Supplementary information

Eligibility Criteria for the study

Eligible patients had measurable or clinically evaluable and progressive disease, were age 18 years or older, had an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1, a life expectancy of ≥3 months, and adequate hematopoietic (hemoglobin ≥100 g/L; white blood cell count ≥3.0 × 109/L; absolute neutrophil count ≥1.5 × 10^9/L; platelet count ≥100 × 10^9/L), hepatic (aspartate aminotransferase and alanine aminotransferase ≤2.5 × upper limit of normal (ULN) or ≤5 × ULN if hepatic metastases present; bilirubin ≤1.5 × ULN; alkaline phosphatase ≤5.0 × ULN), and renal functions (serum creatinine <1.5 × ULN). All anti-cancer therapies must have been completed 6 weeks (mAbs) or 4 weeks (all other agents, including investigational drugs) before start of study treatment, and patients must have recovered from all prior therapy toxicities to at least National Cancer Institute Common Toxicity Criteria (NCI-CTCAE) v3.0 grade 1. Exclusion criteria included concurrent immunosuppressive therapy and prior allergic reactions to mAb therapy, concurrent uncontrolled disease, any concurrent malignancy other than basal cell carcinoma or carcinoma in situ of the cervix, and clinical signs of brain metastasis or leptomeningeal involvement.

Assay for the determination of tomuzotuximab in serum

Tomuzotuximab serum levels were measured with a newly developed electrochemiluminescence immunoassay (ECLIA) using Meso Scale Discovery (MSD® technology, Rockville, MD, USA), based on the specific recognition of the soluble fraction of EGFR (sEGFR) by tomuzotuximab, Briefly, sEGFR (Sigma-Aldrich) was immobilized onto 96-well plates. After blocking and washing, calibration, control (pooled human serum with and without spiked tomuzotuximab) and study samples diluted in dilution buffer were added and incubated. Unbound antibody was removed by washing and bound tomuzotuximab was detected using a sulfo-Tag labeled goat-anti-human antibody. After washing, wells were filled with read buffer and chemiluminescence measured at a SectorImager® SI2400 (MSD®). The measured light signal is proportional to the concentration of tomuzotuximab in the sample, which is extrapolated from an eight-point tomuzotuximab calibration curve. The lower limit of quantification (LLOQ) of the assay is 103 ng/mL at a minimally required dilution of 1:1000.

Assay for the detection of anti-tomuzotuximab antibodies (ADA) in serum

The assay was performed at Glycotope as an ECLIA in a homogenous bridging format on the MSD® platform. Briefly, positive controls, negative controls, and test samples were diluted 1:10 (MRD), treated with acetic acid to release any anti-tomuzotuximab antibodies potentially bound to tomuzotuximab, neutralized and then incubated with biotinylated and sTAG-labeled Fab fragments of tomuzotuximab (= master mix) for two hours. For the confirmatory assay, 150 µg/mL tomuzotuximab was added to the master mix. ADA-tomuzotuximab complexes present in the samples are immobilized on a streptavidin-coated microtiter plates and, after washing and filling the wells with read buffer, chemiluminescence was measured at the SECTOR® Imager 2400 (MSD®). The measured light signal is proportional to the concentration of anti-Tomuzotuximab antibodies in the sample.

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Legends to the figures

Figure S1: Example of ADCC activity of tomuzotuximab in comparison to cetuximab on the human colorectal adenocarcinoma cell line LS174T as target and primary human PBMC from donors with different FcγRIIIa receptor allotype as effector cells: A) homozygous VV, B) heterozygous FV, and C) homozygous FF. Mean values of specific lysis (%) and standard deviation of triplicates are given. The horizontal arrows indicate the decrease in concentration of tomuzotuximab in relation to cetuximab needed to obtain the same lysis. The vertical arrows indicate the increase in maximal lysis with tomuzotuximab in relation to cetuximab at equal concentrations of antibody. A similar maximal specific lysis is obtained with tomuzotuximab for all three donors. The relative increase of maximal specific lysis in relation to cetuximab is highest with the lower affinity binding F/V and FF donor allotypes. The assay used was an europium release assay; measurements were carried out in triplicate, the incubation time was 5h, the effector:target (E:T) cell ratio was 80:1. The percentage of specific cytotoxicity was calculated as (experimental release - spontaneous release) / (maximal release - spontaneous release) x100.

Fig S2 Dose linearity of A) Cmax, and B) $AUC_{0\text{-tlast}}$ after intravenous infusion of tomuzotuximab (12 to 1370 mg).

Figure S3. Scatterplot of (A) terminal half-life (t½), and (B) clearance (CL) of tomuzotuximab at first infusion in relation to dose (12 to 1370 mg) illustrating the dose dependency of $t_{1/2}$ and CL at low tomuzotuximab doses and independence from dose at doses ≥ 480 mg.

Figure S4: Box and whisker plots of cytokine serum levels, grouped by sampling time point, measured over time in hours since start of infusion (0 h) of tomuzotuximab in relation to duration of Infusion. A) no planned break (N 17, doses 12 to 480 mg); B) planned break and extended infusion administration (N 17, doses 480 to 990 mg). In the group of patients with no planned infusion break peak cytokine levels are observed 2 h after start of infusion, and are significantly higher than those observed at the same time-point in the group of of patients with a planned infusion break and extended infusion administration. In the group of patients with a planned infusion break and extended infusion administration peak cytokine levels were observed at 6 h. With the exception of TNF-α, levels at 24 h do not differ from baseline levels (Mann-Whitney U test, 2-tailed *P*, see table).

Figure S5. Box and whisker plots of cytokine serum levels at first infusion measured over time in patients (N 6) receiving 1370 mg of tomuzotuximab administered split over two days (60 mg and 1310 mg), Significantly elevated cytokine levels are observed on the first day; on the second day cytokine levels (with the exception TNF-α) do not differ from baseline levels.