TJ102: A Promising Bispecific Dual-Payload ADC Targeting CDH6 and Folate Receptor Alpha (FRα) for the Treatment of Ovarian and Kidney Cancers

Poster # LB021

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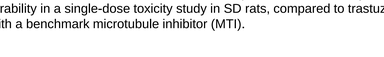
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Introduction

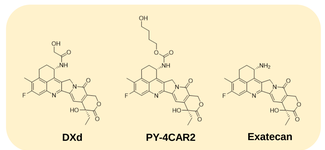
- CDH6 is a calcium-dependent adhesion protein mediating cell-cell binding. CDH6 is overexpressed in various solid tumors, including ovarian and kidney cancers, while exhibiting limited expression in most normal tissues.
- Folate receptor alpha (FRα), encoded by the FOLR1 gene, facilitates folic acid uptake and supports cell survival and proliferation. FRα is frequently overexpressed in epithelium-derived tumors, such as ovarian, endometrial, kidney, pancreatic, breast and lung cancers, with minimal expression in normal tissues.
- CDH6 and FRα are co-expressed in the majority of human ovarian and kidney cancers (TCGA database), making them attractive targets for bispecific ADCs.
- TJ102 is a bispecific, dual linker-payload ADC designed to target CDH6 and FRα. It consists of a natural IgG1-format bispecific antibody, heterodimerized through knobs-into-holes (KIH) technology, conjugated in a single step with two distinct cytotoxic payloads: PY-4CAR2, a novel topoisomerase I inhibitor, and PE-E2K, a microtubule inhibitor, via a branched cleavable linker.

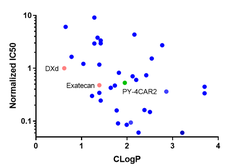
Two by two ADC



Bispecific dual-payload ADCs (2by2 ADCs) offer the advantage of dual antigen targeting, enhancing tumor specificity while reducing off-tumor toxicity compared to monoclonal ADCs. In addition, the simultaneous delivery of two payloads with distinct mechanisms of action (MOAs) has demonstrated synergistic efficacy in animal models while maintaining a manageable safety profile. The branched linker design enables a simple, one-step conjugation of dual payloads via thiol-maleimide chemistry.

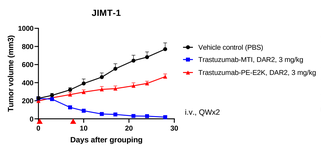
PY-4CAR2 is a topoisomerase I inhibitor with a greater potency than DXd

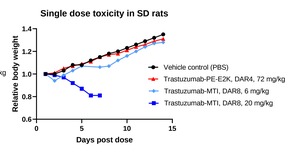




- Thirty-three camptothecin-derived compounds were synthesized and evaluated for cytotoxicity in NCI-N87 cells. Normalized IC_{50} values and CLogP, a hydrophobicity index, were plotted.
- PY-4CAR2 demonstrated greater potency than DXd and comparable efficacy to Exatecan, while displaying higher hydrophobicity than both compounds.

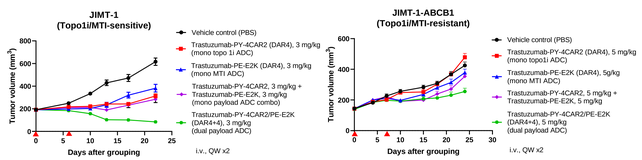
PE-E2K is a microtubule inhibitor with an optimized potency for dual-payload use





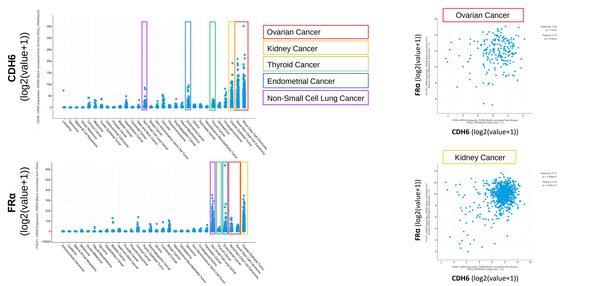
- PE-E2K was potency-attenuated to match the activity level of topoisomerase I inhibitor payloads in dual-payload settings.
- Trastuzumab-PE-E2K showed reduced anti-tumor activity in the JIMT-1 xenograft model and improved tolerability in a single-dose toxicity study in SD rats, compared to trastuzumab conjugated with a benchmark microtubule inhibitor (MTI).

Dual-payload anti-HER2 ADC achieves synergistic efficacy in HER2-low CDX models



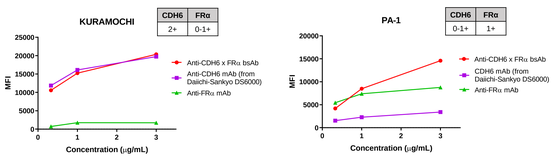
- ABCB1, which encodes an efflux pump (also known as P-gp), was overexpressed in JIMT-1 breast cancer cells via lentiviral transduction to establish a cell line resistant to topoisomerase I inhibitors and microtubule inhibitors.
- JIMT-1 or JIMT-1-ABCB1 cells were subcutaneously inoculated into the flanks of female CB17-SCID mice. Trastuzumab-based ADCs, carrying either single or dual payloads, were administered intravenously once weekly. Data are presented as mean ± SEM.
- The dual-payload trastuzumab ADC demonstrated superior anti-tumor activity compared to ADCs conjugated with either PY-4CAR2 or PE-E2K alone, or their combination.

CDH6 and FRα are frequently co-expressed in ovarian, kidney, and other cancers



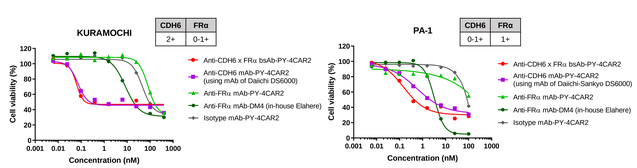
- mRNA expression levels of CDH6 and FRα across different tumor types were obtained from The Cancer Genome Atlas (TCGA) via the cBioPortal database.
- (Left) CDH6 and FRα are frequently overexpressed in ovarian, kidney, thyroid, endometrial, and non-small cell lung cancers. (Right) A majority of ovarian and kidney cancer samples exhibit co-expression of CDH6 and FRα.

CDH6 x FRα bispecific antibody exhibits improved internalization compared to monoclonal antibodies



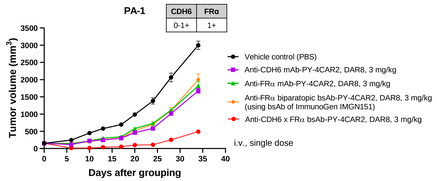
- The internalization capability of the anti-CDH6/FRα bispecific antibody (bsAb) was evaluated using flow cytometry. KURAMOCHI and PA-1 ovarian cancer cells were incubated with fluorescently labeled antibodies for 2 hours, followed by digestion of surface-bound antibodies prior to flow cytometric analysis.
- The anti-CDH6/FRα bispecific antibody exhibited improved internalization compared to anti-CDH6 or anti-FRα monoclonal antibodies.

CDH6 x FRα bispecific ADC induces potent cytotoxicity in CDH6/FRα expressing cells



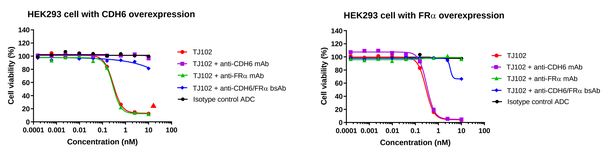
- KURAMOCHI and PA-1 ovarian cancer cells were treated with ADCs for 144 hours, and cytotoxicity was determined using a CellCounting-Lite 2.0 viability assay.
- The anti-CDH6/FRα bispecific ADC generally induced greater in vitro cytotoxicity compared to anti-EGFR and anti-B7-H3 monoclonal ADCs.

CDH6 x FRα bispecific ADC exhibits better efficacy than monoclonal ADCs in an antigen-low CDX model



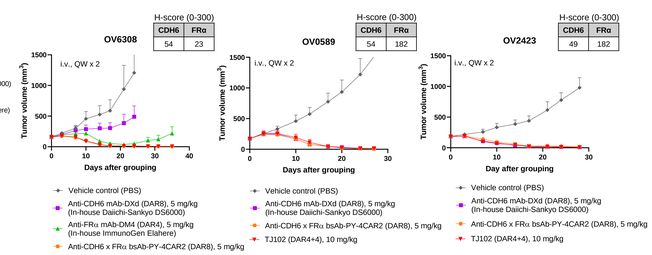
- PA-1 ovarian cancer cells were subcutaneously inoculated into right flanks of female CB17-SCID mice. The tumor volumes are presented as mean ± SEM.
- The anti-CDH6/FRα bispecific ADC exhibited superior anti-tumor activity compared to anti-CDH6 or anti-FRα monoclonal ADCs, or the anti-FRα biparatopic ADC.

TJ102 mediates antigen-specific cytotoxicity in CDH6 or FRα overexpressing cells



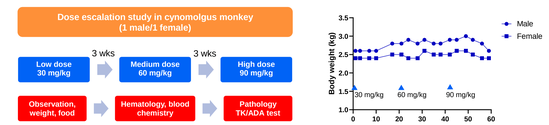
- HEK293 cells overexpressing human CDH6 or FRα were generated via lentiviral transduction. Cells were pre-treated with 20x concentration of the anti-CDH6/FRα bispecific antibody, or individual anti-CDH6 or anti-FRα monoclonal antibodies for 1 hour, followed by a 144-hour incubation with 1x concentration of TJ102. Cell viability was assessed using the CellCounting-Lite 2.0 viability assay.
- TJ102-mediated cytotoxicity was effectively blocked by co-incubation with the anti-CDH6/FRα bispecific antibody or anti-EGFR and anti-B7-H3 monoclonal antibodies, indicating that TJ102 mediates antigen-specific cytotoxicity.

CDH6 x FRα bispecific dual-payload ADC (TJ102) exhibits robust anti-tumor activity in ovarian PDX models



- In vivo efficacy of TJ102 was assessed in patient-derived xenograft (PDX) models of ovarian cancer inoculated in female BALB/c Nude mice (n=3 per group).
- Both the anti-CDH6/FRα bispecific ADCs with dual payloads (TJ102) and that with a single payload (PY-4CAR2) exhibited superior anti-tumor activity compared to anti-CDH6 or anti-FRα monoclonal ADCs (DS6000 and Elahere) in CDH6/FRα-low ovarian PDX models.

TJ102 is well tolerated in non-human primates (NHP)



- TJ102 (DAR4+4) administration exhibited no evidence of weight loss, reduced food uptake, or intestinal abnormalities.
- At 30mg/kg and 60 mg/kg, TJ102 caused reversible reductions in white blood cell, red blood cell, neutrophil, and reticulocyte counts, which recovered before the subsequent dose.
- The highest non-severely toxic dose (HNSTD) in cynomolgus monkey was ≧ 60mg/kg.

Conclusions

- The dual-payload ADC with PY-4CAR2 and PE-E2K demonstrated greater in vivo efficacy than single payload ADCs carrying either payload alone.
- TJ102 exhibited superior internalization and cytotoxicity compared to anti-CDH6 or anti-FRα monoclonal antibodies or ADCs.
- TJ102 produced potent and durable anti-tumor activity in ovarian cancer CDX and PDX models, outperforming anti-CDH6 or anti-FRα monoclonal ADCs even in tumor models with low antigen expression.
- In a non-GLP toxicity study in cynomolgus monkeys, TJ102 was well-tolerated, with the HNSTD exceeding 60 mg/kg.
- These findings highlight TJ102 as a promising bispecific, dual-payload ADC with robust anti-tumor efficacy and a favorable safety profile. Its dual-targeting approach not only improves the therapeutic index but may also mitigate potential tumor resistance in future clinical applications.