Preclinical development of TJ101, a potent bispecific ADC targeting EGFR and B7-H3 for the treatment of solid cancers

Poster # 2955

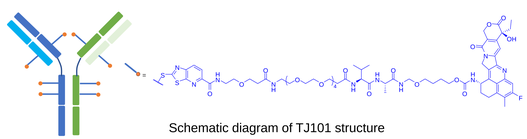
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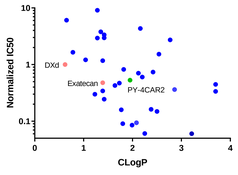
Introduction

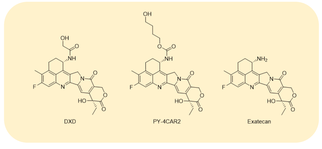
- EGFR (also known as ErbB-1 or HER1) is a member of the ErbB receptor family and a well-established cancer therapeutic target. EGFR is upregulated in various solid tumors, including lung, head and neck, breast, and kidney cancers.
- B7-H3 (also known as CD276) is an immune checkpoint molecule broadly expressed across multiple tumor types, such as melanoma, leukemia, prostate, pancreatic, breast, colorectal, ovarian and lung cancers. Notably, B7-H3 expression in normal tissues is highly restricted.
- EGFR and B7-H3 are frequently co-expressed in solid tumors, particularly in lung cancer and head and neck cancer.
- TJ101 is a bispecific antibody-drug conjugate (ADC) targeting both EGFR and B7-H3. Its backbone is a natural IgG1 bispecific antibody heterodimerized using knobs-into-holes (KIHs) technology. To minimize on-target toxicity, the affinity of the anti-EGFR arm is attenuated, while the anti-B7-H3 arm maintains high affinity.
- TJ101 is conjugated with PY-4CAR2, a novel camptothecin-derived cytotoxic payload with bystander killing activity, via a cleavable linker at a drug-to-antibody ratio (DAR) of 8.



Schematic diagram of TJ101 structure

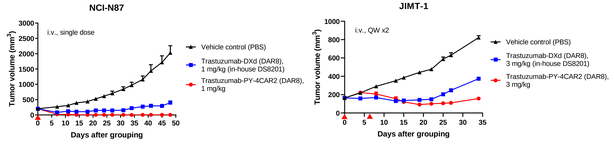
PY-4CAR2 is a camptothecin-derived payload that is more potent and hydrophobic than DXd





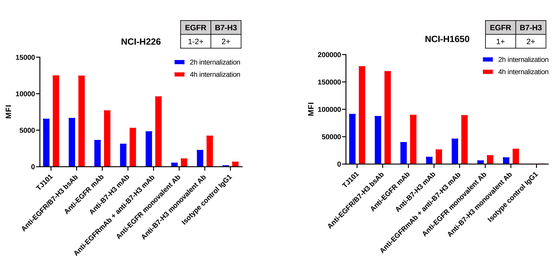
- Thirty-three camptothecin-derived compounds were synthesized, and their IC_{50} values were determined in a cytotoxicity assay using NCI-N87 cells. Normalized IC_{50} values were plotted against CLogP (calculated LogP), an index of hydrophobicity, and compared with those of DXd and Exatecan.
- PY-4CAR2 exhibited greater potency than DXd and comparable activity to Exatecan, while displaying higher hydrophobicity than both compounds.

PY-4CAR2 exhibit better anti-tumor potency than DXd in trastuzumab-sensitive or -resistant xenograft models



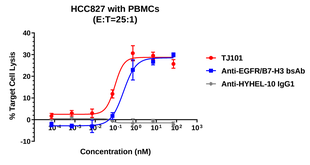
- NCI-N87 (trastuzumab-sensitive gastric cancer cell line) and JIMT-1 (trastuzumab-resistant breast cancer cell line) were engrafted subcutaneously into female CB17-SCID mice (n=6 per group).
- Trastuzumab conjugated with PY-4CAR2 via a dipeptide linker exhibited superior anti-tumor activity in both NCI-N87 and JIMT-1 xenograft models, compared to trastuzumab-DXd (DS8201).

TJ101 and anti-EGFR/B7-H3 bsAb exhibit enhanced internalization over monoclonal antibodies



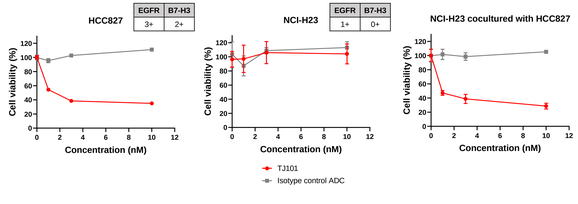
- The internalization of TJ101 and its naked bispecific antibody (bsAb), along with parental anti-EGFR and anti-B7-H3 monoclonal antibodies (mAb) was evaluated using flow cytometry. NCI-H226 and NCI-H1650 NSCLC (non-small cell lung cancer) cells were incubated with fluorescently labeled antibodies for 2 or 4 hours, followed by digestion of surface-bound antibodies prior to flow cytometric analysis.
- TJ101 and the anti-EGFR/B7-H3 bsAb demonstrated superior internalization compared to anti-EGFR and anti-B7-H3 monoclonal antibodies or their combination.

TJ101 mediates potent ADCC activity



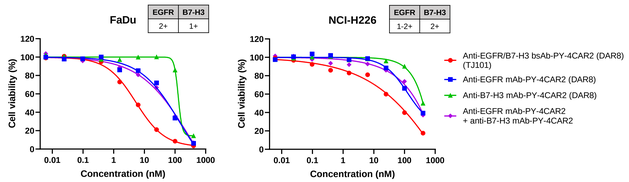
- ADCC (antibody-dependent cell-mediated cytotoxicity) activity of TJ101 and its naked anti-EGFR/B7-H3 bsAb were evaluated using HCC827 cells as target cells and human peripheral blood mononuclear cells (PBMCs) as effector cells, at an E/T ratio of 25:1. Cells were co-cultured with TJ101, anti-EGFR/B7-H3 bsAb, or anti-HYHEL-10 IgG1 (negative control) for 6 hours.
- TJ101 and the anti-EGFR/B7-H3 bsAb induced significant ADCC-mediated cytotoxicity against HCC827 cells.

TJ101 induces robust bystander killing



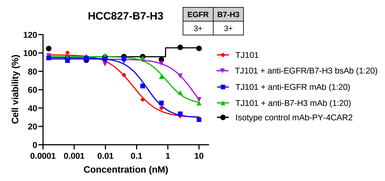
- The bystander killing activity of TJ101 was evaluated using firefly luciferase-labeled NCI-H23 cells, cultured either alone or co-cultured with HCC827 cells. NCI-H23 express low levels of EGFR and B7-H3, whereas HCC827 cells express both antigens at high levels. Cell viability was assessed after 144 hours of culture.
- TJ101 induced dose-dependent cytotoxicity in TJ101-resistant NCI-H23 cells when co-cultured with HCC827 cells.

TJ101 exhibits improved in vitro cytotoxicity compared to monoclonal ADCs



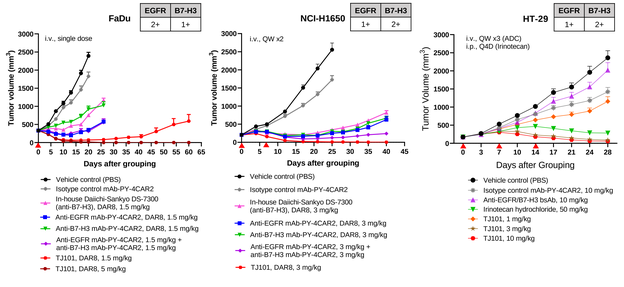
- FaDu head and neck cancer cells and NCI-H226 NSCLC cells were treated with ADCs for 144 hours, and cytotoxicity was assessed using CellCounting-Lite 2.0 viability assay.
- TJ101 exhibited improved in vitro cytotoxicity compared to anti-EGFR and anti-B7-H3 monoclonal ADCs, or their combination.

TJ101-mediated cytotoxicity is antigen-specific and blocked by anti-EGFR and B7-H3 monoclonal antibodies



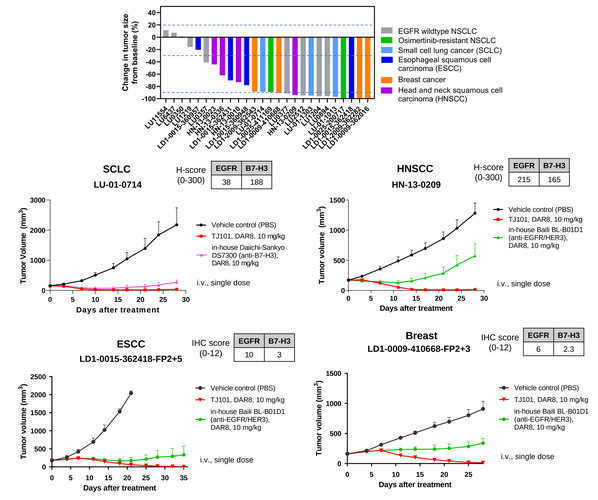
- An HCC827-B7-H3 cell line was generated via lentiviral transduction of human B7-H3. Cells were pre-treated with 20x concentration of the anti-EGFR/B7-H3 bispecific antibody or individual anti-EGFR or anti-B7-H3 monoclonal antibodies for 1 hour, followed by a 144-hour incubation with 1x concentration of TJ101. Cell viability was assessed using the CellCounting-Lite 2.0 reagent.
- TJ101-mediated cytotoxicity was effectively blocked by co-incubation with the anti-EGFR/B7-H3 bispecific antibody and anti-EGFR and anti-B7-H3 monoclonal antibodies, indicating TJ101 mediates antigen-specific cytotoxicity.

TJ101 exhibits potent in vivo anti-tumor activity in EGFR/B7-H3 expressing xenograft models



- In vivo efficacy of TJ101 was assessed in xenograft models of FaDu head and neck cancer, NCI-H1650 NSCLC, and HT-29 colon cancer, established in female BALB/c nude or CB17-SCID mice (n=8 per group). Tumor volumes are presented as mean ± SEM.
- TJ101 induced potent anti-tumor activity, including complete tumor regression in the FaDu and NCI-H1650 models. It also demonstrated superior efficacy compared to anti-EGFR or anti-B7-H3 monoclonal ADCs, administered either alone or in combination.

TJ101 induces durable tumor regression in EGFR/B7-H3 expressing PDX models



- In vivo efficacy of TJ101 was evaluated in patient-derived xenograft (PDX) models of NSCLC, SCLC, HNSCC, breast, and esophageal cancers using female immunodeficient mice (BALB/c Nude, NU/NU, or NOD-SCID; n=3 per group).
- TJ101 induced tumor regression (>30% reduction from baseline) in 21 out of 26 models.
- TJ101 exhibited superior anti-tumor activity compared to the benchmark anti-B7-H3 monoclonal ADC (Daiichi-Sankyo DS7300) and anti-EGFR/HER3 bispecific ADC (Baili BL-B01D1) even in the PDX models with low EGFR or B7H3 expression.

TJ101 is well tolerated in non-human primates (NHP)

- In a non-GLP toxicity study in cynomolgus monkeys, TJ101 was administered at 10, 30, and 60 mg/kg every 21 days for a total of three doses (1 male and 1 female per group).
- Following the 1st and 2nd doses, a decreasing trend in white blood cell, neutrophil, and lymphocyte counts was observed in the 30 and 60 mg/kg groups, which recovered by Day 43. On Day 14 after the 3rd dose, a decreasing trend in neutrophil counts was noted across all dose groups.
- Blood biochemistry and coagulation parameters remained within normal ranges in all groups. Body weight and food consumption were stable, and no gastrointestinal side effects were observed.
- After the 2nd dose, skin discoloration and dark spots (considered target-related toxicity) were observed in the animals receiving 30 and 60 mg/kg.

Conclusions

- TJ101 demonstrated synergistic internalization and cytotoxicity in cancer cell lines co-expressing EGFR and B7-H3.
- TJ101 induced potent, dose-dependent anti-tumor activity in NSCLC, HNSCC, and colon cancer CDX models, and exhibited improved efficacy compared to monoclonal ADCs.
- A single administration of TJ101 produced durable tumor regressions in PDX models of NSCLC, SCLC, HNSCC, breast cancer, and esophageal cancers.
- In a non-GLP toxicity study in cynomolgus monkeys, TJ101 was well-tolerated, with the HNSTD exceeding 30 mg/kg.
- In summary, TJ101 is a promising bispecific ADC with potent anti-tumor efficacy across multiple solid tumor types and a favorable safety profile. TJ101 is currently under IND application.