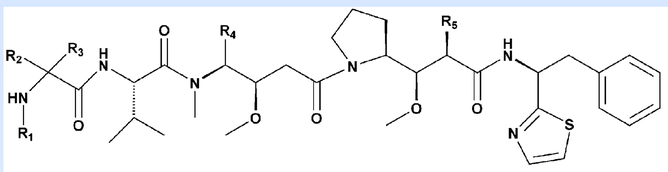
Abstract

This presentation described pre-clinically the *in vitro* and *in vivo* therapeutic efficacy of Herceptin-acestatin™ ADCs in the treatment of subcutaneous HCC1954 human Her2 positive breast cancer xenograft model in Balb/c nude mice.

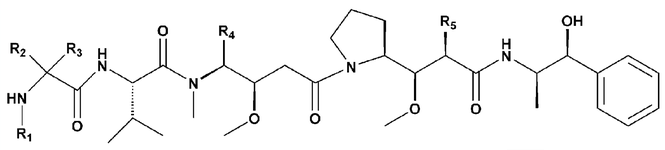
The Herceptin-acestatin ADC at different dose levels produced a moderate or significant antitumor activity, which is dose-effect dependent and H-vc-acestatin at 3 mg/kg cured the tumor bearing mice in subcutaneous HCC1954 human breast cancer xenograft model. Acestatin ADCs demonstrated the same efficacy and better safety profiles than MMAE ADC.

Structure

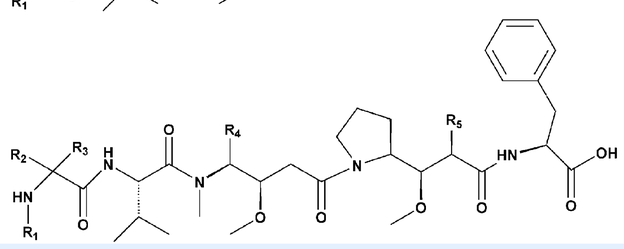
Structures of Acestatins E, F or D



Acestatin D



Acestatin E



Acestatin F

Linker: vc, mc-val-cit-PAB

Experimental Methods and Procedures

1. Cell Culture

The HCC1954 tumor cells were maintained in vitro as monolayer culture in RPMI-1640 medium supplemented with 10% fetal bovine serum at 37ºC in an atmosphere of 5% CO_2 in air. The tumor cells growing in an exponential growth phase were harvested and counted for tumor inoculation.

2. Tumor Inoculation

Each mouse was inoculated subcutaneously at the right flank region with HCC1954 tumor cells (5x 10^6) in 0.1 ml of PBS(1:1 matrigel) for tumor development. The treatments were started when the mean tumor size reached 189 mm^3. The test article was administered to the tumor-bearing mice according to the predetermined regimen as shown in the experimental design.

3. Group assignment

By using randomized block design to assign experimental animals, we ensured that each animal had the same probability of being assigned to any given treatment groups and therefore systematic error was minimized.

Study endpoints: The major endpoints of the study included the followings:

Tumor growth inhibition (TGI): TGI(%) is an indication of antitumor effectiveness, and expressed as: TGI (%)=100 x (1-T/C). T and C were the mean tumor volume (or weight) of the treated and control groups, respectively, on a given day.

Tumor growth delay (TGD): expressed as T-C where T was the time (in days) required for the mean tumor size of the treatment group to reach a predetermined size (e.g., 1000 mm^3), and C was the time (in days) for the mean tumor size of the control group to reach the same size.

Results

- Acestatins E showed lower potency than the correspondent MMAE (IC50: 0.33 nM vs 0.11 nM)
- H-vc-acestatin-E ADC showed slightly better efficacy and safety profile than H-vc-MMAE *in vitro* for several Her2 positive cancel cell lines.

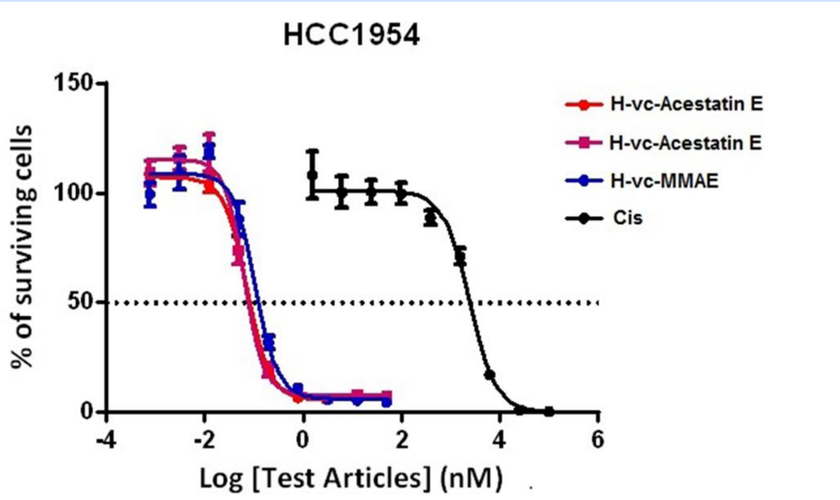


Fig.1 IC_{50} and Dose-responsive curve of Herceptin-vc-Acestatin E on HCC1954 (120-hour assay)

	H-vc-acestatin E (Lot 1)	H-vc-acestatin E (Lot 2)	H-vc-MMAE	Acestatin E	MMAE	Cispatin
IC50 (nM)	0.084	0.078	0.13	0.33	0.11	2500

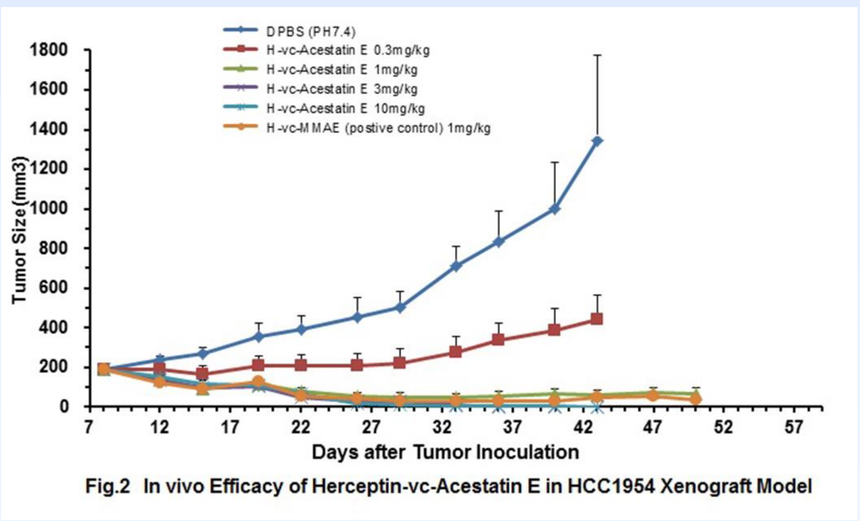


Fig.2 In vivo Efficacy of Herceptin-vc-Acestatin E in HCC1954 Xenograft Model

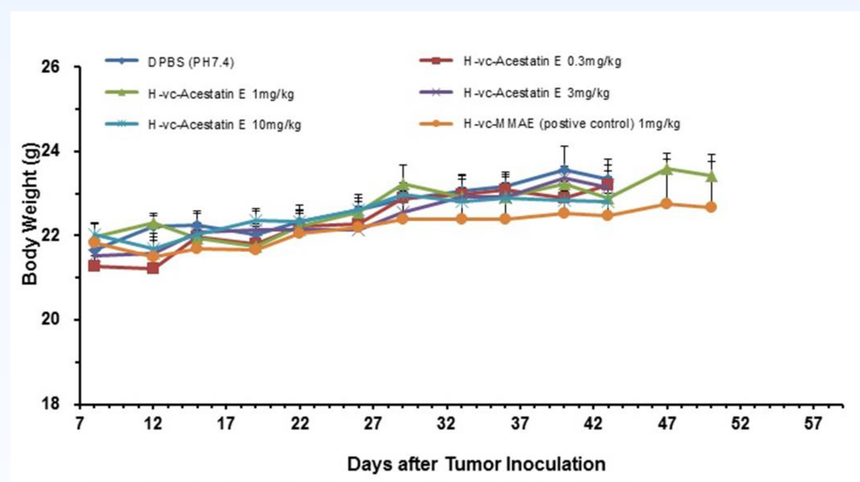


Fig.3 In vivo Efficacy and safety of Herceptin-vc-Acestatin E

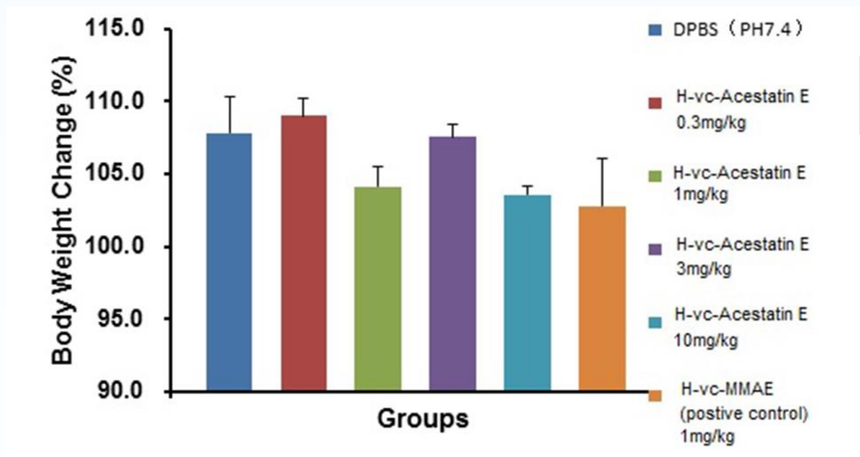


Fig.4 The Body Weight Changes (%) of the Mice in the Different Groups at Day 43 post Tumor Inoculation

Results (cont.)

- The results of tumor sizes in different groups at different time points after tumor inoculation are shown in Figure 3.
- The mean tumor size of the vehicle treated group reached 1342mm^3 on day 43 after tumor inoculation.
- Treatment with H-vc-acestatin E at the lowest dosage (0.3 mg/kg) produced a moderate antitumor activity, its mean tumor size was 439mm^3 (TGI value =67.3%) and had a tumor growth delay of 19 days at the tumor size of 400mm^3 compared with the vehicle group.
- Treatment with H-vc-acestatin E at 1 mg/kg and positive control H-vc-MMAE at 1 mg/kg both produced a significant antitumor activities, tumor regressed, the mean tumor sizes were 50mm^3 and 45mm^3 at the same time (TGI value =96.3% and 96.6% respectively, p =0.017 and 0.019).
- Tumors were completely cured in the 3 mg/kg and 10 mg/kg of H-vc-acestatin E , tumor sizes were both 0 and TGI value were both 100% on day 43 after tumor inoculation. Tumor regrowth was not observed till study termination.
- The results of tumor weight were consist with tumor volume. All the test articles were tolerated well by the tumor bearing mice, no body weight loss was observed during the study.

Conclusion

In summary, the test article H-vc-acestatin E at different dose levels produced a moderate or significant antitumor activity, which is dose-effect dependent and H-vc-acestatin E at 3 mg/kg cured the tumor bearing mice in subcutaneous HCC1954 human breast cancer xenograft model in this study.

Acestatin ADCs demonstrated the same efficacy and better safety profiles than MMAE ADC.

Acknowledgement

We thank *Crown Bioscience (Beijing)* for animal model experiments.

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