The antibody-drug conjugate GENA-111 conjugated to auristatin F shows therapeutic potency in BCAM positive epithelial cancer

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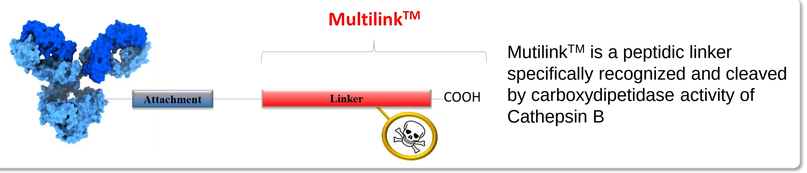
Introduction

Antibody-drug conjugates (ADCs) are promising cancer treatment modalities that combine the selectivity of antibodies and the cytotoxic properties of payloads using chemical linkers. However, despite their success, ADCs still suffer from drawbacks such as systemic toxicity, limiting their potential clinical applications. The systemic toxicity of ADCs is mainly related to its linker's stability and its antibody's selectivity towards the targeted antigen expressed on cancer cells. Lu/BCAM (Lutheran/basal cell adhesion molecule) is a member of the immunoglobulin superfamily and is a receptor for laminin, a protein that facilitates cell adhesion, migration, and invasion. A growing number of studies show that BCAM plays an essential role in tumor progression and is overexpressed on epithelial cancers, e.g., skin cancer (Schön *et al.*, J Invest Dermatol, 2000). Moreover, BCAM has been introduced as a promising ADC target for breast cancer (Kikkawa *et al.*, Sci Rep, 2018).

Here we describe the ADC GENA-111-Multilink™-auristatin F (hereafter GENA-111-AF), wherein the GENA-111 antibody has been armed with an auristatin F derivative using Debiopharm's Multilink™ technology and a stabilized thiol maleimide conjugation. Multilink™ technology comprises a cleavable peptidic sequence that facilitates multidrug attachment and the production of ADCs with high Drug Antibody Ratio (DAR). In this presentation, GENA-111-AF was prepared with one auristatin F per linker at a DAR of 4.26. We investigated the anti-tumor effects of GENA-111-AF on several cancer cell lines expressing both BCAM and HER2 at different levels, and compared it with the efficacy of trastuzumab-Multilink™-DM1 prepared in-house (hereafter trastuzumab-DM1) as a positive control for HER2-positive cancer cell lines. We show here that BCAM is highly expressed in various cancer cell lines, including breast cancer, and GENA-111-AF has significant cytotoxic activity against BCAM expressing cell lines even in the cells not responding to trastuzumab-DM1. In addition, GENA-111-AF shows complete remissions in A431, BCAM-positive human skin cancer, xenograft model.

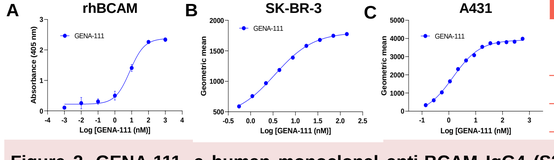
ADC preparation



Mutilink™ is a peptidic linker specifically recognized and cleaved by carboxydipetidase activity of Cathepsin B

Figure 1. ADC preparation. The linker Multilink™ was prepared by standard chemistry methods and convergent strategies. Auristatin F was conjugated to Multilink™ using standard peptidic coupling in solution. The resulting drug-linker was then conjugated to GENA-111 and to the isotype control hIgG4 (Sinobiologicals, China) via cysteines resulting from reduction of interchain disulfide bonds with TCEP (2.3 eq.) in DPBS at 40°C. The reaction mixture was stirred at room temperature for 70 min. It was then diluted with pH8 DPBS and purified on gel-column followed by concentration using an Amicon cell.

Binding affinity of GENA-111 against BCAM



Interaction \ Method, EC_{50}	Method, EC_{50}
GENA-111 / rhBCAM	ELISA, 7.7 nM
	Octet, K_D,0.084 nM
GENA-111 / SK-BR-3	FACS, 3.5 n M
GENA-111 / A431	FACS, 1.4 nM

Figure 2. GENA-111, a human monoclonal anti-BCAM IgG4 (S228P) antibody, binds strongly to recombinant human BCAM (rhBCAM) and cancer cell lines expressing BCAM. The binding affinity of GENA-111 to rhBCAM was determined by ELISA (A) and Octet, and to cancer cell lines expressing BCAM, SK-BR-3 and A431, was determined by flow cytometry (B and C), respectively.

BCAM expression on human cancer cells

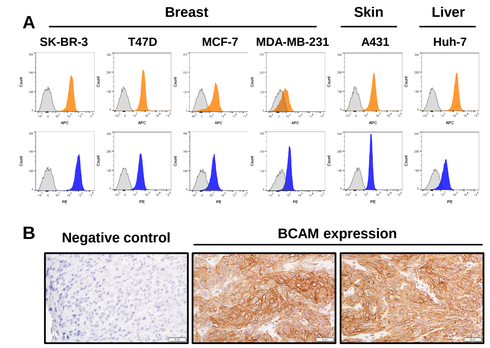


Figure 3. BCAM and HER2 membrane expressions on cancer cell lines. (A) BCAM and HER2 expression on SK-BR-3, T47D, Huh7, MCF-7, MDA-MB-231, A431 and Huh-7 cells was detected by flow cytometry with GENA-111 (filled orange histograms), anti-HER2 antibody (filled blue histograms) and their respective isotype hIgG4 controls (filled grey histograms). (B) Membrane expression of BCAM was confirmed in treatment-naïve A431 xenografted tumor tissue by immunohistochemical staining with GENA-111 (X400).

Internalization of GENA-111 in BCAM expressing tumor cells

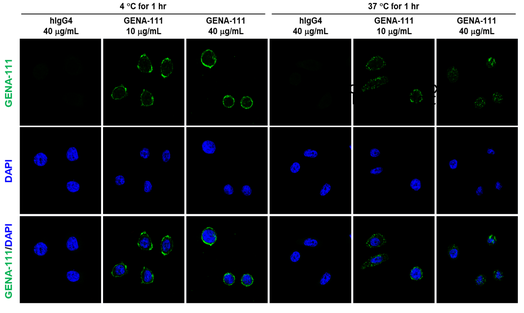
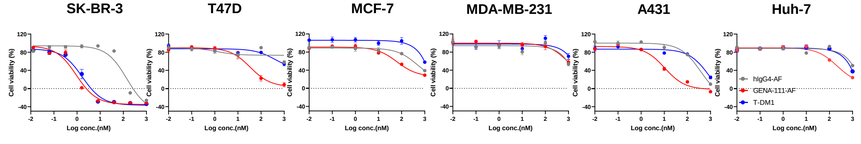


Figure 4. GENA-111 binding and internalization are observed in SK-BR-3 human breast cancer cell line. SK-BR-3 cells were incubated with hIgG4 control (40 μg/mL) or GENA-111 (10 μg/mL or 40 μg/mL) at 4°C and 37°C for 1 hr, followed by Alexa Flour®-488 conjugated secondary antibody. Sub-cellular localization of GENA-111 (green) in cells was determined by confocal microscopy (X400). The nuclei were counterstained with DAPI (blue).

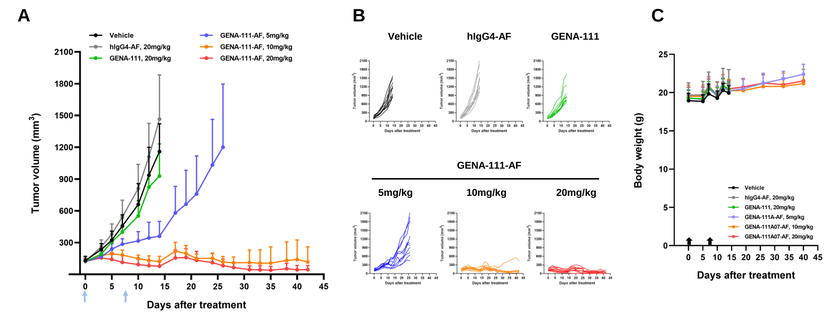
In vitro cytotoxicity of GENA-111-AF on human cancer cell lines



GI_{50} (nM)	SK-BR-3	T47D	MCF-7	MDA-MB-231	A431	Huh-7
GENA-111-AF	**0.40**	**28**	**127**	**>1,000**	**7.8**	**201**
Trastuzumab-DM1	**0.03**	**982**	**>1000**	**>1,000**	**289**	**614**
hIgG4-AF	54	>1,000	504	>1,000	216	>1,000

Figure 5. GENA-111-AF inhibits BCAM-expressing cancer cell growth with a positive correlation between cytotoxicity and BCAM level. The six human cancer cell lines (SK-BR-3, T47D, MCF-7, MDA-MB-231, A431 and Huh-7) were treated with hIgG4-AF, GENA-111-AF, and trastuzumab-DM1 at different concentrations, ranging from 10 pM to 1 μM, for 3 days. The viable cells were measured with CellTiter-Glo® assay. The GI_{50} values of each ADC on human cancer cells were determined by non-linear regression analysis. The graph of cell viability (%) are displayed as mean values ± S.D., and all experiments were performed in triplicate.

In vivo efficacy of GENA-111-AF in A431 xenograft model



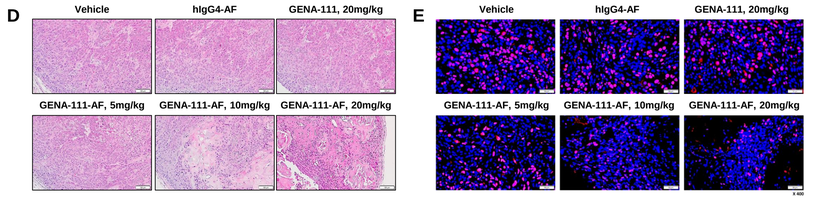


Figure 6. GENA-111-AF significantly inhibits tumor growth in A431 xenograft model. A431 cells ($5x10^6$) were injected subcutaneously into BALB/c nude mouse. Tumor-bearing mice were randomly assigned into 6 groups (n=10/group) and treated with (1) vehicle, (2) hIgG4-AF 20 mg/kg, (3) GENA-111 20 mg/kg, and (4~6) GENA-111-AF 5, 10 and 20 mg/kg by intravenous injection weekly (QW x 2, indicated by sky blue arrows in Fig. 6A). (A) Tumor growth curves were obtained by plotting mean tumor volume (± SD, mm^3) at each time point. (B) Tumor growth curves of individual mouse. (C) Body weight changes in each treatment group. (D and E) Representative H&E (D) and Ki67 (E) stainings of A431 tumor tissues collected on day 27. Scale bars, 100 μm for H&E and 50 μm for Ki67, respectively.

Summary

- GENA-111-AF is comprised of a human monoclonal BCAM antibody, GENA-111, conjugated to auristatin F using Debiopharm's Multilink™ technology and a stabilized thiol maleimide conjugation with a DAR of 4.26.
- As a carrier of the toxic drug to the cancer cells, GENA-111 binds to both recombinant BCAM protein and BCAM-positive cancer cells with high affinity and is efficiently internalized into the BCAM expressing cancer cells after binding.
- GENA-111-AF exhibits potent growth inhibitory effect on BCAM/HER2 double positive cancer cells, including SK-BR-3, T47D, MCF-7, A431 and Huh7, with a positive correlation between cytotoxicity and BCAM level. GENA-111-AF shows better efficacy against T47D, MCF-7, A431 and Huh-7 than trastuzumab-DM1.
- In A431 xenograft model, GENA-111-AF treatment on a weekly schedule (QW x 2) significantly inhibits tumor growth in a dose dependent manner. Moreover, 10 and 20 mg/kg GENA-111-AF treatment results in extensive necrosis and sustained tumor regression with decreased proliferative cancer cells.
- The present study demonstrates that GENA-111-AF has a potent anti-tumor activity, which suggests that an ADC targeting BCAM might be a promising treatment strategy for BCAM positive epithelial cancer patients.

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