Development and characterization of an antibody-drug conjugate (ADC) utilizing PB-223, a novel monoclonal antibody (mAb) specifically targeting core 2 O-glycans on human carcinomas

Abstract #2878

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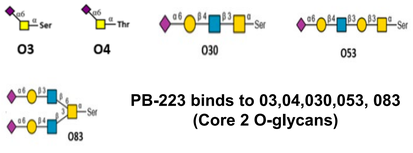
Introduction

Antibody-drug conjugates (ADCs) represent a cutting-edge approach in cancer therapy. The three essential components of an ADC include the mAb, the linker, and the cytotoxic payload. The mAb is designated to target specific tumor-associated antigens that are overexpressed on the surface of cancer cells. The mAb PB-223 was developed through the affinity maturation of mAb NEO-102 (Ensituximab), a chimeric human IgG1 mAb that specifically targets truncated core 2 O-glycans, commonly found in colorectal and pancreatic cancers. The binding affinity of PB-223 for its target was improved, compared to NEO-102, by optimizing its VH and VL sequences through Fast Screening for Expression Biophysical Properties and Affinity. PB-223 demonstrated a binding affinity (KD) at least 4-fold lower than NEO-102, indicating stronger tumor binding. Immunohistochemistry analysis also revealed that PB-223 binds to a wider spectrum of tumor tissues compared to NEO-102, but not to normal tissues. PB-223 can be internalized into human cancer cell lines expressing its target. The objective of this study is to develop an ADC utilizing PB-223 for treatment of various human malignancies.

Experimental Design

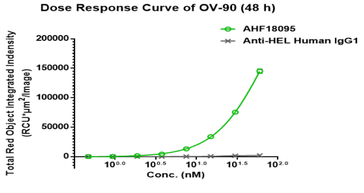
PB-223 (AHF 18095) was used for the development of the ADC. Monomethyl auristatin E (MMAE) was used as payload, with mc-vc-PABc serving as the linker. PB-223 was conjugated to the linker-payload through a cysteine-based conjugation method. Drug-to-antibody ratio (DAR) was measured using Size Exclusion Chromatography (SEC)-MS. Flow cytometry was used for binding assessment of three ADC clones using the ovarian cancer cell line OV-90 as the target. The three ADCs were named PB-vcMMAE-2 (U566-vcMMAE-2); PB-vcMMAE-5 (U566-vcMMAE-5); PB-vcMMAE-6 (U566-vcMMAE-6). Cytotoxicity of the three ADC clones was evaluated through cell viability assays (5-days) using CellTiter-Glo cell viability Kit using OV90 as target. Stability of ADC in human plasma was also evaluated. PB-vcMMAE-5 concentration in human plasma was detected by ELISA at the following time points: 0h, 24h (1 day), 48h (2 days), 96h (4 days), 168h (7 days), 240h (10 days), 336h (14 days). Free paylod release in human plasma was detected by LC-MS/MS method at the same time points. *In vivo* ADC toxicity was evaluated in rats. The ADC PB-vcMMAE-5 in rats was administered intravenously at concentration of 2,3mg/kg.
The efficacy of ADC was assessed in OV-90 subcutaneous xenograft model established in NOD-SCID mice. The ADC PB-vcMMAE-5 was administered intravenously at doses 1 mg/kg and 3 mg/kg, once per week for three weeks. Treatment began when the average tumor volume reached approximately 100-150 mm^3. MMAE were used as control. Tumor volumes were measured twice per week.

PB-223 Binds to Core 2 O-Glycans



PB-223 binds to 03,04,030,053, 083 (Core 2 O-glycans)

PB-223 internalizes into OV-90



48 hours incubation with PB-223 and the isotype control antibody, anti-HEL human IgG1

Results

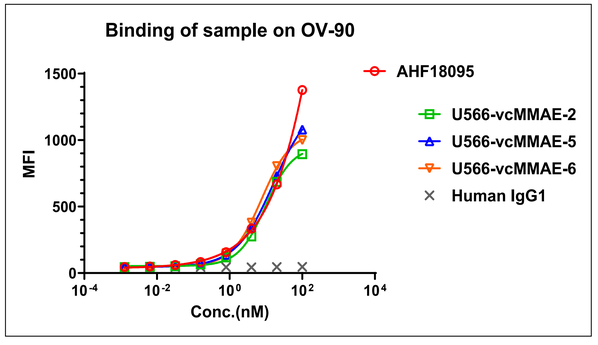
1. ADC DAR

ADC clone	Conc. (mg/ml)	Purity SEC-HLPC%	DAR	Endotoxin (EU/mg)
PB-vcMMAE-2	1.421	95.11	3.72	<3
PB-vcMMAE-5	1.482	99.48	3.92	<3
PB-vcMMAE-6	1.428	99.50	4.15	<3

We developed three ADCs: PB-MMAE-2, PB-MMAE-5 and PB-MMAE-6.
The DAR for these ADCs was 3.72, 3.92 and 4.15, respectively.
It is generally believed that a DAR between 2 and 4 is the best choice for ADC drugs

2. Flow cytometry: ADC binding to OV-90

All three ADCs exhibit similar binding affinity to OV-90 compared to PB-223



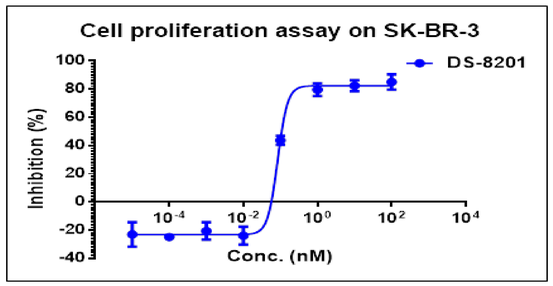
Binding of ADCs and PB-223 to OV-90 was tested using different concentrations (100, 20, 4, 0.8, 0.16, 0.032, 0.0064, 0.00128 nM). Human IgG1 was used as negative control for the binding. AHF 18095 is PB-223.
U566-vcMMAE-2 is PB-vcMMAE-2; U566-vcMMAE-5 is PB-vcMMAE-5; U566-vcMMAE-6 is PB-vcMMAE-6

ADC (100 nM)	% cell positive	MFI
PB-223 (AHF 18095)	30.07	1377
PB-vcMMAE-2	19.52	894
PB-vcMMAE-5	23.56	1079
PB-vcMMAE-6	21.86	1001
Human IgG1	1.03	47.3

The table depict the percentage of positive cells and MFI when PB-223 and ADCs were used at the concentration of 100 nM

3. Killing assay: ADC kills OV-90

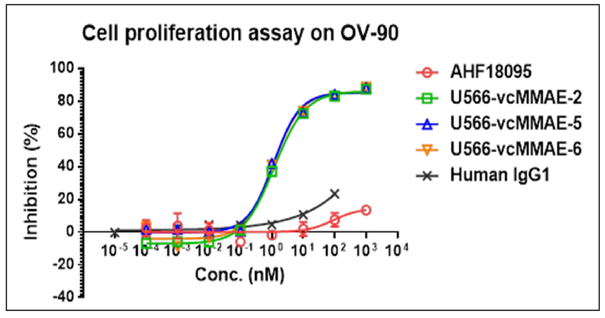
Positive control: Trastuzumab deruxtecan ADC (DS-8201)



The cytotoxicity of the ADCs was evaluated using the breast cancer cell line SK-BR-3 with Trastuzumab deruxtecan ADC (DS-8201) as positive control. SK-BR-3 cells were treated with serially diluted system control (DS-8201, highest concentration 100 nM) for 5 days before measuring the cell viability. Each data point represents mean ± SEM (n = 2).

At a concentration of 10nM, DS-8201 inhibited cell proliferation by 82.39%.

PB-ADCs killing of OV-90 cells



OV-90 cells were treated with serially diluted test samples (highest concentration 1000 nM) or the negative control human IgG1 (highest concentration 100 nM) for 5 days before measuring the cell viability. Each data point represents mean ± SEM (n = 2).

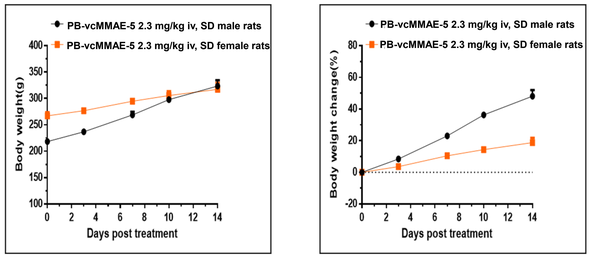
AHF 18095 is PB-223. U566-vcMMAE-2 is PB-vcMMAE-2; U566-vcMMAE-5 is PB-vcMMAE-5; U566-vcMMAE-6 is PB-vcMMAE-6

All three PB- ADCs effectively killed OV-90 cells.

At a concentration of 333nM, the percentage of cell killing for PB-vcMMAE-2, PB-vcMMAE-5 and PB-vcMMAE-6 was 82.91%, 84.04% and 83.16%, respectively.

In contrast, both human IgG1 and naked PB-223 mAb showed no killing of OV-90 cells.

4. ADCs toxicity *in vivo*: rats body weight

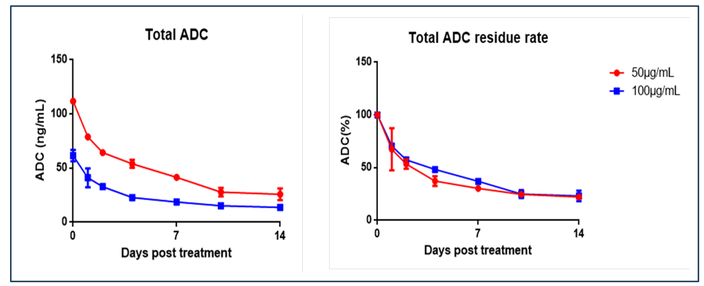


The ADC PB-vcMMAE-5 in rats was administered intravenously at concentration of 2,3mg/kg as single dose. Animal body weight was measured at different time points until 14 days after ADC administration.

The ADC PB-vcMMAE-5 was well tolerated in rats. No sign of distress and loss of body weight were observed after administration

5. ADC stability in human plasma

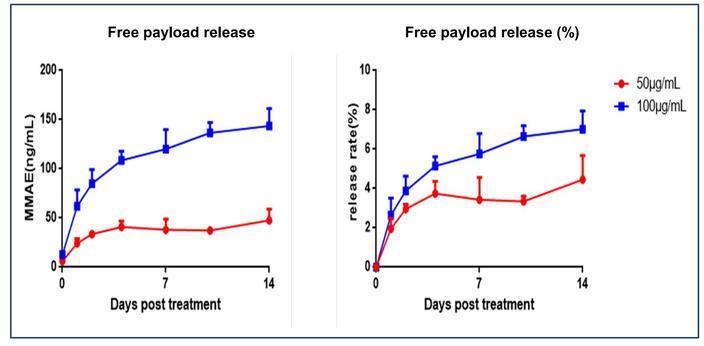
PB-vcMMAE-5 ADC is stable in human plasma



PB-vcMMAE-5 ADC was incubated with male and female human plasma at concentration of 50 μg/mL and 100 μg/mL. Concentration in human plasma was detected by ELISA at the following time points: 0h, 24h (1 day), 48h (2 days), 96h (4 days), 168h (7 days), 240h (10 days), 336h (14 days).

Total ADC residue rate is calculated as ADC $quantity_{0h}$-ADC $quantity_{detection\ time}$)/ADC $quantity_{0h}$*100%

After 14 days, mean residual rate of PB-vcMMAE-5 ADC in human plasma was 23% for ADC at 100 μg/mL and 22% for ADC at 50 μg/mL



PB-vcMMAE-5 ADC was incubated with male and female human plasma at concentration of 50 μg/mL and 100 μg/mL. Free paylod release in human plasma was detected by LC-MS/MS method at the following time points: 0h, 24h (1 day), 48h (2 days), 96h (4 days), 168h (7 days), 240h (10 days), 336h (14 days).

Release rate is calculated as Payload quantity/theoretical payload quantity0h*100%

After 14 days, mean free payload release rate of PB-vcMMAE-5 ADC in human plasma was 7.02% for ADC at 100 μg/mL and 4.46% for ADC at 50 μg/mL

Conclusions

ADC leverages the specificity of mAbs to deliver a potent payload. In this study, we have developed three ADCs using the anti-core 2 O-glycans anti-human carcinoma mAb PB-223. PB-223 recognizes specifically cancer cells, sparing healthy tissues. The three PB-223 ADCs generated in this study were PB-vcMMAE-2 (U566-vcMMAE-2), PB-vcMMAE-5 (U566-vcMMAE-5) and PB-vcMMAE-6 (U566-vcMMAE-6). Our findings indicate that these ADCs can efficiently kill *in vitro* cancer cells expressing the PB-223 target antigen. Further analysis focusing on PB-vcMMAE-5 (U566-vcMMAE-5) demonstrates that the ADC is stable in human plasma and non-toxic in rats. Additional PK/PD evaluations are ongoing. *In vivo* efficacy of PB-vcMMAE-5 is being evaluated using an OV-90 subcutaneous xenograft model established in NOD-SCID mice. Preliminary data suggest that PB-vcMMAE-5 exhibits anti-tumor activity in the NOD-SCID mice tumor model.
Two days after second dose of PB-vcMMAE-5 at 3mg/kg, treated mice showed a reduction of 59.78% and 56.23% in tumor volume compared to mice treated with PBS (109mm^3 vs 271mm^3) or payload alone (109mm^3 vs 249mm^3), respectively. Our data demonstrated that PB-vcMMAE-5 has potential as a therapeutic option for a range of human malignancies expressing core 2 O-glycans.