Poster #: B068

In vitro and *in vivo* efficacy of the antibody-drug-conjugate (ADC) PB-vcMMAE-5 against human ovarian cancer expressing truncated core 2 O-glycans

PRECISION BIOLOGICS

Kwong Y. Tsang[1], Massimo Fantini[1], Anjum Zaki[1], Sharon A. Mavroukakis[1], Christina M. Annunziata[2], Philip M. Arlen[1]

[1]Precision Biologics, Inc., Bethesda, MD, 20814; [2]American Cancer Society, Glen Allen, VA, 23058

Introduction

Ovarian cancers remain largely unresponsive to immune checkpoint inhibitors, in part due to their ability to suppress the cytotoxic activity of immune cells infiltrating the tumor microenvironment. One of the disrupted pathways in these cancers is O-glycosylation, a feature particularly associated with ovarian cancer progression, metastasis and poor prognosis. This underscores the urgent need for alternative immunotherapeutic strategies. The monoclonal antibody (mAb) PB-223 was developed through affinity maturation of NEO-102, a chimeric human IgG1 mAb that targets tumor specific truncated core 2 O-glycans. PB-223 exhibits 4-fold improvement in binding affinity (KD) compared to NEO-102 and recognizes a broader range of tumor tissues while sparing normal tissues. We developed an ADC, designated PB-vcMMAE-5, composed of the mAb PB-223 conjugated via the mc-vc-PABc linker to the microtubule-inhibitor MMAE. PB-vcMMAE-5 has a drug-to-antibody ratio of 3.92 and demonstrates stability in human plasma. This study aimed to evaluate the therapeutic efficacy of PB-vcMMAE-5 *both in vitro* and *in vivo* in ovarian cancer models.

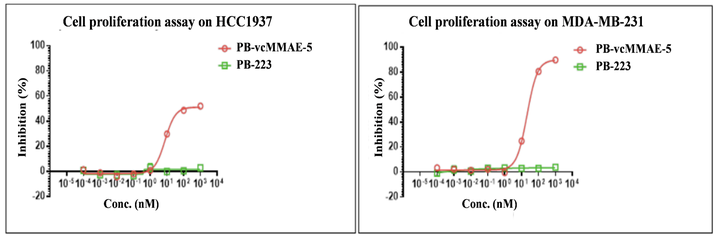
Experimental Design

The *in vitro* cytotoxicity of PB-vc-MMAE-5 was tested in four human cancer cell lines: triple-negative breast cancer (HCC1937, MDA-MB-231), ER$^+$/PR$^+$/HER2$^+$ breast cancer (BT474), and ovarian cancer (OV-90). Cells were treated with varying ADC concentrations for 5 days, and cytotoxicity was measured by luminescence assay. *In vivo* efficacy was assessed in NOD-SCID mice bearing OV-90 xenografts. Mice (n = 6 per group) received weekly doses of PBS, MMAE alone, or PB-vc-MMAE-5 (1, 3, 6, or 9 mg/kg) for five weeks. Tumor volume, hematology, clinical chemistry, and viable tumor cell content were monitored. On day 31, tumors were excised for histological analysis using Ki-67 staining to assess proliferating viable tumor cells. To further assess systemic toxicity, three mice each from the 6 and 9 mg/kg groups were followed to day 45.

Results

1. Killing assay: *in vitro* efficacy of PB-vcMMAE-5 against tested cancer cell lines

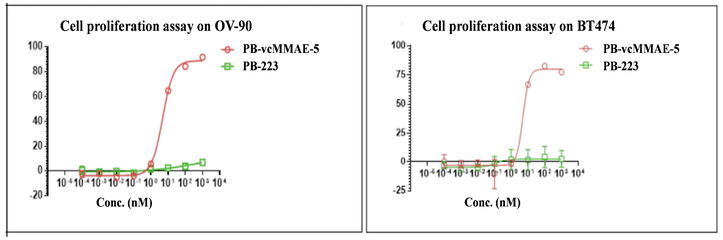
Human triple negative cancer cell lines



At the highest concentration, the percentage of cell killing for PB-vcMMAE-5 was 52.72% in HCC1937 and 88.36% in MDA-MB-231.

In contrast, naked PB-223 mAb showed no killing in both cell lines.

Human ovarian (OV-90) and ER$^+$/PR$^+$/HER2$^+$ breast (BT47) cancer cell lines



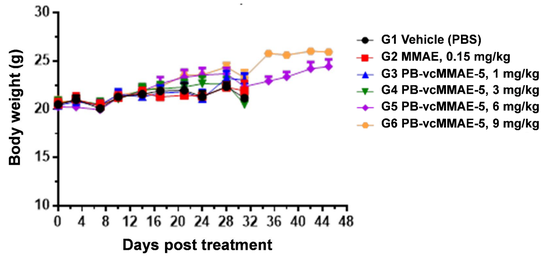
At the highest concentration, the percentage of cell killing for PB-vcMMAE-5 was 92.51% in OV-90 and 83.22% in BT474.

In contrast, naked PB-223 mAb showed no killing in both cell lines.

All cancer cell lines were treated with serially diluted ADC (PB-vcMMAE-5) or the naked mAb PB-223 (highest concentration 1000 nM) for 5 days before measuring the cell viability.

Each data point represents mean ± SEM (n = 2).

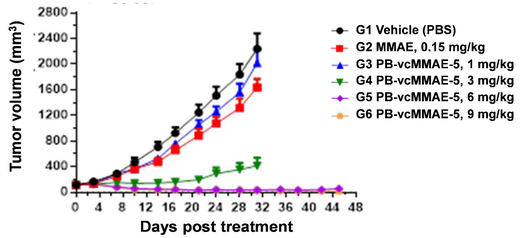
2. ADC toxicity *in vivo*: mice body weight



Animal body weight was monitored regularly, twice a week, as an indirect measure of toxicity. Data points represent group mean body weight

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

3. ADC efficacy *in vivo*: NOD-SCID mice

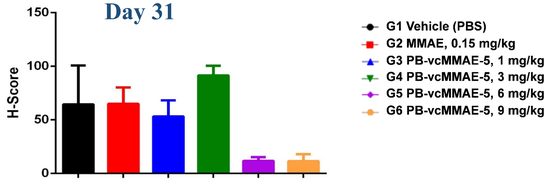


Tumor growth curve of different treatment groups in female NOD-SCID mice bearing OV90 tumors. Data points represent group mean until day 31. Three mice from G5 and G6 were followed to day 45.

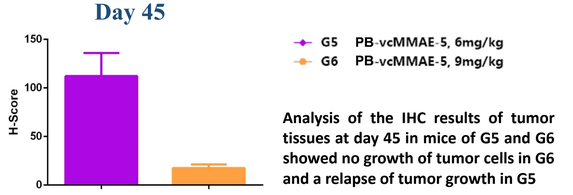
PB-vcMMAE-5 at 1 mg/kg did not significantly reduce tumor volume compared with PBS, whereas PB-vcMMAE-5 at 6 and 9 mg/kg induced robust reduction of tumor volume.

On day 31, tumor growth inhibition (TGI) vs control group (G1) was 28.37, 10.40, 85.91, 103.90 and 103.77 for G2,G3,G4,G5,G6, respectively. TGI is calculated as [1- (change in tumor volume of treated group/change in tumor volume of control group)] x 100%

4. Ki-67 staining in excised tumors



The H-scores of groups G5 and G6 decreased significantly compared with groups G1-G4.



Analysis of the IHC results of tumor tissues at day 45 in mice of G5 and G6 showed no growth of tumor cells in G6 and a relapse of tumor growth in G5

Conclusions

PB-vc-MMAE-5 demonstrated potent anti-ovarian cancer activity in both *in vitro* and *in vivo* models, with good tolerability. *In vivo*, PB-vc-MMAE-5 at 9 mg/kg produced the most potent and durable anti-tumor response, while maintaining a favorable safety profile. H-score of Ki-67 staining of excised tumors at day 45 showed absence of viable tumor cells in G6. No significant hematological or pathological changes were detected in the liver, spleen, brain, or heart of mice treated with efficacious doses of the ADC compared with controls. These results highlight the potential of PB-vc-MMAE-5 as a therapeutic candidate for ovarian tumors expressing truncated core 2 O-glycans.