**Supplemental Methods**

**Characterisation of ADCT-301 by SEC, HIC and RPLC**

Characterisation of synthesised ADC was performed by size exclusion chromatography (SEC), hydrophobic interaction chromatography (HIC) and reduced reverse phase liquid chromatography (RPLC). SEC was carried out by injection of undiluted ADCT-301 across an isocratic gradient (mobile phase 200 mM Potassium Phosphate, 250 mM Potassium Chloride, 10% v/v isoPropanol (iPA), pH 6.95) at a flow rate of 0.5 ml/min on a TOSOH Bioscience TSK-Gel® GW3000SWxl , 250A, 5µm column at room temperature with detection at 280 nm. For HIC DAR determination, ADCT-301 (1-5 mg/ml) was injected across a gradient established by using mobile phase A buffer (1.5M (NH4)2SO4, 25mM NaPi, pH=6.95±0.05) and mobile Phase B buffer (75% 25mM NaPi, pH=6.95±0.05; 25% iPA). A TOSOH Butyl-NPR 4.6mm x 3.5cm, 2.5μm column was used for the analysis with a flow rate of 0.8 ml/min and detection at 214 nm. DAR was calculated according to the formula:

DAR = (sum of (n x % DAR(n))/(sum of % DAR(n)); {n= 0-8}

 For DAR determination by reduced RPLC, prior to injection ADCT-301 was reduced using DTT in sodium borate buffer pH 8.4 for 15 minutes. The column used for the analysis was an Agilent PLRP-S 1000A, 5μm, 50 x 2.1 mm at a column temperature of 80 ˚C. Changes in gradient across Mobile Phase A buffer (0.1% TFA in Water) and Mobile Phase B buffer (0.1% TFA in 10% Water, 90% Acetonitrile) were used for analysis with a flow rate of 1 ml/min and detection at 214 nm. DAR was calculated using the following formula:



**Assessment of ADCT-301 efficacy in Karpas 299 and Ramos *in vivo* disseminatedmodels**

Karpas 299 or Ramos xenografts were established in female Fox Chase SCID® (C.B-17/Icr-*Prkdcscid*, Charles River) by injecting 107 Karpas 299 or 5 x 106 Ramos cells, respectively, intravenously into the lateral tail vein. 12 days after Karpas 299 injections, mice were randomly allocated into groups to receive 0.1, 0.2, 0.4, 0.6 mg/kg of ADCT-301, 0.6 mg/kg non-binding ADC, PBS (vehicle) or 0.6 mg/kg Adcetris intravenously on Day 1 or Adcetris (0.5 mg/kg qd4 x 4). Treatment of Ramos xenografted mice with PBS (vehicle), or 1 mg/kg of non-binding ADC or ADCT-301 injections was initiated 7 days after tumor inoculation. The day of death or euthanasia represented the time to endpoint (TTE). Animals that did not reach the endpoint were euthanized at the end of the study, and assigned a TTE value equal to the last study day. Any animal classified as having died from treatment-related (TR) causes was assigned a TTE value equal to the day of death. Any animal classified as having died from non-treatment-related (NTR) causes was excluded from the analysis. A TTE value was recorded for each assessable animal, and the median TTE was calculated for each group. The median TTE of treated mice was expressed as a percentage of the median TTE of the control mice (%T/C), and the increase in life span (ILS) was calculated as:

ILS = %T/C – 100%

where T = median TTE treated, and C = median TTE control. Thus, if T = C, ILS = 0%.

The logrank test was employed to determine the significance of the difference between the overall survival experiences (survival curves) of two groups, based on their TTE values.

**Supplemental Tables**

**Table S1.** High molecular weight, low molecular weight and monomer designation by size exclusion chromatography showing area and percentage of each fraction occupying total area under curve at 280 nm

|  |  |  |  |
| --- | --- | --- | --- |
| **Retention Time (min)** | **Designation** | **Area280nm** | **% Area280nm** |
| 14.537 | HMW Aggregates | 154826 | 1.457 |
| 17.178 | Monomer | 10300767 | 96.951 |
| 18.353 | LMW Fragment 1 | 169138 | 1.592 |
|  |  | 10624730 | 100.000 |

**Table S2.** Heavy and light chain measurements by reduced Reverse Phase Liquid Chromatography showing area and percentage of each fraction occupying total area under curve at 214 nm

|  |  |  |  |
| --- | --- | --- | --- |
| **Retention Time (min)** | **Designation** | **Area214nm** | **% Area214nm** |
| 8.258 | L0 | 12276612 | 19.834 |
| 10.121 | L1 | 7687771 | 12.42 |
| 11.072 | H0 | 18622737 | 30.087 |
| 12.009 | H1 | 16547265 | 26.734 |
| 13.512 | H2 | 5871591 | 9.486 |
| 14.641 | H3 | 890155 | 1.438 |
| Total |  | 61896131 | 100.000 |

**Table S3.** Individual DAR components as determined by Hydrophobic Interaction Chromatography showing percentage of each fraction occupying total area under curve at 214 nm

|  |  |  |
| --- | --- | --- |
| **Retention Time (min)** | **Designation** | **% Area214nm** |
| 4.71 | DAR 0 | 18.80 |
| 5.15 | DAR 1 | 3.41 |
| 6.71 | DAR 2 | 50.43 |
| 8.43 | DAR 4 | 24.71 |
| 9.60 | DAR 6 | 2.65 |
| Total  |  | 100.00 |