Effective Collaborations Between CRO and Clients Lead to Breakthrough Innovations. Identification of New Classes of Maytansinoid Payloads for ADCs that Display *In Vivo* Activity

Francisco Velázquez[a],* Thomas Nittoli[b],* Frank Delfino[b], Marcus Kelly[b], Serena Carosso[a], Thomas Markotan[a], Arthur Kunz[b], Zhaoyuan Chen[b], Shu Mao[b], Jing Shan[b], Elizabeth Navarro[b], Feng Zhao[b], Sosina Makonnen[b], Carlos Hickey[b], Jan Spink[a], William Olson[b], Jessica Kirshner[b], Gavin Thurston[b], Nicholas Papadopoulos[b].

[a] Abzena, 360 George Patterson Boulevard, Bristol PA 19007, USA. [b] Regeneron Pharmaceuticals, Inc. 777 Old Saw Mill River Road, Tarrytown, NY 10591, USA

ABZENA + REGENERON

Introduction

Abzena is the leading Partner Research Organization for ADC research, development, and manufacturing. Having worked on hundreds of projects with leading pharmaceutical and biotech companies in the ADC field, Abzena has become an extension of its partners' research teams leading to the successful delivery of the innovative ADCs needed to address unmet medical needs.

In the R&D space, Abzena is the best equipped CRO to support research efforts in the design, synthesis and evaluation of payloads, linker-payloads, and ADCs. The close collaboration with our partners, coupled with the skill and ingenuity of our scientists, have resulted in the discovery of novel cytotoxic payloads for all different families including maytansines, auristatins, camptothecins, duocarmycins, pyrrolobenzodiazepines, and others.

The maytansine scaffold has been used to generate ADCs which are at different stages of clinical trials, including an ADC that has been approved by the FDA. Our talented team of scientists has generated several series of maytansine-based payloads through internal research efforts and in collaboration with numerous partners (Fig 1). Herein, we present the results of a successful collaboration between Abzena and Regeneron Pharmaceuticals. The main goal of the project was the design and synthesis of novel tubulin inhibitors based on the maytansine core. These payloads were designed to complement the cell impermeable amino-alkyl maytansinoids that had previously been developed. The new designs were intended to be capable of cell permeation to increase the so-called "by-stander killer effect". Payloads that showed acceptable potency profile were later attached to cleavable linkers and further conjugated to mAbs specific for MUC16 (an ovarian cancer antigen).

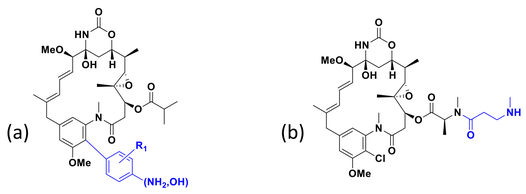
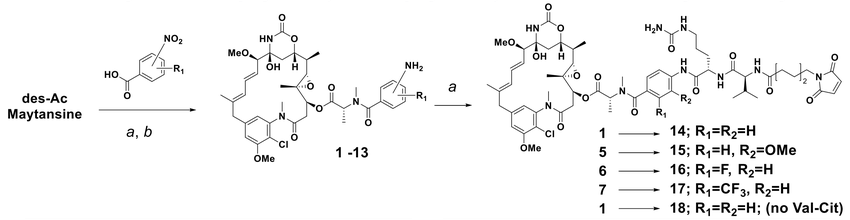


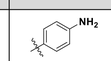
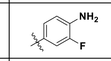
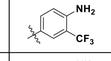
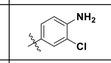
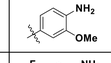
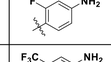
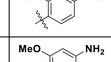
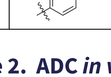
Figure 1. Novel maytansinoid payloads. (a) AP3 bi-aryl maytansinoids (see ref 1); (b) Amino–alkyl maytansinoids (see ref 2).

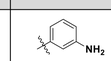
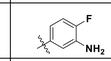
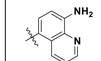
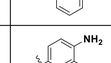
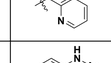
(a) AP3 bi–aryl maytansinoids
- Developed at Abzena through internal research efforts
- Abzena's proprietary scaffold
- ADC containing a maytansinoid in this class is being advanced into clinical development through collaborations

(b) Amino–alkyl maytansinoids
- New series developed in collaboration with Regeneron Pharmaceuticals
- EGFRvIII targeting ADCs were made bearing maytansinoids from this series
- Those ADCs showed excellent *in vivo* activity

Table 1. Synthesis of payloads, linker-payloads, and their *in vitro* cytotoxicity.



Cmpd #	R_1	cLogP	Ovcar3 IC_{50} (nM)
1		4.1	0.167
2		4.2	0.029
3		4.9	0.030
4		4.7	0.043
5		3.9	0.064
6		4.2	0.177
7		4.9	0.123
8		3.9	0.305

Cmpd #	R_1	cLogP	Ovcar3 IC_{50} (nM)
9		4.1	0.214
10		4.2	0.109
11		4.2	0.080
12		4.2	1.060
13		4.2	1.120
MMAE	–	3.5	0.102

Reagent and conditions: (a) HATU, DIPEA; (b) Zn, AcOH.

Table 2. ADC *in vitro* cytotoxicity (IC_{50} values).

ADC	DAR	Ovcar3 IC_{50} (nM)	HEK293 IC_{50} (nM)
3A5-mc-VC-PAB-MMAE	2.2	0.818 (100)**	>100
Isotype Control-mc-VC-PAB-MMAE	2.2	59.2 (100)	>100
3A5-**14**	1.5	1.40 (97)	>100
Isotype Control-**14**	2.8	>100	>100
3A5-**15**	1.5	1.18 (100)	>100
Isotype Control-**15**	3.1	>100	>100
3A5-**18**	1.0	>100	-
Isotype Control-**18**	1.0	>100	-
MUC16-**14**	2.0	0.796 (100)	>100
MUC16-**15**	1.6	0.828 (97)	>100
Cell Surface Expression		~176K copies	na

** Values in parenthesis are the percent of cell kill

Experimental Methods

In vitro cytotoxicity: Ovcar3 (MUC16+) and HEK293 (MUC16–) cells were seeded in 96 well plates at 3000 cells per well and grown overnight.

Conjugation and characterization: Three antibodies were conjugated to L-P generated in this study. Targeting antibodies (Regeneron generated Ab, anti-MUC16 Ab from literature, 3A5. mAbs were expressed in CHO cells. Ab (10 mg/mL) in 50 mM HEPES, 150 mM NaCl, pH 7.5, was treated with 1mM dithiothreitol at 37 °C for 30 min. L-P (10 mg/mL in DMSO) were added (1.2 eq/SH group) for 1h. Purified by SEC. >95% monomeric purity by HPLC-SEC, DAR determined by UV.

In vivo efficacy: Anti-tumor efficacy was assessed in intraperitoneal tumor model using MUC16 endogenously expressing OVCAR-3 cells transfected with luciferase. 1 x 10^6 OVCAR3/luc cells were implanted IP. Day 5 post implantation, IV dosed 5 mg/kg with ADCs. All doses were monitored by detection of tumor bioluminescence signal.

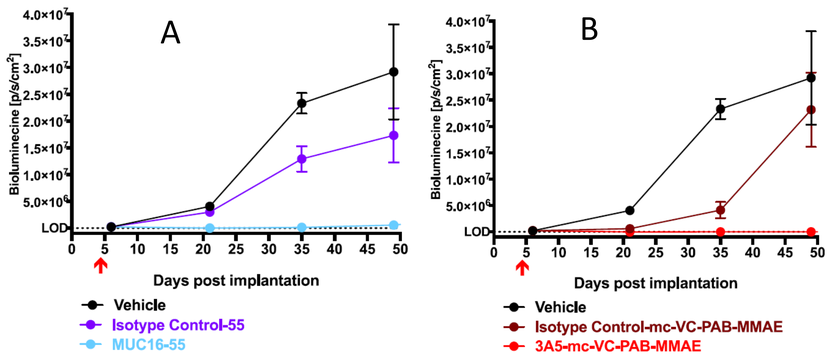


Figure 2. In vivo efficacy of MUC16 targeting ADCs in the Ovcar3 tumor model. Graph A shows the MUC16-**55** ADC and graph B shows the 3A5-mc-VC-PAB-MMAE ADC dosed at 5 mg/kg (in each study with the exact dosing for control) in SCID mice.

Conclusions

The successful execution of this project demonstrated how a close collaboration between Abzena and its partner Regeneron delivered a new series of maytansine-based cytotoxic payloads. Regeneron's designs were carefully executed by Abzena's scientist to make ADCs that displayed *in vivo* activity in the Ovcar3 tumor model. Interestingly, the novel Muc16 targeting ADC was able to suppress tumors with similar efficacy as the clinical positive control (mc-VC-PAB-MMAE).

References

1. Jain, N.; Ghone, S.; Smith, S.; Glassford, I.; Degrado, S.; Kang, F.; Zhao, S. **2018**, *Novel Cytotoxic Agents and Conjugates Thereof*; WO 2018051109.
2. Nittoli, T.; Kelly, M.; Delfino, F.; Rudge, J.; Kunz, A.; Markotan, T.; Spink, J.; Chen, Z.; Shan, J.; Navarro, E.; Tait, M.; Provoncha, K.; Giurleo, J.; Zhao, F.; Jiang, X.; Hylton, D.; Makonnen, S.; Hickey, C.; Kirshner, J.; Thurston, G.; Papadopoulus, N. *Bioorg. Med. Chem*. **2018**, *26*, 2271 – 2279.