In vivo Efficacy of a Novel Peptide-Conjugated Drug in Patient-Derived Xenograft Models of Breast Cancer

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INTRODUCTION

- Peptide-drug conjugates (PDCs) are a novel class of agents entering clinical development for solid tumors.
- Novel targets are being evaluated to optimize payload delivery to achieve maximum tumor cytotoxicity while minimizing clinical toxicity.
- Sortilin (*SORT1*) is a member of the Vacuolar Protein Sorting 10 protein (Vps10p) family of receptors which regulate peptide trafficking between the plasma membrane and lysosomes. It is also able to mediate efficient endocytosis of extracellular ligands (Figure 1).
- *SORT1* is frequently over expressed in breast cancer; overexpression is associated with aggressive disease biology.[1,2]
- ARB-1-6 (ProteinQure) is a computationally designed sortilin-engaging peptide conjugated to the cytotoxic agent monomethyl auristatin E (MMAE) which has demonstrated pre-clinical activity and tolerability in cell line xenografts of triple negative breast cancer.[3]
- The anti-tumor activity of ARB-1-6 in patient-derived xenografts (PDX) of breast cancer is unknown.

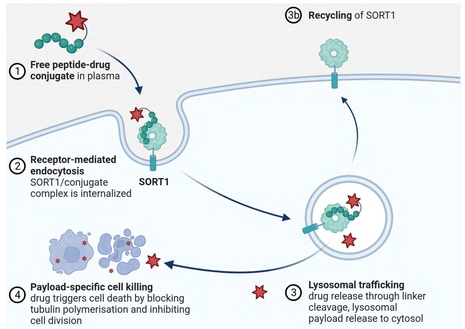


Figure 1. Sortilin-engaging PDC Mechanism of Action.

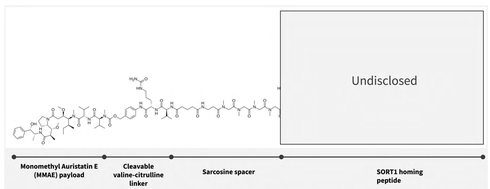


Figure 2. Chemical Structure of ARB-1-6, a Sortilin Engaging PDC. Sortilin engaging homing peptide is conjugated to a Monomethyl Auristatin E (MMAE) payload via a cleavable valine-citrulline linker.

METHODS

- Breast cancer PDX models were generated and propagated under an IRB approved protocol at the Princess Margaret Cancer Centre [14-8358].
- ARB-1-6 and Scrambled PDC Control (ARB-1-6 biological control, non-binding peptide conjugated to MMAE) was dosed intravenously in tumor-bearing SCID mice by tail vein injection at 3 mg/kg weekly for 4 weeks.
- Animals were assigned to treatment in a modified n=1 protocol when tumors reached 100-200 mm^3.[3,4]
- Tumor volume was measured twice weekly until day 28-30 or until models reached humane endpoints, after which tumors were collected.
- Animals were weighed and assessed for treatment-related toxicity regularly.

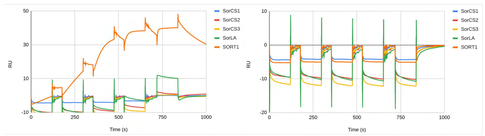
METHODS

- Immunohistochemistry (IHC) optimization was performed on historical formalin-fixed, paraffin-embedded blocks representing models with high and low *SORT1* expression based on RNA-seq.
- A commercial antibody (ProteinTech, 12369-1-AP) was used per manufacturer guidelines and a standard TRIS-EDTA protocol for IHC assessment of sortilin staining intensity.
- PDX response assessment was calculated using XEVA DB.[5]
- Data presented includes efficacy assessment as of March 1st, 2024.

RESULTS

- 92 individual animals from 16 unique PDX models have been treated in this cohort [33 ARB-1-6, 23 Scrambled PDC control, 36 H2O]
- PDX models derived from patients with triple negative (TNBC) and estrogen receptor-positive/HER2-negative (ER+) breast cancer were prioritized for treatment.

A. ARB-1-6: **B. Scrambled PDC:**



C.

Test Compound	ARB-1-6	Scrambled Control
Receptor		
Sortilin (K_D, nM)	0.42	>10,000
SorCS1 (K_D, nM)	>10,000	>10,000
SorCS2 (K_D, nM)	>10,000	>10,000
SorCS3 (K_D, nM)	>10,000	>10,000
SorLA (K_D, nM)	>10,000	>10,000

D.

Figure 3. ARB-1-6 Binds to Sortilin and is Trafficked to the Lysosomes. Surface plasmon resonance (SPR) results of (A) ARB-1-6 and (B) scrambled PDC control interaction with sortilin. (C) Interaction results summary with representative equilibrium dissociation constant (K_D). ARB-1-6 binds to sortilin with high affinity **(K_d=0.42 nM)**. (D) ARB-1-6 localization. MDA-MB-231 cells were stained with AF488 labeled ARB-1-6 (green), lysotracker (red), and hoechst nuclear stain (blue) for 2 hours. Intracellular trafficking and lysosomal colocalization of ARB-1-6 probe was observed in agreement with the proposed mechanism of action.

A.

PDX Model	Clinical Receptor Subtype	Treatments Received by Patients Prior to PDX Generation	
		(Neo)adjuvant	Metastatic
PDX-1	TNBC	Dose-dense AC-Taxol	None
PDX-2	TNBC	FEC-D	Gemcitabine and Carboplatin
PDX-3	TNBC	None	None
PDX-4	ER+	None	None
PDX-5	ER+	TC, Tamoxifen, Letrozole	Capecitabine, Gemcitabine and Cisplatin, TROP2-ADC (topoisomerase inhibitor)
PDX-6	TNBC	Dose-dense AC-Taxol	None
PDX-7	TNBC	None	None
PDX-8	TNBC	None	None
PDX-9	TNBC	Dose-dense AC-Taxol	None
PDX-10	TNBC	None	None
PDX-11	TNBC	Dose-dense AC-Taxol	None
PDX-12	ER+	FEC-D, Letrozole	Fulvestrant and Palbociclib, Phase I BET-inhibitor
PDX-13	TNBC	Dose-dense AC-Taxol	None
PDX-14	ER+	None	None
PDX-15	TNBC	Dose-dense AC-Taxol, Capecitabine	None
PDX-16	TNBC	KEYNOTE-522, Capecitabine	None

B.

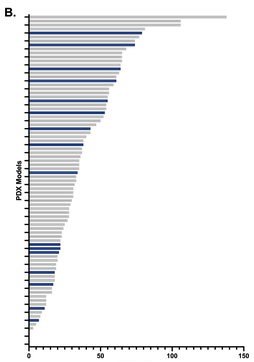


Figure 4. Characteristics of PDX Models. (A) PDX model characteristics including previous treatment(s) received by patients prior to PDX generation. (B) *SORT1* expression amongst all profiled PDX (RNA-seq). Models included in this cohort are highlighted in blue.

RESULTS

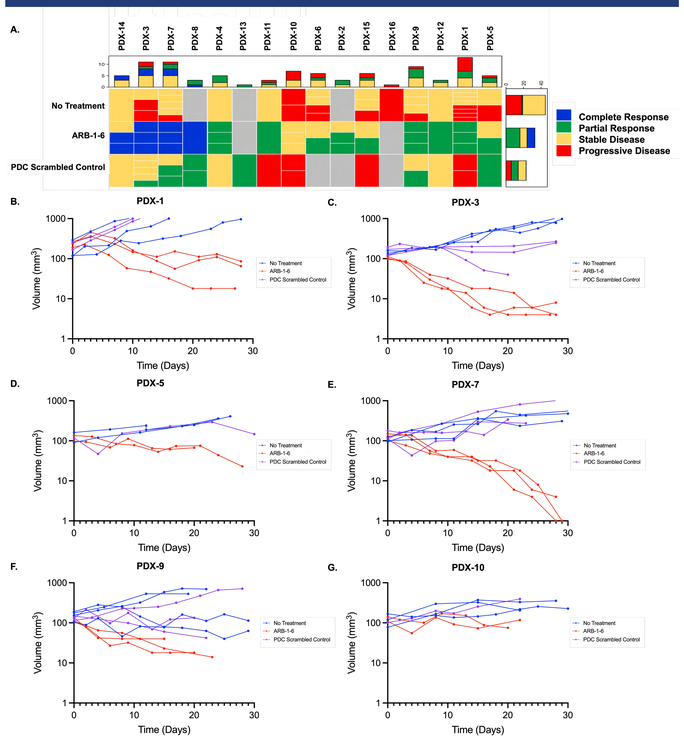


Figure 5. PDX Response Metrics to ARB-1-6 and Controls. (A) mRECIST summary of individual animal and model response to H2O, ARB-1-6, and PDC Scrambled control. (B-G) Representative growth curves of 6 models included in the study with n≥2 biological replicates. Y-axis plotted in log scale. Blue = No Treatment, Red = ARB-1-6, Purple = PDC Scrambled Control.

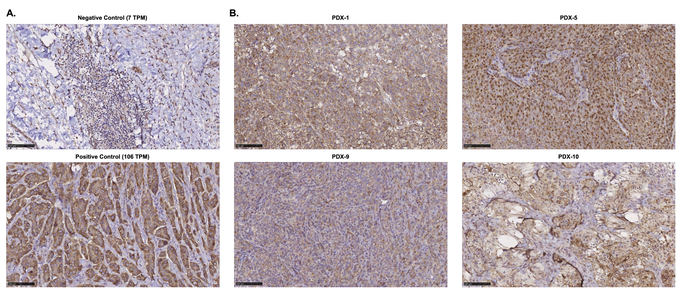


Figure 6. Sortilin IHC Optimization. (A) Representative images of negative and positive control blocks for antibody optimization for the assessment of sortilin staining via IHC (ProteinTech, 12369-1-AP). (B) Representative images of sortilin staining as assessed by IHC on donor PDX tumors across models of interest. *SORT1* expression, RNA-seq: PDX-1 (38 TPM), PDX-5 (42 TPM), PDX-9 (11 TPM), PDX-10 (8 TPM).

RESULTS

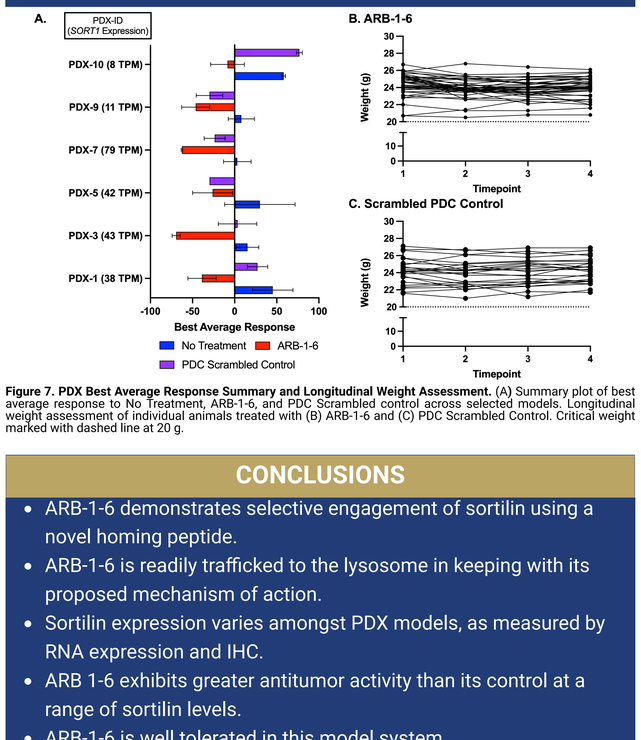


Figure 7. PDX Best Average Response Summary and Longitudinal Weight Assessment. (A) Summary plot of best average response to No Treatment, ARB-1-6, and PDC Scrambled control across selected models. Longitudinal weight assessment of individual animals treated with (B) ARB-1-6 and (C) PDC Scrambled Control. Critical weight marked with dashed line at 20 g.

CONCLUSIONS

- ARB-1-6 demonstrates selective engagement of sortilin using a novel homing peptide.
- ARB-1-6 is readily trafficked to the lysosome in keeping with its proposed mechanism of action.
- Sortilin expression varies amongst PDX models, as measured by RNA expression and IHC.
- ARB 1-6 exhibits greater antitumor activity than its control at a range of sortilin levels.
- ARB-1-6 is well tolerated in this model system.
- ARB 1-6 demonstrates substantial anti-tumor activity in breast cancer PDX models, supporting clinical development in this indication.

FUTURE DIRECTIONS

- Treatment of additional animals is underway to complete biological and technical replicates across all models.
- *SORT1* expression is being assessed between PDX passages to assess the stability of target expression.
- Antitumor activity of ARB-1-6 is being assessed in models with acquired resistance to Sacituzumab Govitecan.
- Clinical development of ARB-1-6 is planned.

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