ABSTRACT
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Unique Pharmacological Properties of Dolaflexin-Based ADCs- A Controlled Bystander Effect.

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Abstract

Antibody-drug conjugates (ADCs) are designed to bind tumor-associated antigens and deliver conjugated cytotoxic payloads to antigen-positive cells. Some ADCs also kill neighboring cells (including antigen-negative cells) by a mechanism referred to as the bystander effect. This effect can be beneficial when the antigen has heterogeneous expression among cells in a solid tumor, but it can also increase off-target toxicity of ADCs. Herein, we report on a unique pharmacological property of the Dolaflexin platform which provides a controlled bystander effect that retains the benefits of the bystander effect with respect to anti-tumor cytotoxicity but reduces the off-target toxicity.

The controlled bystander effect, termed “DolaLock”, was achieved through design of a payload, auristatin F-hydroxypropylamide (AF-HPA), that is membrane-permeable and capable of bystander killing but is further catabolized to membrane-impermeable auristatin F (AF). This catabolism of the payload “locks” the highly potent AF in the cell. Using Dolaflexin-based ADCs, we investigated the extent of intracellular AF-HPA and AF release, tumor cell retention and bystander activities *in vitro* and *in vivo*. We observed both auristatin species within cells. Co-culture assays with HER2-positive and HER2-negative cells confirmed the cell permeability and bystander killing capabilities of AF-HPA released from a Dolaflexin-based ADC. Biodistribution studies of Dolaflexin-based ADCs revealed time-dependent concentrations of AF-HPA and AF as well as significant accumulation of AF in xenografted tumor cells, consistent with the Dolalock mechanism. An additional benefit of AF formation was seen in multidrug resistant transporter studies which demonstrate that AF, in contrast to AF-HPA, is not a P-glycoprotein 1 (Pgp) substrate. This property may offer additional benefit in Pgp-expessing tumors.

In summary, we have shown that the proprietary AF-HPA payload used in the Dolaflexin platform allows for a controlled bystander effect which likely contributes to the enhanced efficacy and lack of neutropenia we have observed with Dolaflexin-based ADCs in nonclinical models.

DolaLock – Controlled Bystander Effect

Targeted Delivery

- Intracellular drug release in antigen-expressing cells

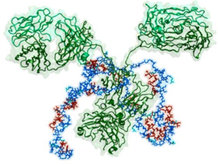
Bystander Killing

- AF-HPA diffusion to antigen-negative cells
- Greater efficacy.

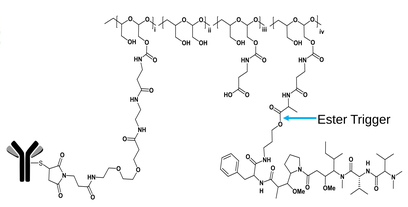
No Bystander Killing

- AF is not cell membrane permeable
- Greater tolerability.

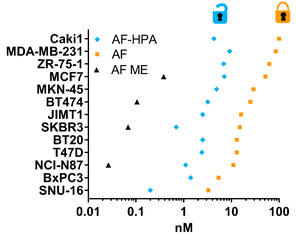
The Structure of a Dolaflexin ADC



Green = mAb
Blue = Fleximer polymer
Red = Auristatin F-HPA payload

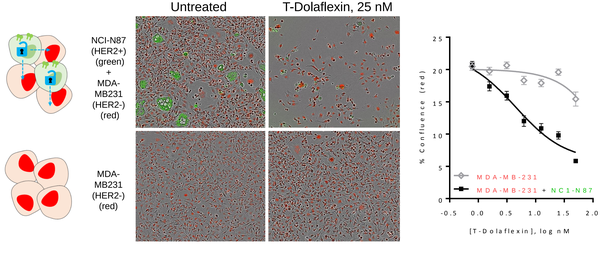


AF is Highly Potent When Formed Intracellularly

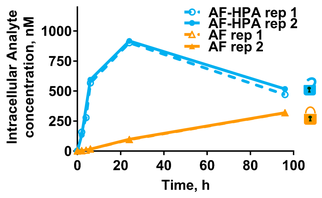


- The cell permeable, AF-HPA, exhibits potent toxicity across a broad range of cancer cells.
- AF has limited cell permeability resulting in a decrease in potency in cell based assays.
- The methyl ester (ME) derivative of AF restores cell permeability; demonstrating the true potency of the released AF.

Trastuzumab-Dolaflexin Exhibits Bystander Killing

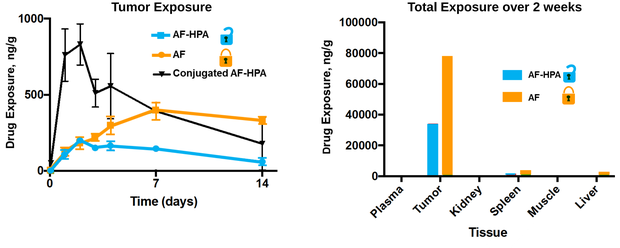


AF-HPA is Converted into AF within Tumor Cells



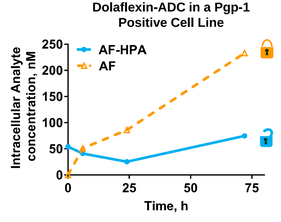
- At appropriate timepoints, N87 cells treated with 150 nM AF-HPA were collected and intracellular metabolites were identified by LC-MS.
- The intracellular formation and retention of AF is consistent with DolaLock.

AF Accumulates in Tumors in Vivo



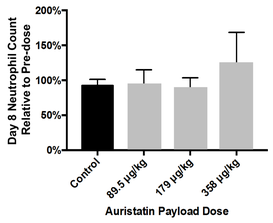
AF is Not a Substrate of Pgp-1 Drug Efflux Pump

Test Article	Direction	Efflux Ratio	Pgp substrate classification
AF	A-to-B	1.8	Negative
	B-to-A		
AF + 1 uM Valspodar	A-to-B	1.4	
	B-to-A		
AF-HPA	A-to-B	32	Positive
	B-to-A		
AF-HPA + 1 uM Valspodar	A-to-B	4.7	
	B-to-A		



- Even in the presence of a major drug efflux pump, AF is still locked in the cell.

Dolaflexin ADCs Show No Neutropenia



- No Neutropenia was observed in NHP treated with Dolaflexin ADC, even at twice the auristatin dose associated with fatal neutropenia.
- Neutropenia is a major nonclinical and clinical toxicity of vc-MMAE cleavable auristatin
- vc-MMAE exhibits neutropenia in NHP at auristatin payload doses **> 60 µg/kg**[1,2].

Discussion and Conclusions

- The controlled bystander effect, termed “DolaLock”, was achieved with a proprietary auristatin derivative by incorporating a hydrolysable amide (AF-HPA) to allow for intracellular metabolism to the corresponding carboxylate (AF).
  - AF-HPA is cell permeable and can cause the bystander killing observed in co-culture assays.
  - AF is highly potent when formed intracellularly but has limited cell permeability.
- Treatment of N87 cells with AF-HPA resulted in the intracellular formation and retention of AF.
- Multi-drug resistance transporter studies demonstrated the AF, in contrast to AF-HPA, is not a P-glycoprotein 1 substrate. This was confirmed in a P-glycoprotein positive cell line, where AF continued to accumulate while AF-HPA was effluxed.
- Bio-distribution studies of a Dolaflexin-based ADC revealed the in vivo formation of AF-HPA and AF and significant accumulation of AF in xenografted tumor cells, consistent with the DolaLock mechanism.
- The proprietary auristatin payload, AF-HPA, used in the Dolaflexin platform, results in a controlled bystander effect which contributes to the enhanced efficacy and lack of neutropenia observed with Dolaflexin-based ADCs in nonclinical models.

References

1. *De Goeij BE, Lambert JM. New developments for antibody-drug conjugate-based therapeutic approaches. Curr Opin Immunol 2016 40:14-23.*
2. *Lin K, Rubinfeld B, Zhang C, Firestein R, Harstad E, Roth L, Tsai SP, Schutten M, Xu K, Hristopoulos M, Polakis P. Preclinical development of an anti-Napi2b (SLC34A2) antibody-drug conjugate as a therapeutic for non-small cell lung and ovarian cancers. Clin Cancer Res 2015 21):5139-50.*