Developing a CD71-Targeting Probody™ Drug Conjugate (PDC) for Activity in Multiple Solid Tumor and Lymphoma Models and for Tolerability in Non-human Primates

Shweta Singh[1], Laura Serwer[1], Niharika Chauhan[2], Amy DuPage[1], Michael Krimm[1], Ken Wong[1], Yuanhui Huang[1], Andrew Jang[1], Eric Ureno[1], Adam Miller[1], Sarah Patrick[1], Shanti Duvur[1], Fritz Buchanan[2], Matthew Ravn[2],
Rob Leanna[2], Ilaria Badagnani[3], Tracy Henriques[2], Shouchun Liu[1], Luc Desnoyers[1], Claus Krebber[1], Sridhar Viswanathan[1], Jennifer Richardson[1], Susan Morgan-Lappe[2] and W. Michael Kavanaugh[1]
[1]CytomX Therapeutics, Inc., South San Francisco, CA; [2]AbbVie, North Chicago, IL, [3]Former Abbvie Employee

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ABSTRACT

Probody™ therapeutics are antibody prodrugs designed to remain largely inactive until proteolytically activated in the tumor microenvironment (TME), potentially enabling the safer targeting of antigens that are highly expressed in both tumor and normal tissue. CD71 (transferrin receptor) is an example of an ideal Probody Drug Conjugate (PDC) target not only because it efficiently internalizes and can deliver a cytotoxic payload intracellularly, but also because it is expressed at high levels both in many different tumor types as well as in dividing normal cells. We have previously demonstrated that while an anti-CD71 antibody drug conjugate (ADC) is highly toxic, a CD71-targeting PDC is both efficacious in mouse tumor models and well tolerated in non-human primates.

Two key components of a Probody therapeutic prodomain that reduce its binding to normal tissue and allow for its tumor-specific activation are 1) a mask that reduces the ability of the antibody binding site to interact with target antigen and 2) a protease-activatable substrate that is cleaved in the TME resulting in removal of the mask. Here, we demonstrate how modulating mask strength and substrate cleavability can impact efficacy and safety of a CD71-targeting PDC in preclinical models. Through this process, we have selected a lead molecule, CX-2029, for further development. CX-2029 is a CD71 targeting PDC conjugated to vcMMAE with a Drug to Probody Ratio (DPR) of 2, achieved by purification.

At comparable dose levels, a more strongly masked PDC, CX-2018, was less efficacious in a mouse xenograft tumor model compared to PDC CX-2016, which has a weaker mask, demonstrating that mask strength affects anti-tumor activity. Further, PDC CX-2019, which has the same mask but a less cleavable substrate than CX-2016, was similarly efficacious in a mouse xenograft tumor model, demonstrating that both substrates are sufficiently cleaved in the TME to activate the PDCs. However, CX-2019 was better tolerated in NHP at 6 mg/kg than CX-2016, suggesting that the less cleavable substrate in CX-2019 leads to a better therapeutic index. Using a LC/MS/MS method, we showed lower levels of circulating activated CX-2019 compared with circulating activated CX-2016, which is consistent with CX-2019's improved tolerability. Lead CX-2029 contains the same mask and substrate as CX-2019 but differs in having a DPR of 2 versus ~3 for CX-2019. Up to 6 mg/kg of CX-2029 as a single dose produced complete regressions and durable responses in mouse xenograft tumor models encompassing multiple indications, and was tolerated in monkeys at doses of up to 12 mg/kg.

These data demonstrate that, in preclinical models, changes in mask strength and substrate cleavability can enhance the efficacy and tolerability of Probody Therapeutics and have the potential to enable the safe and effective targeting of highly expressed tumor antigens like CD-71. CX-2029 is currently under development, with an IND filing expected in 2018.

CD71 BACKGROUND

- CD71, TfR, TRFC (transferrin receptor) is a transmembrane glycoprotein that exists as a 180 kDa homodimer whose function is to primarily bind transferrin
- The receptor is continuously re-cycled through endocytosis
- CD71 is ubiquitously expressed on dividing cells
- CD71 is highly expressed in almost all primary and metastatic cancers evaluated

CD71 is Highly Expressed in Many Metastatic Cancers

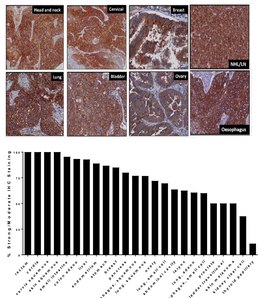


Figure 1: Panel of IHC images represent strong staining in the stated indications. Graph represents 2/3+ staining at metastatic sites in patient tumors.

Potent *in vitro* Cytotoxicity of the parental CD71 ADC in Multiple Tumor Cell Types

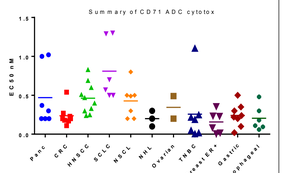
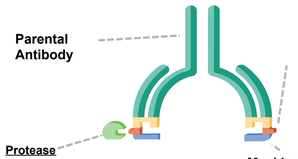


Figure 2: EC_{50} values represented for 5 day cell death assay across multiple cell lines where each point represents one cell line. A fully activated CD71 PDC shows the same activity as the ADC.

Figure 3: Probody Therapeutics are Protease-Activatable Antibody Prodrugs



- Probody therapeutics mitigate on-target toxicity by minimizing target binding in normal tissues
- CD71 is an attractive but undruggable ADC target due to lethal on-target toxicity
- We have developed a PDC, CX-2029, which enables the safe and effective targeting of CD71
- CX-2029 was selected based on mask strength and substrate cleavability of the Probody Tx

LEAD SELECTION

Test Article	MMAE Conjugate	Mask Strength	Substrate cleavability	DPR or DAR
CX-2018	PDC	Higher	More	~3
CX-2016	PDC	Lower	More	~3
CX-2019	PDC	Lower	Less	~3
CX-2029	PDC	Lower	Less	~2
CX-2030	ADC	NA	NA	~2

Table 1. Description of molecules presented. CX-2029 has the same mask and substrate as CX-2019 but differs in having a DPR of ~2 versus ~3 for CX-2019. CX-2030 is a DAR 2 purified ADC.

DPR or DAR; drug to Probody or antibody ratio

Higher affinity mask is less efficacious than a lower affinity mask

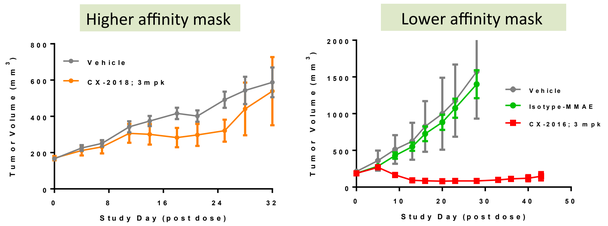


Figure 4: Single dose of CX-2018 and CX-2016, at 3mpk in HT-29 cell line derived xenograft (CDX). The lower affinity mask PDC gives complete regressions and the high affinity mask results in marginal TGI after a single dose. Both CX-2018 and CX-2016 have the same, more cleavable, substrate. Same result were observed with a less cleavable substrate (data not shown).

A less cleavable substrate is equally efficacious than a more cleavable substrate

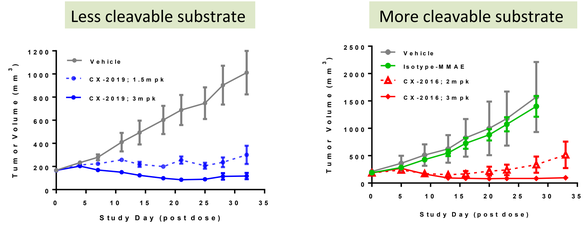


Figure 5: Single dose of CX-2016 or CX-2019 (same, lower affinity, mask) are equally efficacious in HT-29 CDX. Note that the contribution of substrate to efficacy is less apparent in the context of this mask.

Substrate cleavability and DPR influence tolerability of CD71 PDCs in cynomolgus monkeys

Test Article/ DPR or DAR	Dose mpk	*Body weight loss	*Average ^Neutrophil count +/- SD (per ul)	Relative stability of Probody Tx in circulation	Tolerability
CX-2016 DPR~3	6 mpk	~10%	98 ± 60	Lower	Not tolerated
CX-2019 DPR~3	6 mpk	~8%	290 ± 290	Higher	Tolerated
CX-2029 DPR~2	12 mpk	0%	180 ± 210	Higher	Tolerated
CX-2030 DAR~2	2 mpk	~8%	70	NA	Not tolerated

Table 2: Cynomolgus monkeys were treated IV, scheduled q3wx2, with CD71 PDCs (n=2). The same, lower affinity, mask with different substrates or different DPR format or an E2 containing ADC (n=1) were dosed. Only one dose was tolerated for CX-2016 and only one dose was administered for the ADC. Stability of Probody Tx was measured using an LC/MS/MS method to monitor intact vs. total Pb (**visit poster B103**)
*Lowest measured value after 1st dose ^baseline neutrophil count ≥1000/ul

- CD71-ADC demonstrates lack of tolerability within 8 days of a single dose of 2 mpk of DAR 2 purified ADC
- CX-2019 is better tolerated and is more stable in circulation in cyno than CX-2016
- CX-2029 demonstrates improved tolerability when administered at a higher dose compared to CX-2019

CX-2029 is as highly and equally efficacious as CX-2019 in multiple CDX models

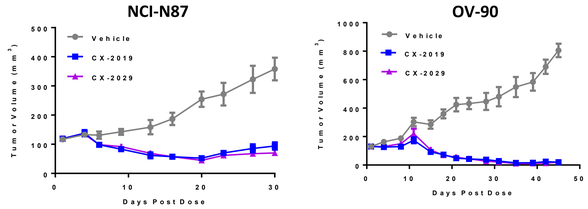


Figure 6: Examples of single dose of 3mpk CX-2019 or CX-2029, at a toxin matched dose, resulted in regressions in the above CDX models.

CX-2029 is as highly efficacious in multiple CDX and PDX models

Tumor Type	Cell line or Patient derived xenograft	Regressions or Stasis	Growth Inhibition	No response
Gastric	CDX	1		
Ovarian	CDX	2	1	
MM	CDX		1	
Prostate	CDX	1		
Mesothelioma	CDX		1	
Colorectal	CDX	2		1
Ovarian	CDX	2	1	
Esophageal	CDX	3	1	
	PDX	2		
NSCLC	CDX	3	1	
	PDX	2	1	1
Breast	CDX	3	2	
	PDX	3		
DLBCL	PDX	4	1	
HNSCC	PDX	2		
Total	***CDX***	***17 (65%)***	***8 (31%)***	***1 (4%)***
	PDX	***13 (81%)***	***2 (13%)***	***1 (6%)***

Table 3. More than 40 CDX or PDX models were tested; CDX models were agnostically run with no prior evaluation of CD71 expression. No correlation between CD71 expression and efficacy was observed in these models. PDX models were selected for high CD71 expression. Overall, >90% of models responded, resulting in regressions/ stasis or tumor growth inhibition. Protease activation status unknown in these models. Dosing schedules varied: q2wx2, q1wx2 or single dose at ≤6mpk.

SUMMARY/CONCLUSIONS

- Probody Therapeutics have the potential to enable the safe and effective targeting of highly expressed tumor antigens, regardless of expression on normal tissue, like CD71, thus expanding the utility of ADCs
- We have developed an efficacious and well tolerated PDC targeting CD71, CX-2029, by modifying mask, substrate and drug Probody ratio (DPR) properties
  - The lower affinity mask significantly improved efficacy in mouse tumor models
  - The less cleavable substrate resulted in improved tolerability in NHP and improved stability of circulating Probody therapeutics without compromising efficacy in mouse tumor models
  - The lower DPR 2 purified PDC resulted in improved tolerability in NHP whilst maintaining potent activity in mouse tumor models
- CX-2029 was better tolerated in monkeys than the parental ADC with matching DAR, which was not tolerated after a single dose at 2 mg/kg
- CX-2029 is currently under development with an IND filing expected in 2018