

Evidence for a Therapeutic Window of a T Cell-engaging BiTE Antibody Based on Monoclonal Antibody Cetuximab

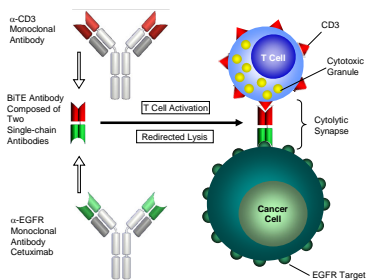
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Background

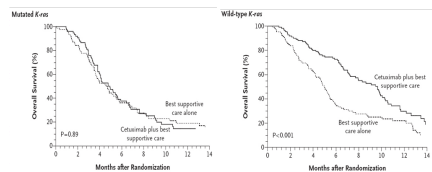
Treatment with the chimeric monoclonal antibody cetuximab (Erbix) has become the standard of care for patients with advanced colorectal cancer (CRC). Cetuximab and other EGFR-specific monoclonal antibodies predominantly inhibit cancer growth by interfering with receptor signalling. Research has shown that CRC patients with mutated KRAS and BRAF oncogenes do not respond to treatment with such antibodies.

Here we have used the binding domains of cetuximab to construct a BiTE (bispecific T-cell engager) antibody with specificity for CD3 on T cells and EGFR on the surface of target cancer cells. By transiently bridging the two cell types, the cetuximab-based BiTE antibody (C-BiTE) can directly engage and activate T cells and mount a polyclonal T cell response that is not limited by T cell specificity, presence of MHC Class I, generation of peptide antigen, or the need for T cell costimulation.



Generation and mode of action of EGFR/CD3-bispecific BiTE Antibody based on chimeric monoclonal antibody cetuximab

Treatment failure of cetuximab (Erbix®) in colorectal cancer patients with mutated KRAS



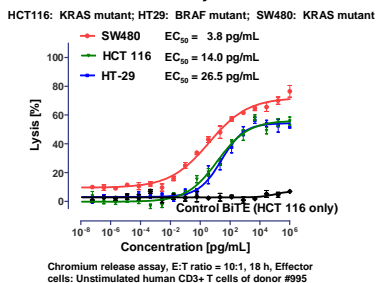
Taken from Karapetis C.S. et al. *NEJM* 359: 1757-65; 2009

Safety profile of cetuximab in Cynomolgus monkeys

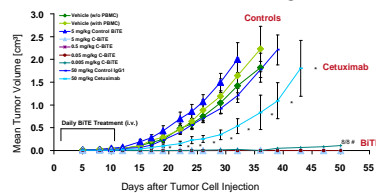
(Source: EPAR, EMA)

- Skin was the primary target organ as indicated by clinical and necropsy findings
- Skin alterations of dose-related severity and incidence
- Two intercurrent deaths at 120 mg/kg cetuximab weekly dosing due to sequelae of skin lesions
- Histopathology revealed epidermal lesions (circumscribed to multifocal) of the skin and squamous epithelium of tongue, nasal cavity and esophagus of all monkeys treated with cetuximab
- Onset of the skin toxicity (scaling) at day 15 in high-dose groups
- Diarrhea or soft feces in majority of high-dose treated monkeys

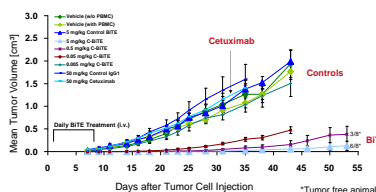
Potent redirected lysis of human CRC lines by C-BiTE antibody



Activity of C-BiTE and cetuximab parental antibody in KRAS-mutated HCT116 human xenograft model



Activity of C-BiTE and cetuximab parental antibody in BRAF-mutated HT-29 human xenograft model



HCT116, KRAS-mutated CRC cell line, or HT-29, BRAF-mutated CRC cell line, were subcutaneously injected with or without untreated human PBMC. At, as applicable, an E:T ratio of 1:2 in the right dorsal flank of female NOD/SCID mice (n=8 per group). Mice were treated daily with C-BiTE antibody from the day of tumor/PBMC injection with 5, 0.5, 0.05 or 0.005 mg/kg for eight (HT-29) or ten (HCT116) consecutive days, respectively by intravenous bolus injection in the lateral tail vein. Controls included two vehicle-treated groups (tumor cells injected with or without human PBMC) and one group of animals treated with an irrelevant control BiTE antibody (at 5 mg/kg). Cetuximab was administered by intraperitoneal (i.p.) injection at 50 mg/kg per dose twice weekly for a total of eight (HT-29) or ten (HCT116) injections, respectively. Animals in the corresponding control group were treated i.p. with human IgG (50 mg/kg). Progress of tumors was determined by external caliper measurements, and tumor volumes were calculated using a standard hemi-ellipsoid formula: (length [mm] x width [mm]²)/2.

Study of tolerability of cetuximab-based BiTE antibody in Cynomolgus monkeys

Objectives

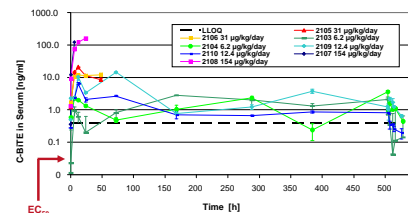
- Evaluation of toxicity of Cynomolgus cross-reactive C-BiTE over 21 consecutive days of continuous i.v. infusion
 - Mortality/morbidity, clinical signs, body weight and temperature, food consumption, ophthalmology, cardiovascular function, haematology, blood chemistry, urinalysis, necropsy, organ weights, histopathology
- Pharmacokinetics
 - Immunogenicity samples also taken
- Pharmacodynamics
 - Lymphocyte subtyping (FACS), cytokine release

Dose levels used in 3-week continuous infusion study of C-BiTE in Cynomolgus monkeys

Dose Group	Treatment	Human Therap. Dose Multiples	Human Equivalent Dose		Cynomolgus Equivalent Dose
			µg/Patient	µg/kg	µg/kg/day
1	Control	--	--	--	0
2	C-BiTE	2x	120	2	6.2
3	C-BiTE	4x	240	4	12.4
4	C-BiTE	10x	600	10	31
5	C-BiTE	50x	3,000	50	154

Two weeks before the start of treatment, a catheter was implanted for continuous infusion into the posterior vena cava of animals via a femoral vein and tunnelled subcutaneously to exit at the inter-scapular region. The catheter was attached to the delivery system via a tether system and a swivel joint. Groups of two male animals were administered either C-BiTE antibody or vehicle as stated above.

Serum levels of C-BiTE antibody in Cynomolgus monkeys

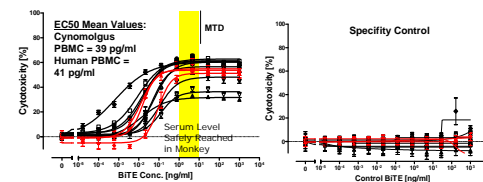


C-BiTE serum concentrations based on electro-chemoluminescence detection technology-based assay. C-BiTE in serum was immobilized by soluble EGFR protein coated to high-bind microtiter plates. Blocking solution (5% BSA) was added to plate followed by incubation for 1 h at RT. Plates were washed (PBS and 0.05% Tween-20) before serum addition. Concentrations of C-BiTE were determined in duplicate by addition of penta-His-biotin Ab (1 µg/ml) and streptavidin SULFO-TAG (2 µg/ml) after incubation for 1 h at RT and measurement with an MSD S12400 analyser. LLOQ was 0.5 ng/mL; LLOD was 0.01 ng/mL; ULOQ was 1000 ng/mL.

Safety results

- C-BiTE was well tolerated at dose levels of 6.2 and 12.4 µg/kg/day; for comparison, the BiTE binatumomab shows complete and partial responses in lymphoma patients at 1.6 µg/kg/day (Bargou et al., 2008)
- C-BiTE was not tolerated at dose levels of 31 or 154 µg/kg/day
- Unlike cetuximab, C-BiTE did not show any macroscopic evidence of skin toxicity after 3 weeks of treatment
- C-BiTE-related findings, which regressed during continued infusion, were:
 - Reduced activity during first week at 6.2 µg/kg/day only
 - Slight body temperature increase (of 0.5-1.0 °C) during first 24 hours of infusion in 3/4 animal
 - Slight body weight decrease (of 100-200 g) during first (at 12.4 µg/kg/day) or third week (at 6.2 µg/kg/day)
 - Leukopenia, specifically reductions in absolute neutrophil and lymphocyte counts in all animals to approx. 25-50% of baseline
 - Transient effect on hepatobiliary parameters (bilirubin, ALP and ALT) during first week in one 12.4 µg/kg/day animal
- Microscopic findings involved increased lymphocyte infiltration into tissues known to express EGFR

Potent redirected lysis of EGFR-expressing cell line by C-BiTE antibody engaging human or Cynomolgus T cells --Evidence for a therapeutic window--



FACS-based 24-h assay. Effector Cells were stimulated Cyno PBMC (black) or stimulated human PBMC (red). Target cells were EGFR-positive KATO III Gastric-Ca line at E:T ratio of 10:1

Conclusions

- C-BiTE antibody is well tolerated by primates at serum concentrations between 1 and 10 ng/ml that were maintained by continuous i.v. infusion for up to 3 weeks
- Safety profile is distinct from cetuximab: No evidence for skin toxicity by C-BiTE after 3 weeks
- Serum concentrations of 1-10 ng/ml [0.02 - 0.2 nM] support a high level of lysis of EGFR-positive cells *in vitro* by cynomolgus and human PBMC (EC >90%)
- First data support a therapeutic window for a cetuximab-based BiTE antibody
- C-BiTE antibody shows potent redirected lysis of KRAS- and BRAF-mutated human CRC lines HCT116 and HT-29, respectively, with EC₅₀ values in range of 10-30 µg/mL
- C-BiTE antibody is highly efficacious in xenograft mouse models preventing the outgrowth of HCT116 and HT-29 tumors at doses as low as 0.005 mg/kg/day. These cell lines are resistant to cetuximab, which was administered in the mouse models twice weekly at 50 mg/kg for up to 5 weeks
- C-BiTE antibody is crossreactive with cynomolgus monkey; it binds to CD3 and EGFR antigens of macaque origin resulting *in vitro* in potent redirected lysis with an EC₅₀ value of 20 µg/mL [0.4 pM]