#2608 Hu2G10 antibody-drug conjugates selectively target cleaved-activated Trop-2 in cancer cells and show therapeutic efficacy against multiple human cancers

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ABSTRACT

Trop-2 is a transmembrane calcium signal transducer, which activates growth-signaling networks that converge on Akt, ERK, Cyclin D1, NFkB (1). Trop-2 cleavage by ADAM10 was shown to be an activator switch for induction of cancer growth and metastatic diffusion (2). We devised a strategy to obtain monoclonal antibodies (mAbs) capable of recognizing ADAM10-exposed sites in cancer cells. We succeeded at obtaining mAbs that efficiently bound Trop-2 expressing cancer cells and were able to inhibit cell growth in vitro. We humanized the 2G10 mAb as the IgG1/k Hu2G10 antibody. Hu2G10 binds cancer-specific, cleaved/activated Trop-2 with Kd <10-12 M. On the other end, it binds uncleaved/wtTrop-2 in normal cells with Kd 3.16x10-8 M, thus promising an unprecedented therapeutic index in patients. Antibody-drug conjugates (ADC) of Hu2G10 were obtained using stable linkers, multiple conjugation chemistries and different payloads, among them topoisomerase I inhibitors, bleomycin, RNA polymerase inhibitor, MMAE and calicheamicin. Hu2G10-ADC selectively killed Trop-2-expressing cells in vitro. Dose-response curves were obtained for the BxPC-3 pancreatic cancer, DU-145 prostate cancer, HT29 colon cancer cell lines and KM12SM/Trop2 colon cancer transfectants, reaching EC50 values as low as 5.05x10-10 M. Hu2G10-ADC drove high anticancer effectiveness on cell- and patient-derived xenografts from colon, pancreas, prostate and ovarian cancer. Effectiveness followed a gradient of payload potency, with correspondingly high therapeutic indexes. Consistently, neither detectable toxic effects nor weight loss were observed in any subgroup of treated xenotransplant-bearing mice. Taken together these findings candidate anti-Trop-2 Hu2G10-ADC for best-in-class anticancer therapy.

(1) Guerra E. et al., The Trop-2 signalling network in cancer growth. Oncogene 32, 1594-1600 (2013).

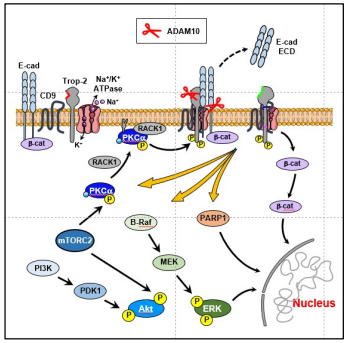
(2) Trerotola M. et al., Trop-2 cleavage by ADAM10 is an activator switch for cancer growth and metastasis. Neoplasia 23, 415-428 (2021).

BACKGROUND

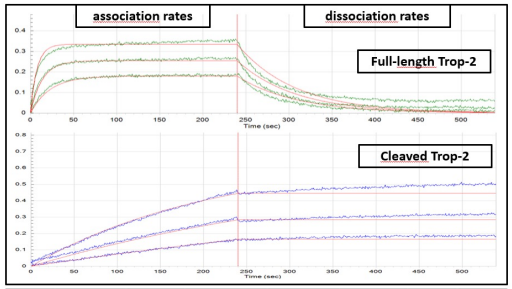
Trop-2 is a type-I transmembrane protein encoded by the tumor-associated calcium signal transducer 2 (TACSTD2/TROP2) gene, which is widely overexpressed in the majority of human carcinomas. Here, Trop-2 is cleaved by ADAM10 to drive tumor cell growth and metastasis, through the activation of a dormant, ubiquitously-expressed signaling super-complex and downstream effectors.

The Hu2G10 mAb targets this cancer vulnerability by binding with high efficiency and specificity the cleaved/activated Trop-2 (c-Trop-2).

Multiple Hu2G10 antibody-drug conjugates targeting c-Trop-2 were obtained and assayed against human cancers in vitro and in vivo.



Top: cartoon depicting Trop-2 signaling cascade activation by ADAM10 cleavage between R87 and T88 in the first loop of Trop-2 extracellular domain (adapted from JCO 2023 41:4688).



Ligand	Analyte	Conc. (nM)	KD (M)	k on (1/Ms)	k dis (1/s)	χ^2	R^2
Hu 2G10	T2 –full length	250-62.5	3.16E-08	4.68E+05	1.48E-02	1.2872	0.9682
Hu 2G10	T2-Cleaved	25-6.25	<1.0E-12	1.90E+05	<1.0E-07	1.4568	0.9772

Left: Hu2G10 absolute affinity to full length and cleaved Trop-2 as measured by bio-layer interferometry-based label-free binding. Association/dissociation kinetics are shown (Mol Cancer Ther 2023 22:790).

RESULTS

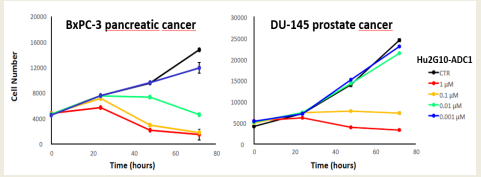


Figure 1. Time course of Hu2G10-ADC1 toxicity on c-Trop-2 expressing human cancer cells (payload=α-amanitin). Bars: +SEM

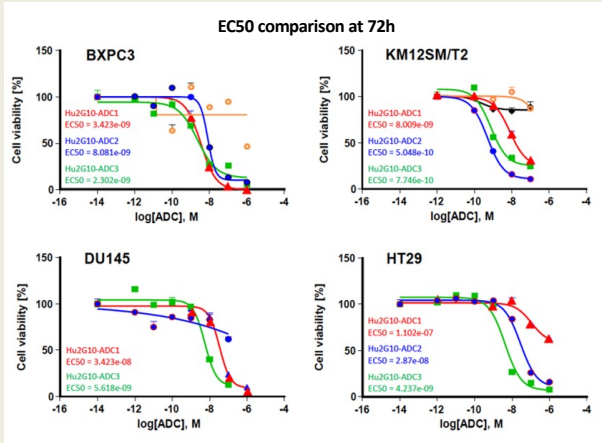


Figure 3. In vitro toxicities of Hu2G10-ADCs bearing different payloads on c-Trop-2 expressing human cancer cells, as log-dose versus % cell viability, measured after 72 h of exposure. Calculated EC50s are indicated.

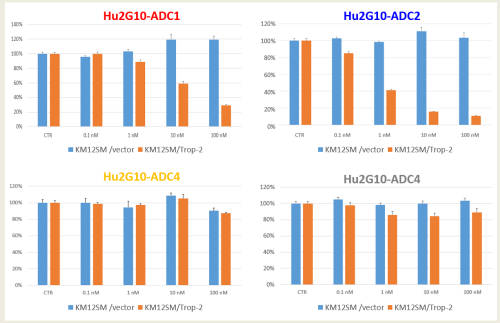


Figure 2. No off-target toxicities of Hu2G10-ADCs in vitro. KM12SM/Trop-2 and /vector only mock transfectants were assayed in parallel. Normalized values are shown. Bars: +SEM

SUMMARY OF Hu2G10-ADCs IN VITRO TOXICITIES

	Hu2G10-ADC1 α-amanitin, RNA polymerase inhibitor	Hu2G10-ADC2 MMAE, microtubule disrupting agent	Hu2G10-ADC4 SN-38 topoisomerase I inhibitor	Hu2G10-ADC5 Bleomycin-DNA damaging agent
DU-145 Prostate cancer	$3.42x10^{-8}$ M -7.47	Not Found (> $1x10^{-6}$ M)		
HT29 Colon cancer	$1.10x10^{-7}$ M -6.96	$2.87x10^{-8}$ M -7.54		
BXPC3 Pancreatic cancer	$3.42x10^{-9}$ M -8.47	$8.08x10^{-9}$ M -8.09	Not found	
KM12SM/Trop-2 Colon cancer transfectants	$8.00x10^{-9}$ M -8.10	$5.05x10^{-10}$ M -9.30	$2.32x10^{-4}$ M -3.63	$2.11x10^{-2}$ M -1.68
KM12SM/vector Colon cancer mock transfectants	Not found	Not found	Not found	Not found

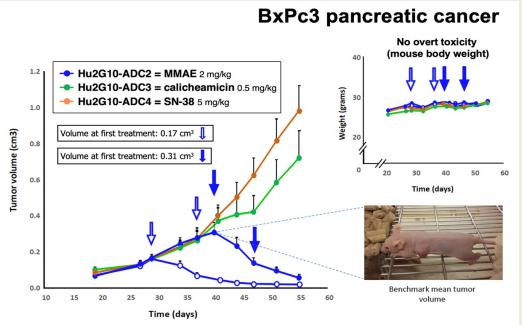


Figure 4. In vivo anticancer activity of Hu2G10 ADCs. Athymic mice subcutaneously (SC) injected with BxPC-3 pancreatic cancer cells were randomized (n=16 per group) and treated as indicated (arrows). Mice in the control groups received an irrelevant isotype-matched mAb. Tumor volumes and body weights were plotted against time. The inset shows a mouse bearing a 0.3 cm^3 tumor. Error bars: +SEM

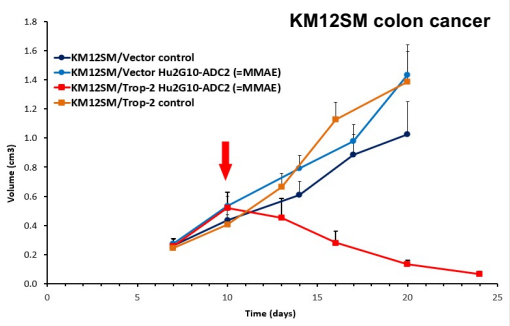


Figure 5. In vivo anticancer activity and specificity of Hu2G10-ADC. Athymic mice SC injected with cancer cell transfectants (n=16 per group) were treated with 4mg/kg of the Hu2G10-ADC2 or with an equal dose of an irrelevant isotype-matched mAb as control. KM12SM/Trop-2 and /vector only (mock) transfectants were tested in parallel. Tumor volumes were plotted against time. Arrow: time at treatment. Error bars: +SEM

CONCLUSIONS

Hu2G10-ADCs showed specific killing of c-Trop-2 expressing cancer cells in vitro. Among the payloads that were tested, monomethyl auristatin E (MMAE) showed the highest potency, with the notable exception of prostate cancer. No off-target toxicities were observed.

MMAE-based Hu2G10-ADCs showed high efficacy and specificity in vivo against cancer cells expressing c-Trop-2. No adverse effects were observed in treated mice.

Our findings candidate the anti-c-Trop-2 Hu2G10 for best-in-class ADC anticancer therapy.