Preclinical Development of a Probody™ Drug Conjugate (PDC) Targeting CD71 for the Treatment of Multiple Cancers

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ABSTRACT

The targets of Antibody Drug Conjugates (ADCs) have typically been selected by identifying transmembrane antigens that are highly expressed in tumors but are low or absent in normal tissues. The number of potential ADC targets meeting these requirements is limited, either because expression in tumors is not high enough for optimal efficacy, or because expression in normal tissues is too high, leading to toxicity. Probody therapeutics are antibody prodrugs designed to remain inactive until proteolytically activated in the tumor microenvironment. Probody technology therefore has the potential to enable targeting of more desirable tumor antigens with higher, more persistent and more homogeneous expression in tumors, while limiting toxicity due to interaction with these antigens in normal tissues.

CD71 (transferrin receptor) is an example of a highly desirable ADC target, because of its well-characterized ability to efficiently internalize and deliver an ADC payload intracellularly. Further, CD71 is expressed at homogeneously high levels (3+ expression by IHC) in almost all tumor types, including in metastatic disease. However, because CD71 is also expressed on multiple normal cell types, including many progenitor hematological cells, we reasoned that a CD71-targeted ADC would be challenging to develop. To enable targeting of CD71, we have developed an anti-CD71 Probody Drug Conjugate (PDC), which can be activated by multiple proteases in the tumor microenvironment, but which remains in a relatively inactive form while in circulation and in normal tissues. The CD71 PDC produces complete tumor regressions at therapeutic doses in mouse models of lymphoma, breast cancer and lung cancer. Consistent with our hypothesis that it would be difficult to develop an anti-CD71 ADC, treatment of cynomolgus monkeys with an anti-CD71 ADC at doses that were efficacious in mouse tumor models caused life-threatening depletion of CD71-expressing hematopoietic cells, including neutrophils, lymphocytes and RBCs. In contrast, these toxicities were not observed in monkeys treated with the same dose of the anti-CD71 PDC, consistent with the Probody therapeutic avoiding interaction with these normal cells.

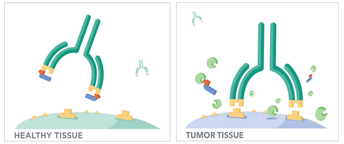
Our data demonstrate that, in preclinical studies, Probody drug conjugates can safely and effectively target attractive tumor antigens like CD71 which have been difficult to address with traditional ADCs due to their expression on critical normal tissues. Further, our data support the development of Probody therapeutics directed against CD71 in multiple different cancers.

BACKGROUND: CD71

- Ubiquitously expressed on dividing, normal (hematological precursors) and malignant cells
- Mediates iron uptake required for cell division: cannot be down regulated by tumors
- A professional internalizing protein: often used as a positive control in ADC experiments
- Expression in normal dividing cells prohibits development of a traditional ADC

INTRODUCTION

Probody Therapeutics are Designed to Bind to Tumors & Protect Healthy Tissue



Masking Peptide
- Reduces antigen binding
- Released from Probody therapeutic upon cleavage of substrate linker, producing a fully active antibody

Protease
- Dysregulated activity of specific proteases in cancer tissue
- Cleaves mask to enable Probody therapeutic binding

Substrate Linker
- Stable *in vivo* in circulation
- Preferentially cleaved by tumor proteases

Probody Drug Conjugates are Designed to Target a Densely Concentrated Tumor Antigen, Regardless of Expression on Normal Tissue

		ADCs	PDCs
Desired Properties	Expression in healthy tissue	Low	Low or High
	Expression of target in tumor	High	High
Resulting Properties	Homogeneity of expression in tumor	Often Modest	High
	Tumor types addressed	One/Few	Many
	Prevalence per cancer type	Lower	Very High

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RESULTS

Figure1: Comparable Binding by Antibody to Human & Cynomolgus CD71

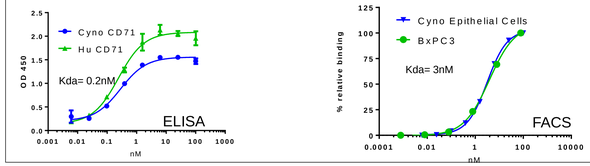
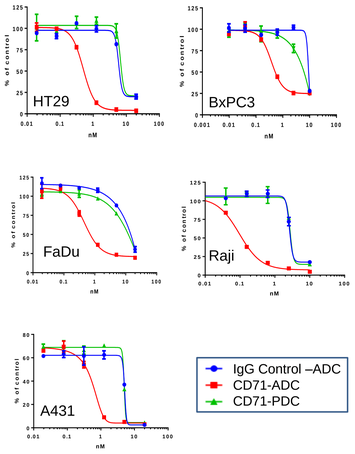


Figure 2: Potent *in vitro* Cytotoxicity of CD71 ADC in Multiple Tumor Cell Types



		EC50 (nM)
Pancreatic	Miapaca2	0.2
	HPAF 2	0.2
	BxPC3	0.37
CRC	SW480	0.11
	SW1417	0.17
	HT29	0.18
	Ls411N	0.2
	SW48	0.2
	Ls174T	0.23
	Lovo	0.27
	HCT-116	0.54
	DLD1	1.76
HNSCC	SCC25	0.24
	BHY	0.25
	SAS	0.33
	Fadu	0.40
	KYSE 150	0.47
	SCC1	0.51
	KYSE 70	0.6
	SCC9	0.69
Lung	A253	0.83
	A431	0.22
	NCI H292	0.3
	Detroit 562	0.3
	A549	0.49
	NCI H2141	0.5
	NCI-H69	0.57
	NCI H526	0.7
	HS766T	1.02
	NCI H727	1.29
	NCI H889	1.3
NHL	Ramos	0.2
	Raji	0.3
Ovary	OVCAR3	0.20
	ET201	0.49
Breast	HCC1806	0.2
	MDA MB231	1.1

Figure 3: CD71 is Highly Expressed in Many Metastatic Cancers

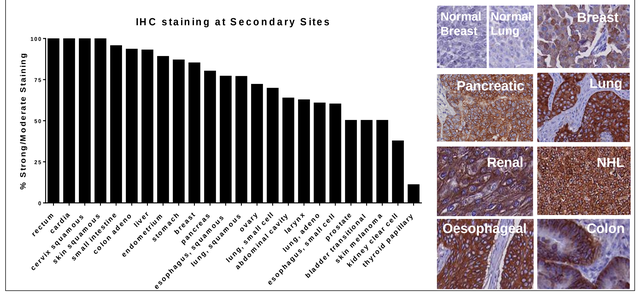


Figure 4: CD71 PDC Renders Complete and Durable Responses in Human Xenograft Tumors At or Below Clinically Relevant Doses

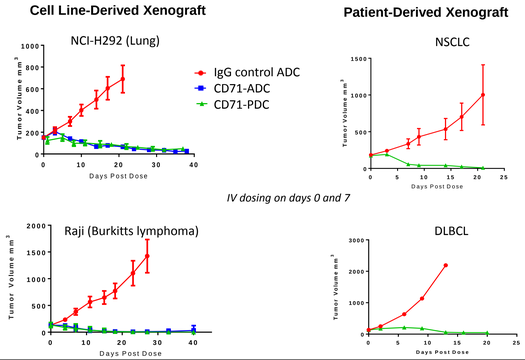
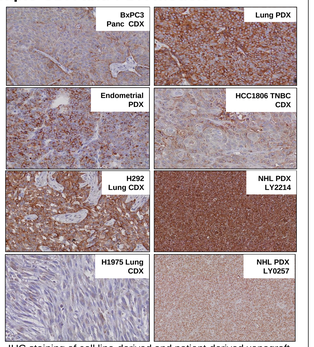


Figure 5: CD71 PDC is Efficacious in Multiple CDX and PDX models

Model	CD71
Pancreatic BxPC3	CR
Endometrial PDX	PR
Lung H292	CR
Lung H1975	PR
Lung PDX	CR
TNBC HCC1806	CR
NHL Raji	CR
NHL PDX 2214	CR
NHL PDX 0257	CR

CR complete response; PR partial response



IHC staining of cell line-derived and patient-derived xenograft models run with our CD71-ADC and or CD71 PDC

Figure 6: CD71 Probody Therapeutic Shows Extended Exposure in Cynomolgus Monkeys, Consistent with Reduced Binding in Normal Tissues

- ELISA PK assay shows increased exposure of CD71 Probody therapeutic (total IgG) compared with CD71 Ab (single dose, 5mpk, i.v., n=2)
- Extended exposure is consistent with CD71 Probody Tx avoidance of the antigen sink in normal tissues

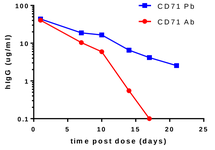
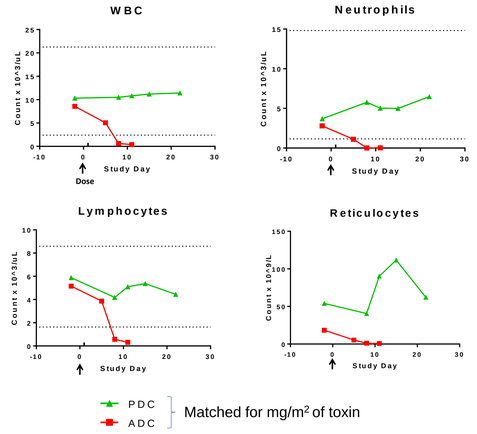


Figure 7: CD71 PDC Safety in Cynomolgus Monkeys at a Clinically Relevant Dose



- Cynomolgus monkeys were treated IV with a single dose of CD71 ADC (n=1) or PDC (n=2)
- ADC-treated monkey demonstrated life-threatening hematological toxicity predicted by CD71 expression profile
- PDC was well-tolerated at a toxin-matched dose. No treatment-related adverse effects were observed

SUMMARY/CONCLUSIONS

- PDCs have the potential to safely target highly expressed tumor antigens, regardless of expression on normal tissue, thus expanding the utility of ADCs
- We have developed a PDC targeting CD71, a highly expressed antigen present in many cancers but also in hematologic precursors and other normal tissues
- CD71 PDC is efficacious in cell line-derived and patient-derived xenograft tumors at doses equal to or below the predicted clinical therapeutic dose
- In monkeys, CD71 PDC protects against leukopenia and severe toxicity associated with the ADC
- CD71 Probody PK is consistent with protection from binding to normal monkey tissue sink
- The safety and efficacy profiles of CD71 PDC are supportive of further development