**Supplementary Data**

**Materials and Methods**

**Biomarker analysis**

During dose escalation, newly obtained tumor or archival tumor were collected at screening/baseline to assess CDH6 expression, surface expression markers, characterization of TIL phenotypes, myeloid cell populations, and to assess other biomarkers of immunologic response. Newly obtained tumor were the newly obtained formalin fixed tumor biopsy samples in ethanol (3-6 passes). Archival tumor were the FFPE block preferred or newly cut slide.

In addition to the biomarkers specified above, exploratory biomarker analysis may be conducted on any remaining tumor samples. For safety related investigation, cytokine panel analysis were performed using back up PK samples.

*CDH6 expression (IHC and qPCR) in patient tumor samples*

Tumor samples were available for retrospective CDH6 expression testing. CDH6 mRNA and protein expression of clinical samples were measured by immunohistochemistry (IHC) and quantitative polymerase chain reaction (qPCR) by ICON Specialty Laboratories (formerly MolecularMD). The sample collection was mandatory and the biopsy could be either primary or metastatic lesion. For IHC, slides were stained on the Ventana Benchmark ULTRA platform. Slide-mounted FFPE sections were dewaxed (EZ Prep, Ventana/Roche), subjected to head-induced epitope retrieval (ULTRA Cell Conditioning 1 (CC1), Ventana/Roche), quenched with endogenous peroxidase blocker (UltraView Ventana/Roche), and washed (Reaction Buffer, deionized water, Ventana/Roche #808-0021) prior to application of the primary antibody. The primary antibody was a mouse monoclonal antibody (mAb) to CDH6/K (clone 2B6, ThermoFisher Scientific). A mouse IgG1 isotype control mAb (clone MOPC-21, Abcam) was used to discriminate specific staining from background. The bound primary antibody was detected using an anti-mouse IgG-HRP conjugate (UltraView DAB kit, Ventana/Roche). DAB was used as the chromogen to visualize the sites of antibody binding within tissue (UltraView DAB, Ventana/Roche). Slides were counterstained with hematoxylin to label cell nuclei. Slides were cover slipped using permanent mounting medium (Cytoseal, Thermo Scientific) and glass coverslips. IHC staining results were reported as positive (any detectable membrane-associated staining within the tumor) or negative (no detectable membrane-associated staining within the tumor). Staining intensity was reported as 0, 1+, 2+, or 3+. Percentage of positively stained tumor cells were reported as 0-100% as well as the computed H-score (Computed H-score was calculated using the following formula: 3×(% tumor cells 3+) + 2×(%tumor cells 2+) + 1×(%tumor cells 1+) for a max total of 300).

For qPCR, the Maxwell FFPE RNA Extraction Kit was employed to extract RNA from FFPE samples. The NanoDrop 2000c instrument was used to estimate RNA concentration (ng/µL). The High Capacity cDNA Kit was used to synthesize cDNA from FFPE RNA templates per manufacturer’s instructions. qPCR was performed on the ABI QuantStudio™ DX Real-Time PCR Instrument. ThermoFisher TaqMan Gene Expression Master Mix was used for qPCR amplification per manufacturer’s instructions. Universal TaqMan gene expression cycling conditions were employed. RNA input per reaction varied depending on the experimental parameters per 50-µL reaction. The validated input for clinical samples was 250ng per 50µL real-time (RT) reaction and 25ng per qPCR reaction. Singleton RT and triplicate qPCR reactions for the CDH6 assay and two reference gene assays were performed. To run controls, the CDH6 RT-qPCR Test included a no template control (NTC), two CDH6 positive controls (i.e., High and Medium CDH6 expression) and a universal human reference (UHR) (low CDH6 expression) control on each plate. The High and Medium controls were comprised of OVCAR-3 and SKOV3 FFPE cell line RNA, respectively. Successful plate control performance validated individual runs. Reverse transcription-No Template Control (RT-NTC) was nuclease-free water used in lieu of template. The RT-NTC was used to detect the presence of potential contamination during the RT assay setup that could result in false positive sample data. QuantStudio software analyses employed automatic baseline and a manual threshold setting of 0.1 for all three-gene expression assays. Average cycle quantification (Cq) values and associated standard deviation (SD) values for reference genes and CDH6 were calculated. Individual samples were scored as evaluable when average reference gene Cq values were ≤35. Only samples with CDH6 Cq values ≤ LoQ (i.e., Cq ≤ 36) were considered valid. Reference genes and CDH6 Cq SD values were required to be ≤0.5 where replicates were collected.

*Cytokine analysis*

The cytokine analysis was performed using backup PK samples for cohorts 1 and 2 (all time points; Refer to Clinical PK Analysis section for time points). Cytokine analysis was performed by Myriad RBM, Inc. using the Human Inflammation Multi Analyte Profile (MAP®) v. 1.0 assay. All samples were stored at less than -70°C until tested. Samples were thawed at room temperature, vortexed, spun at 3700-x g for 5 minutes for clarification and transferred to a master microtiter plate. Using automated pipetting, an aliquot of each sample was added to individual microsphere multiplexes of the selected MAP® and blocker. This mixture was thoroughly mixed and incubated at room temperature for 1 hour. Multiplexed cocktails of biotinylated reporter antibodies were added robotically and after thorough mixing, incubated for an additional hour at room temperature. Multiplexes were labelled using an excess of streptavidin-phycoerythrin solution, thoroughly mixed and incubated for 1 hour at room temperature. The volume of each multiplexed reaction was reduced by vacuum filtration and washed 3 times. After the final wash, the volume was increased by addition of buffer for analysis using a Luminex instrument and the resulting data interpreted using proprietary software developed by Myriad RBM. For each multiplex, both calibrators and controls were included on each microtiter plate. Eight-point calibrators to form a standard curve were run in the first and last column of each plate and controls at three concentration levels were run in duplicate. Standard curve, control, and sample quality control were performed to ensure proper assay performance. Study sample values for each of the analytes were determined using 4 and 5 parameter logistic, weighted and non-weighted curve fitting algorithms included in the data analysis package.

**Translational and non-clinical methodology**

*Molecular localization studies on archived normal and diseased tissues (IHC and ISH)*

IHC studies were conducted using FFPE tissues; these tissues were sectioned at 5μm, bar coded and placed in the Ventana Discovery XT autostainer (Roche). Antigen retrieval, primary antibody dilution, incubation temperature and duration, detection technique and chromogen were optimized on non-study archived tissue and included evaluation of an irrelevant antibody control (mouse IgG1, BD Biosciences, and known negative and positive cell lines and tissues. Staining with the anti-CDH6 antibody (clone 2B6, Invitrogen, # MA1-06305) used a final antibody dilution of 1 µg/ml. Staining was completed on a Ventana XT autostainer using a 60 minutes primary incubation at a concentration of 1.0 µg/ml and a 4 minutes OmniMap anti-Rabbit HRP (Multimer HRP) secondary.

In situ hybridization (ISH) to detect CDH6 mRNA was performed using Advanced Cell Diagnostics (ACDBio) and Ventana Medical Systems (Roche) reagents. The ISH probe for CDH6 (ACDBio, #403019) targeted the region 326-1632 (accession number NM\_005910.5). Positive peptidylprolyl isomerase B (PPIB) (and negative bacterial dihydrodipicolinate reductase (DapB) control probe sets were included for each tissue to ensure mRNA quality and specificity, respectively. The ISH method followed protocols established by ACD Bio and Ventana systems using a 3, 3’-Diaminobenzidine (DAB) chromogen. Briefly, 5 µm sections were baked at 60°C for 30 minutes and used for hybridization. The deparaffinization and rehydration protocol was performed using a Sakura Tissue-Tek DR5 stainer with the following steps: xylene for 3 minutes; 2 times 100% alcohol for 3 minutes; air dried for 5 minutes. Off-line manual pretreatment in 1X retrieval buffer at 98°C to 104°C was optimized by first evaluating PPIB and DapB ISH signal and subsequently using the same conditions for all slides. Following pretreatment, the slides were transferred to a Ventana XT autostainer for finishing the ISH procedure including protease pretreatment; hybridization at 43 degrees C for 2 hours followed by amplification; and detection with HRP and hematoxylin counter stain. For photomicrographs, slides were scanned at 20-x magnification using a Leica Aperio AT2 slide scanner or images captured with an Olympus BX46 microscope coupled to an Olympus DP72 digital camera and DP2-BSW imaging software.

*CDH6 expression on immune cells (flow cytometry and RTqPCR)*

For flow cytometry procedures, on healthy human volunteer samples 50 μl whole blood per well were added to a 96-well deep well plate. RBC were lysed with ammonium-chloride-potassium buffer. The remaining WBC were washed (centrifugation at 300xg, 5 minutes, 4°C) with fluorescence-activated cell sorting (FACS) buffer and transferred to U-bottom 96-well plates. Control cell lines, previously grown as adherent cultures, were also added to the plate with the washed WBC. Human bone marrow aspirates from healthy human volunteers were processed by a brief RBC lysis, which was followed by a washing step, as described above. All cells were subjected to an Fc-blocking step for 20 minutes, on ice, followed by incubation with the respective immunophenotype (IPT) mix and each of the individual CDH6 antibodies/ADCs or their respective IgG controls. IPT mix only (Fluorescence-minus-one controls) or unstained controls were also included. Incubation with the antibodies was carried out for 30 minutes on ice, in the dark. Cells were then washed and fixed with 2% paraformaldehyde for 30 minutes on ice, in the dark. After fixation, samples were washed and stored at 4°C until flow cytometry acquisition.

In addition, compensation controls were prepared for each individual fluorophore with the use of UltraComp beads (eBioscience). In some cases, single color cell-based compensation controls were used. All samples were acquired on the Fortessa flow cytometer. Data was analyzed with FlowJo V.10 software. Expression of CDH6 on immune cell populations was calculated and reported as GMFI (FITC) for CDH6.

RTqPCR was performed on RNA extracted from bone marrow, whole blood, kidney, and control cell lines. For flash-frozen tissues, approximately 30-50 mg tissue per sample was placed in tubes containing ceramic beads (MagNA Lyser Green Beads, Roche) and 900-µl Qiazol reagent (Qiagen RNeasy Universal Plus Kit, Qiagen). Samples were homogenized twice using the MagNA Lyser instrument (Roche) with a setting of 6500 rpm for 30 sec and 90 sec cooling on ice between cycles. The homogenate was processed for subsequent manual RNA isolation according to the manufacturer’s instructions for Qiagen RNeasy Universal Plus Kit. For fresh bone marrow aspirates, extraction was carried out per the manufacturer’s instructions using the Qiagen RNeasy Plus Mini Kit (Qiagen). Whole blood samples in RNA protect tubes were brought to room temperature for 2 h before processing. Extraction was carried out per the manufacturer’s instructions using the Qiagen RNeasy Protect Animal Blood Kit (Qiagen).

Synthesis of cDNA was carried out with 100 ng of total RNA per sample using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, #4368814) per the manufacturer’s instructions. qPCR reactions were performed using the TaqMan Gene Expression Master Mix (Applied Biosystems, #4369016) per the manufacturer’s instructions, using a 40 μl reaction volume and 5 ng cDNA per reaction. RT-PCR amplification of cDNA samples was carried out on the QuantStudio 7 instrument (Applied Biosystems) using commercially available TaqMan PCR probes. Each reaction was performed in triplicate. Raw data were analyzed per individual sample utilizing a relative quantification method assuming equivalent reaction efficiencies of the reference gene (18S ribosomal RNA) and each gene of interest. After analysis, ratios of gene expression over 18S ribosomal RNA expression were graphed in arbitrary units.

*In vitro* *cytokine release assay*

Whole blood from 5 healthy human donors was incubated with the ADC HKT288, the corresponding unconjugated CDH6 targeting antibody (LTV977),a non-binding IgG1 (hIgG1) antibody, or a non-binding IgG1 antibody conjugated to a maytansinoid linker/payload sulfo-SPDB-DM4 (hIgG1-DM4). In parallel, the assay included two separate positive controls: lipopolysaccharide (LPS) and alemtuzumab. LPS was included at 2 concentrations, 10 and 1 μg/mL, concentrations selected to lead to a robust release of cytokines from activated immune cells subsets. This control was included to confirm the functionality of the assay matrix. The anti-CD52 antibody alemtuzumab was included as a positive control that allowed benchmarking to the clinical situation as administration of this drug leads to cytokine release syndrome (CRS) in patients. The concentrations selected, 300 and 100 μg/mL, have been shown to induce robust cytokine release in this in vitro system. As a negative control, whole blood was incubated with phosphate buffered saline alone. After 24 hours, plasma supernatants were collected and the cytokine levels were determined using a bead-based multiplex immunoassay approach (Luminex). An 8-plex panel of proinflammatory cytokines (IL-1β, IL-2, IL-6, IL-8, IL-10, IFN-γ, TNF-α, and MIP-1α) were included in this study.

*Syngeneic mouse model in vivo study*

Renca cells were obtained from ATCC (#CRL-2947) and engineered to express murine CDH6 (Genecopoeia #Mm29955) using an EF1 alpha-driven lentiviral vector. The resulting cell population was sorted twice by FACS to select for CDH6-positive cells that were maintained in Roswell Park Memorial Institute 1640 medium supplemented with 10% fetal bovine serum. All animal studies were performed according to the Novartis Institutes for BioMedical Research (NIBR) Animal Care and Use Committee protocols and regulations. For the Renca-mCDH6 xenograft studies, female Balb/cJ mice from Jackson Laboratories were implanted subcutaneously with 5x105 cells/mouse suspended in Hanks balanced salt solution. Tumors were measured following implant and animals were randomized into groups of equal mean tumor volume (150 mm3) prior to treatment initiation. Treatments were prepared in phosphate buffered saline and performed by single intravenous injection. In this experiment a single dose of 5 mg/kg HKT288 or isotype control, ADC or vehicle was administered. Samples for cytokine analysis were collected 24 hours post dose and processed for Mesoscale Diagnostics (MSD) electrochemiluminescent immuno-assay measurement for murine MCP1 and MIP1β according to the manufacturer’s guidelines.

**Additional investigative safety studies**

*Low CDH6 expression on immune cells*

To investigate the relevance of CDH6 expression and possible HKT288 binding to immune cells, flow cytometry and qPCR were performed on blood and bone marrow cells obtained from healthy human volunteers (Suppl. Fig 4). Flow cytometry analysis demonstrated variable low levels of cell surface expression of CDH6 on myeloid cells and natural killer cells. This near- background CDH6 signal was detected almost exclusively with the unconjugated antibody and was not found or was very low when the cells were stained with the DM4-conjugate. The biological significance of this low CDH6 expression is questionable considering that CDH6 was not found in the same samples (blood and bone marrow aspirates) by qPCR. In addition, the immune cell populations identified in the flow cytometry analysis (bone marrow aspirates) were also CDH6 negative in the IHC analysis. The low levels of CDH6 expression measured by qPCR in a subset of the bone marrow samples are unlikely to represent expression on the immune cells because the CDH6-positive samples were from flash-frozen, cadaver bone marrow collections. It is likely that the low-level expression in these samples is a result of sample contamination with vascular tissue as CDH6 was found to be expressed in this cell type. Vascular tissue is not expected to be found in the fresh bone marrow aspirates collected from living donors, thus the difference in the collection methods could account for the lack of CDH6 expression in these donors.

*No induction of cytokine release in whole blood in vitro by HKT288*

*In vitro* whole blood cytokine release experiments were conducted to determine if HKT288 induces cytokine release from peripheral blood immune cells via potential off-target mechanisms. Whole blood from five healthy human donors was incubated with HKT288, the CDH6 targeting antibody; a non-binding IgG1 (hIgG1) antibody; or a non-binding IgG1 antibody conjugated to a maytansinoid linker/payload sulfo-SPDB-DM4 (hIgG1-DM4). In parallel, LPS and alemtuzumab were included as positive controls to confirm the functionality of the assay system and to act as a clinical benchmark. After 24 hours, plasma supernatants were collected and the cytokine levels were determined using a bead-based multiplex immunoassay approach (Luminex). Test items were included at concentrations 7.5, 67.5 and 150 µg/ml corresponding to the higher range of Cmax at each cohort. No increases in cytokine levels were observed in donors after incubation with HKT288 or unconjugated anti-CDH6 antibody and the findings did not support a direct on- or off-target effect mediated through peripheral blood immune cells obtained from healthy human volunteers.

**Suppl Fig. 1: Individual concentration-time profiles for total ADC and total antibody**

ADC, antibody-drug conjugate; C, cycle; Q3W, every 3 weeks; tAb, total antibody; tADC, total ADC.

**Suppl Fig. 2: Target lesion size evaluation (Subject 4)**

Tumor evaluation were performed every 2 cycles (Screening, C3D1, C5D1, C7D1, C9D1).

C, cycle; D, day.

**Suppl Fig. 3: Tissue cytokine levels for murine MCP1 and MIP1β at 24 hours post dosing with 5 mg/kg HKT288, control IgG-ADC or vehicle**

Tissues analyzed were plasma, liver, brain and tumor. Unpaired t-tests were performed for all groups and statistically significant differences in cytokine levels between groups are highlighted.

The horizontal bars in the figures represent the median values.

ADC, antibody-drug conjugate; IgG, immunoglobulin G; MCP1, monocyte chemoattractant protein 1; MIP1β, macrophage inflammatory protein 1β.

**Suppl Fig. 4: Potential etiologies for neurological AEs – a) CDH6 expression in human bone marrow samples and control cell lines by QPCR; b) CDH6 expression in subsets of immune cells in human bone marrow by FACS**

OVCAR8 and OVCAR3 are cell lines.

The error bars represent the standard deviation across the three technical replicates.

ADC, antibody-drug conjugate; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate GMFI, geometric mean fluorescence intensity; mAb, monoclonal antibody; NK, natural killer; QPCR, quantitative polymerase chain reaction.

**Suppl Fig. 5: Potential etiologies for neurological AEs – CDH6 expression in CNS and blood vessels**

AE, adverse events; CD68, cluster of differentiation 68; CNS, central nervous system; IHC, immunohistochemistry; ISH, *in situ* hybridization; TCR, tissue cross reactivity.

**Suppl Table 1: Subject pretreatment**

**Suppl Table 2: Adverse events regardless of relationship to the study drug**