#LB059

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Multimeric linker exatecan-based ADC targeting Guanylyl cyclase C (GCC) as novel therapeutic modality for treatment of colorectal cancer

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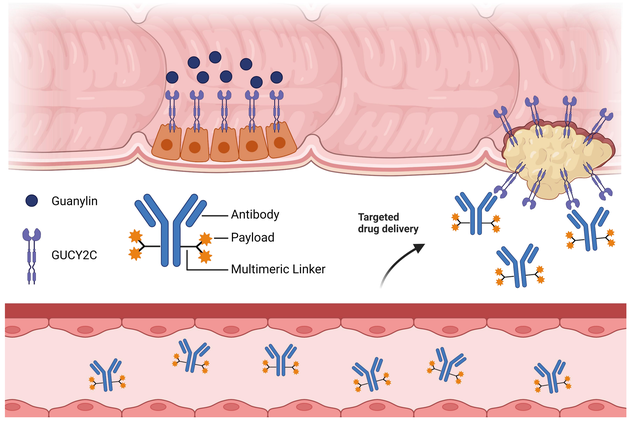
Heidelberg PHARMA
Focused Cancer Therapies

Background

Tumor-specific delivery of cytotoxic small molecules can be achieved by conjugation to monoclonal antibodies which bind with high affinity to a specific target thereby enhancing anti-tumor activity and reducing off-target toxicity.
The approval of Sacituzumab govitecan (Trodelvy®) and trastuzumab deruxtecan (Enhertu®) demonstrated the potential of topoisomerase I inhibitors in cancer therapy (1,2). Both ADCs rely on payload conjugation to interchain cysteines of an unmodified wildtype antibody coming along with unfavorable properties like instability in circulation, premature payload release and unspecific uptake via Fc gamma receptors (3). Heidelberg Pharma uses a **proprietary, site-specific conjugation** to cysteine-engineered and Fc-silenced antibodies to obtain a homogeneous and stable ADC devoid of Fc-mediated uptake (4). By using Heidelberg Pharma's proprietary **multimeric linker** comprising exatecan and a solubility enhancer (ELP01), ADCs with a homogenous degree of labeling (DoL) of 2 are achieved resulting in a **DAR of 4**. In the current study, *in vitro* and *in vivo* data of an ADC targeting GCC (guanylyl cyclase C) for the treatment of colorectal cancer with favorable potency and tolerability are presented.
Guanylyl cyclase C (GCC) is a transmembrane cell surface receptor that has an essential role in the maintenance of intestinal fluid and electrolyte homeostasis. GCC expression is restricted to the luminal site of the intestinal epithelium in healthy tissue. It is found to be overexpressed in >95% of primary and metastatic colorectal tumors and in 60–70% of gastric, esophageal, and pancreatic cancers (5). The tissue-restricted expression and consistent association with GI malignancies make GCC a highly attractive target for ADCs.

Graphical Abstract

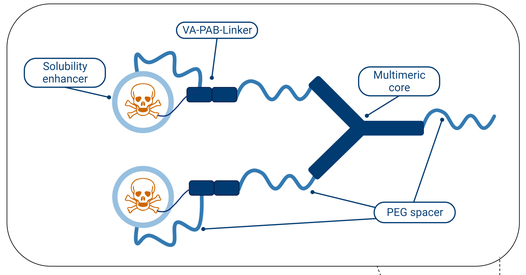


References

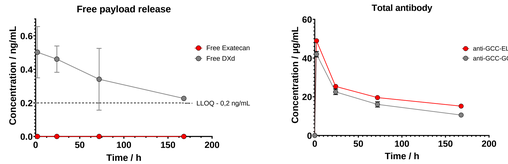
(1) Ogitani Y, Aida T, Hagihara K, Yamaguchi J, Ishii C, Harada N, et. al., Clinical Cancer Research, AACR Journals, 2016
(2) Syed Y, Drugs 2020, 80:1019-1025
(3) Buecheler J.W, Winzer M, Weber C, Gieseler H, J P Pharmsci 2020, 161-168
(4) Pahl A *et al.* in Cytotoxic Payloads for Antibody–Drug Conjugates Chapter 19; The Royal Society of Chemistry 2019
(5) Winn B, Tavares R, Matoso A, Noble L, Fanion J, Waldman SA, et. al., Human Pathol 2010, 41(1):123-128

Results

Multimeric exatecan-based linker payload

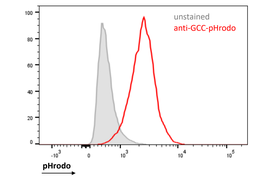


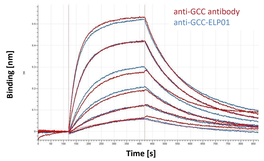
Pharmacokinetic profile of anti-GCC-ELP01 in comparison to anti-GCC-GGFG-DXd-hD



Pharmacokinetics of anti-GCC-ELP01 and anti-GCC-GGFG-DXd-hD (high DAR of ~10) in mouse after single dose intravenous administration (n=3 animals/group). Free exatecan was not detected in serum of mice treated with -ELP01 whereas free DXd was detected in serum of mice treated with –GGFG-DXd-hD (left). Total antibody concentration in serum was similar in both cases as determined by ELISA assay (anti-idiotype Ab, biotinylated anti-idiotype Ab + SA-HRP).

Binding and Internalization of the anti-GCC Antibody

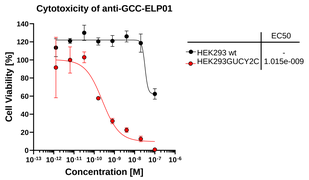


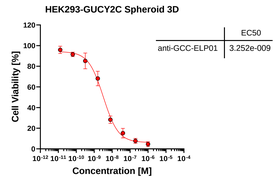


Test item	KD
Anti-GCC-ELP01	9.12E-09
Anti-GCC antibody	9.08E-09

Internalization of anti-GCC antibody in HEK293-GUCY2C cells was tracked using pHrodo in a time-dependent manner and analyzed by flow cytometry (upper panel). Affinity of anti-GCC-ELP01 and anit-GCC antibody to GCC-overexpressing HEK293-GUCY2C cells was measured using Octet (lower panel).

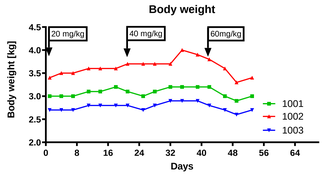
Cytotoxicity of exatecan-based ADCs



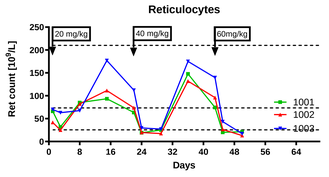


All anti-GCC exatecan-based ADCs tested showed target-specific cytotoxicity *in vitro* on GCC-overexpressing HEK293-GUCY2C cells (left, red), but not on GCC-negative HEK293*wt* (black) by BrdU ELISA 96h after incubation. Target-specific cytotoxicity was also observed in 3D spheroid cultures by CellTiter Glow 120h after incubation (right).

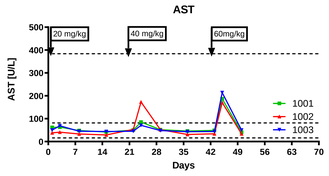
Dose escalation of anti-GCC-ELP01 in cynomolgus monkeys



Tolerability of anti-GCC-ELP01 in cynomolgus monkeys was assessed in a dose escalation study (n=3 animals). Single doses of 20, 40 and 60 mg/kg were given intravenously on day 1, day 22 and day 43, respectively. No changes in body weight were observed after intravenous administration of 40 mg/kg.



Reticulocytes and white blood cells (data not shown) were transiently decreased in a dose-dependent manner (upper panel). Very mild, transient increase of liver damage markers including aspartate aminotransferase (AST) was observed (lower panel).



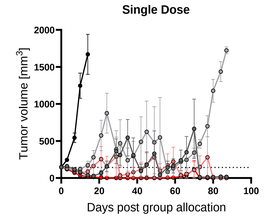
$$TI = \frac{MTD_{monkey}}{MED_{mouse}converted}$$

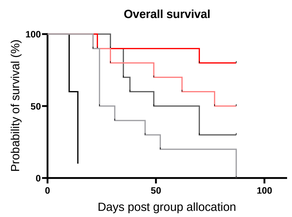
Test item	TI
Anti-GCC-ELP01	>32

Formula for the calculation of the TI with MTD_{monkey} and MED_{mouse}converted (minimal effective dose defined as single dose that leads to tumor volume reduction below the start value for at least two consecutive measurements) in the mouse models, converted to monkey by the body surface area (divided by 4). The maximal tolerated dose in cynomolgus monkeys is ≥ 40 mg/kg resulting in a therapeutic index ≥ 32.

Results

Anti-tumor efficacy of anti-GCC-ELP01 in HEK293-GUCY2C xenografts





Anti-tumor efficacy of anti-GCC-ELP01 was evaluated in mouse HEK293-GUCY2C s.c. xenograft models *in vivo* in female NOD SCID mice. Single dose intravenous administration of 10 mg/kg resulted in transient complete remission in 100% of the animals treated with anti-GCC-ELP01. In contrast, only 50% of animals treated with 10 mg/kg of anti-GCC-GGFG-DXd-hD transiently reached complete remission (left). 80% of the animals treated with anti-GCC-ELP01 were tumor-free and survived until the study end on day 94 post randomization. In contrast, only 30% of animals treated with anti-GCC-GGFG-DXd-hD survived until study end, of which one animal was still tumor-free (right). (n=10 animals/group, mean ± SEM)

PBS (Control)
anti-GCC-ELP01, 5 mg/kg
anti-GCC-ELP01, 10 mg/kg
anti-GCC-GGFG-DXd-hD, 5 mg/kg
anti-GCC-GGFG-DXd-hD, 10 mg/kg

Conclusion

- Anti-GCC-ELP01 shows target-specific cytotoxicity on GCC-overexpressing cells and no activity on target-negative cells
- Anti-GCC-ELP01 shows similar binding affinity to GCC-overexpressing cells as anti-GCC antibody only
- No free exatecan was detected in mice treated with anti-GCC-ELP01
- Complete remission in HEK293-GUCY2C tumor-bearing mice was achieved upon single and multiple dose treatment
- Anti-GCC-ELP01 shows significantly better anti-tumor efficacy as compared to anti-GCC-GGFG-DXd-hD conjugate
- Trastuzumab-ELP01 (DAR4) shows similar anti-tumor activity as Enhertu (DAR8) in a $HER2^+$ gastric cancer model (data not shown)
- Anti-GCC-ELP01 shows good tolerability in cynomolgus monkeys
- The therapeutic index is ≥ 32 based on the MTD_{Cyno} ≥ 40 mg/kg

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