Mechanistic and Pharmacodynamic studies of ADCT-301, a Pyrrolobenzodiazepine (PBD) Dimer-Containing Antibody Drug Conjugate (ADC) Targeting CD25-Expressing Hematological Malignancies

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Results

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Conditioned media transfer MTS absorbance cell viability assays; Early apoptosis (Annexin-V) and viability flow cytometric assays; subcutaneous and disseminated lymphoma models (7).

ADCT-301 has highly potent and targeted cytotoxicity against a panel of human lymphoma cell lines (7). γ-H2AX response (6).

PBD-induced DNA interstrand cross-links elicit a robust, but delayed action via makes up the IL-2R. It plays a key role in signal transduction pathways associated with lymphocytes (1). The IL-2R is comprised of two chains, 

The single cell-gel electrophoresis (comet) assay was carried out on untreated Karpas 299 and cells treated with 10 ng/mL ADCT-301 for 8 hours. A mean reduction in comet tail moment of 70% was observed for cells treated with ADCT-301 compared to untreated cells.

The bar chart shows the mean percentage in each cell cycle phase for each condition in three independent experiments. * p ≤ 0.05 ** p ≤ 0.01.

Figure 3- Cell cycle G2/M arrest and apoptosis

A. Histograms depicting percentage of propidium iodide labeled DNA in each cell cycle phase (G0/G1, S, G2/M) from Karpas 299 cells either untreated or treated with 1 (µg/mL) Karpas 299 cells over a time course to 16 hours at 37 °C. B. The mean fold increase of cells in G2 phase compared to untreated control for cells treated with 1(µg/mL) ADCT-301 in equitoxic (1.5) pM naked warhead at 16 h, 24 h or 48 h.

Figure 4- Bystander killing

A. Percentage of Karpas 299 cells per well with surface Annexin V exposure or loss of viability, as indicated by permeability to propidium iodide dye over stress curve (4, 6, 72 and 72 h) after treatment with ADCT-301 or non-binding ADC. B. The mean fold increase of cells in G2 phase compared to untreated control for cells treated with 1 (µg/mL) ADCT-301 in equitoxic (1.5) pM naked warhead at 16 h, 24 h or 48 h.

Figure 5- In vivo Pharmacological assays

A. The single cell-gel electrophoresis (comet) assay was carried out on untreated Karpas 299 and ADCT-301 or non-binding ADC treated isolated cells. Images panels show DNA labelled with propidium iodide for each treatment condition.

Figure 1- Internalization

A. Merged inverse-fluorescence images of CD25 positive Hodgkin’s lymphoma (lymphoma 6) 24 h after treatment with ADCT-301 10 ng/mL on Karpas 299 cells incubated at 37°C relative to that of cells kept at 4°C or sodium azide.

A. The mean percentage in each cell cycle phase for each condition in three independent experiments. * p ≤ 0.05 ** p ≤ 0.01.

Figure 2- DNA cross-linking, H2AX phosphorylation and cytotoxicity

A. Percentage viability of Ramos cells following ADCT-301 treatment with various concentrations of low and high warhead. B. Percentage viability of Ramos cells following ADCT-301 treatment with 0 vs 10 ng/mL of ADCT-301 in non-binding ADC. C. Percentage viability of Ramos cells following ADCT-301 treatment with various concentrations of low and high warhead.

References