Biological Basis for the Development of CPL410005 as an Antibody-Drug Conjugate

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INTRODUCTION

CPL410005 is a potent UBA1 inhibitor with strong anti-cancer activity in vitro. However, its systemic toxicity and poor tumor selectivity limit its therapeutic potential as a standalone small-molecule drug. To overcome these limitations, this study explores the conjugation of CPL410005 to an anti-HER2 antibody to enhance tumor specificity and reduce off-target effects. HER2-overexpressing cancers, such as breast and ovarian tumors, often exhibit resistance to standard therapies, necessitating novel treatment strategies. Targeting the Ubiquitin-Proteasome System (UPS) with Antibody-Drug Conjugate (ADC) for directed UBA1 inhibition represents a promising strategy for cancer therapy.

MATERIALS AND METHODS

***In vitro* activity assays:** Activity on the cell-line panel was outsourced to ProQinase GmbH. Stability assays were conducted at physiological and tumor-like conditions to evaluate linker integrity and assessed using HPLC-MS/MS, HPLC-TOF-MS). Enzymatic cleavage of colorimetric substrates (X-gluc, B-Gal) were performed to confirm the controlled release of the payload. Antibody binding assay was performed with Biacore S200 SPR system (Cytiva) on a CM5 sensor chip in single-cycle mode. Cytotoxicity of antiHER2-CPL410005 was tested in HER2+ (SKOV-2, SKBR3) and HER2-(HEK292) cell lines; **Conjugation process:** CPL410005 was conjugated to antiHER2 antibodies via sugar-based covalent maleimide linkers CPL410082 (β-galactose) and CPL410077 (β-glucuronide). Conjugation efficiency was assessed using RP-HPLC, SEC-UV, and mass spectrometry (HPLC-MS/MS, HPLC-TOF-MS); **In vitro stability assay:** Conjugates of antiHER2-410-005 were characterized for their stability at 37 °C in culture media and 10 % human plasma and 4 °C, -20, and -80 °C in conjugation buffer. At periodic intervals, aliquots were taken and analyzed by reversed-phase HPLC; **In vivo studies:** PK studies were performed on SCID mice xenografted with HCT-116 cell line; **Pharmacokinetics:** The plasma and tumor concentrations were analyzed using Ultrahigh performance liquid chromatography coupled with mass spectrometry (UHPLC-MS) with electrospray (ESI) ionization detection, quadrupole.

RESULTS

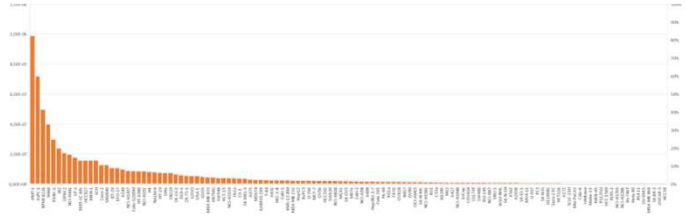

Figure 1. IC50 values of CPL410005 in a broad panel of over 120 cancer cel lines. The majority of cell lines demonstrated high sensitivity to CPL410005, where only 18 cell-lines (15%) showed a lower sensitivity with IC50 > 100 nM.

A

$t_{1/2}$ / min	Cl_{int} / µL·min^{-1}·10^{-6}		$t_{1/2}$ / min	Cl_{int} / µL·min^{-1}·10^{-6}
167.1	8.3		204.6	6.8
hepatocytes: human			hepatocytes: rat	
slope: -0.00415			slope: -0.00339	
R^2: 0.95939			R^2: 0.96452	

B

Compound	C / µM	T_R / min	T_R - T_0 / min	SD / min	RSD	CHI IAM / -	SD / -	CHI IAM criterium	log k (IAM) / -	log K(IAM) / -2	SD / -2
Atenolol	100	2.441	1.209	0.00	0.33%	4.82	0.06	low	0.64	1.25	0.00
Diclofenac	100	4.284	3.052	0.00	0.13%	31.90	0.06	med	1.89	2.61	0.01
410-005-05	100	4.526	3.294	0.00	0.00%	35.46	0.00	med	2.05	2.96	0.00

Figure 2. ADME characterization of CPL410005. (A) CPL410005 presents good permeability parameters on human and rat hepatocytes. **(B)** CPL410005 presents medium affinity to phospholipids, suggesting a good permeability of the compound.

RESULTS

A

pH	T0 [%]	2 h [%]	4 h [%]	7 h [%]	24 h [%]
CPL410077					
4,50	89,16	89,92	89,48	90,93	87,61
6,80	89,16	82,64	74,14	68,07	26,97
7,40	89,16	71,94	52,16	40,31	6,3
8,00	89,16	25,15	6,17	2,28	0,37

pH	T0 [%]	2 h [%]	4 h [%]	7 h [%]	24 h [%]
CPL410082					
4,50	92,52	93,1	93,53	-	90,35
6,80	92,52	88,72	77,47	-	40,72
7,40	92,52	74,58	56,81	-	9,01
8,00	92,52	29,45	8,63	-	0,26

B

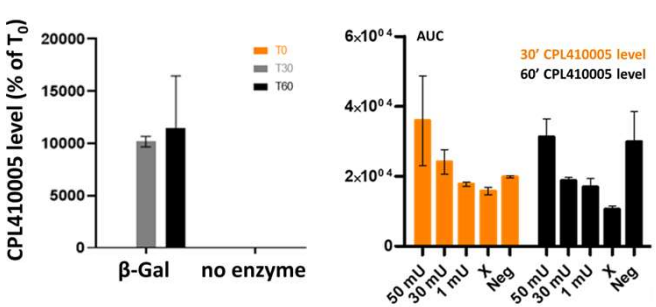

Figure 3. In vitro evaluation of the functionality of the cleavable linkers based on CPL410005. (A) 92% of CPL410005 was released within 24 hours under tumor-mimicking conditions with application of glycosyl hydrolases (pH 7.4; 37ºC). **(B)** Enzymatic cleavage confirms in vitro linker cleavage CPL410005 payload release by β-galactosidase (left) and β-glucuronidase (right).

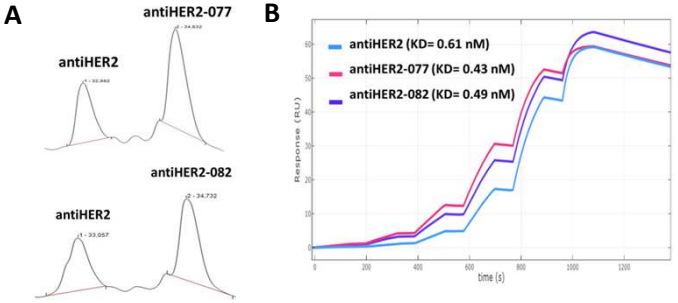

Figure 4. Cleavable linkers conjugate efficiently and do not affect antibody's binding properties. (A) Analytical characterization by denaturing RPLC–MS. Conjugation efficiency of HER2 and CPL-405-077 and CPL-405-082 was confirmed at over 80%, with high sample homogeneity. **(B)** antiHER2-CPL410077 (11) ADC retains excellent binding parameters of the antibody (purple) after conjugation with cleavable linkers (pink), with fast association and long dissociation time.

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NCBR National Centre for Research and Development
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RESULTS

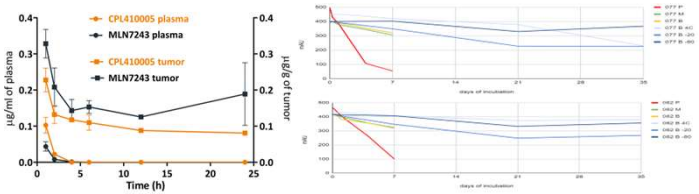

Figure 5. Pharmacokinetics of CPL410005 and its ADCs. (A) PK/PD analysis of free CPL410005 in human colon cancer xenograft model on SCID mice showed half-time in plasma at t1/2 = 2.9 h, with much longer retention time in the tumor tissue.
(B) Analysis of conjugates' stability in medium (M), buffer (B) and plasma (P). Analyzed conjugates were stable for 168h in 37°C in both conjugation buffer and medium, with registered loss of protein at 20%. In lower temperatures both conjugates present high stability in long term storage (up to 35 days).

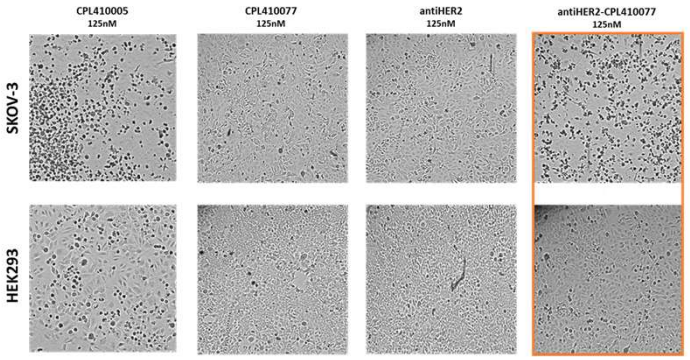

Figure 6. ADC greatly improves CPL410005 selectivity towards cancer cells and reduces toxicity on normal cell-lines. CPL410005 is a potent inhibitor of cell viability of human ovarian adenocarcinoma cell line (SK-OV-3) and the human breast adenocarcinoma cell line (SK-BR-3) at low nanomolar IC50 (17 nM and 40 nM, respectively), and normal human embryonic kidney cell line (HEK293), at IC50 =118 nM. ADC antiHER2-CPL410077 effectively kills cancer cells (SKOV-3) at 125 nM, where unconjugated cleavable linker CPL410077 and antiHER2 antibody show no cytotoxicity. HEK293 control cell-line is not affected by the ADC at 125 nM.

CONCLUSIONS

We developed targeted antibody-drug conjugates of antiHER2 antibody and CPL410005 derivatives, for specific targeting of CPL410005 to solid tumor cells for reduced off-target activity. CPL410005's potent UBA1 inhibition utilized in the form of ADC technology enhances tumor selectivity and reduces systemic toxicity. These findings support further preclinical development of HER2-targeted UBA1 inhibitors as a novel therapeutic strategy for HER2-positive cancers.

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