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DuoBody-EpCAMx4-1BB mediates conditional T-cell co-stimulation and promotes antitumor activity in preclinical models

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Conclusions

DuoBody®-EpCAMx4-1BB (BNT314/GEN1059) is a novel Fc-inert bispecific antibody designed to boost antitumor immune responses through EpCAM-dependent 4-1BB agonistic activity.

In preclinical studies we found that:

- DuoBody-EpCAMx4-1BB induced 4-1BB signaling, thereby enhancing activation, proliferation, cytokine secretion, and cytotoxic activity of human T cells *in vitro* and *ex vivo*.
- 4-1BB agonistic activity of DuoBody-EpCAMx4-1BB was strictly conditional, *i.e.*, dependent on EpCAM binding.
- An Fc-inert hEpCAMxm4-1BB bispecific antibody exhibited antitumor activity *in vivo*, providing proof-of-principle for dual targeting of EpCAM and 4-1BB.
- DuoBody-EpCAMx4-1BB was well-tolerated in cynomolgus monkeys.

Based on these results, DuoBody-EpCAMx4-1BB is hypothesized to boost antitumor immunity in patients with EpCAM⁺ tumors. The clinical safety and preliminary antitumor activity of DuoBody-EpCAMx4-1BB will be investigated in patients with solid tumors in a first-in-human trial.

References

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Disclosures

Fellermeier-Kopf, Imle, Janaitis, Köhne, Muik, Nuermberger, Toker: Employee and stockholder of BioNTech. **Şahin, Türeci:** Management board member, employee and stockholder of BioNTech. **Ahmadi, Breij, Burm, Diks, Gamse, Guelen, Houtkamp, Ioan-Facsinay, Kemper, Satijn:** Employee and stockholder of Genmab.

Introduction

- 4-1BB is recognized as a promising target to enhance antitumor immunity, although classical 4-1BB agonistic antibodies have been associated with the occurrence of dose-limiting liver toxicity or have shown lack of efficacy as monotherapy in clinical trials[1].
- Bispecific antibodies (bsAbs) redirecting 4-1BB agonistic activity to the tumor microenvironment were shown to potently enhance antitumor immune responses in preclinical mouse models[1,2].
- EpCAM is highly expressed on the surface of tumor cells and accessible for antibody binding in many solid tumor types of epithelial origin, whereas in healthy epithelia EpCAM is largely shielded by tight junctions[3,4].
- DuoBody-EpCAMx4-1BB (BNT314/GEN1059) is an Fc-inert bsAb that is generated by controlled Fab-arm exchange (DuoBody platform) of an EpCAM-specific human IgG1 antibody and a 4-1BB-specific humanized IgG1 antibody (**Fig 1**).
- DuoBody-EpCAMx4-1BB binds to immobilized recombinant human EpCAM and human 4-1BB with affinities (K_D) of 56 nM and 0.12 nM, respectively.

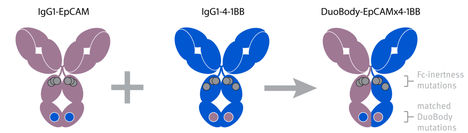

Figure 1: Generation of DuoBody-EpCAMx4-1BB by controlled Fab-arm exchange.

Proposed mechanism-of-action

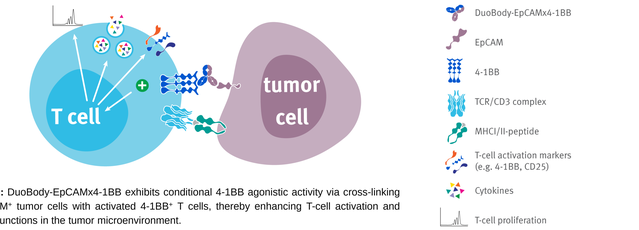

Figure 2: DuoBody-EpCAMx4-1BB exhibits conditional 4-1BB agonistic activity via cross-linking of EpCAM⁺ tumor cells with activated 4-1BB⁺ T cells, thereby enhancing T-cell activation and effector functions in the tumor microenvironment.

Results

EpCAM expression in human solid tumors

- Immunohistochemistry analyses revealed solid tumor indications with high EpCAM expression (as shown by an H-score of >200) and indications with variable EpCAM expression (**Fig 3**).

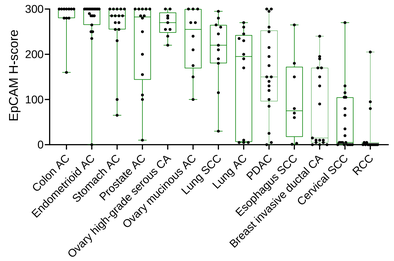

Figure 3: Immunohistochemistry analysis of EpCAM expression in tumor tissue microarrays. H-score was calculated based on manual scoring of EpCAM staining intensity by a certified pathologist (0 = no, 1 = low, 2 = intermediate, 3 = high staining) using the formula: 1 × % low pos. + 2 × % intermediate pos. + 3 × % high pos. cells. Symbols represent tissue cores of individual patients. AC, adenocarcinoma; CA, carcinoma; RCC, renal clear cell carcinoma; SCC, squamous cell carcinoma; PDAC, pancreatic ductal adenocarcinoma.

Conditional 4-1BB agonistic activity *in vitro*

- DuoBody-EpCAMx4-1BB exhibited conditional (*i.e.*, EpCAM-dependent) 4-1BB agonistic activity *in vitro* (**Fig 4A**).
- 4-1BB agonistic activity of DuoBody-EpCAMx4-1BB was observed in the presence of EpCAM⁺ tumor cell lines showing a range of EpCAM expression levels (**Fig 4B**).

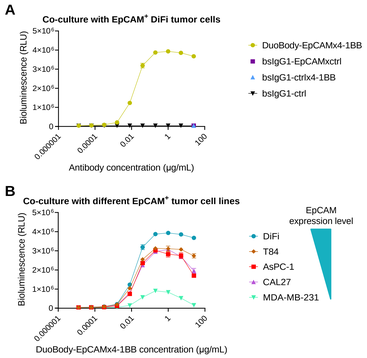

Figure 4: In reporter Jurkat T cells, activation of 4-1BB results in luciferase expression, which is measured by conversion of a bioluminescent substrate. **A.** 4-1BB agonistic activity of DuoBody-EpCAMx4-1BB in co-cultures of reporter Jurkat T cells and EpCAM⁺ DiFi tumor cells. **B.** 4-1BB agonistic activity of DuoBody-EpCAMx4-1BB in co-cultures of reporter Jurkat T cells with a panel of five EpCAM⁺ tumor cell lines. Data shown are mean bioluminescence ± SD from duplicate measurements. RLU, relative light units.

T-cell proliferation, activation, and cytokine secretion *in vitro*

- In co-cultures of healthy donor PBMCs and different EpCAM⁺ tumor cells, DuoBody-EpCAMx4-1BB dose-dependently enhanced proliferation of polyclonally stimulated T cells, except for MDA-MB-231 cells which had the lowest EpCAM expression levels (**Fig 5A**).
- In co-cultures of healthy donor PBMCs with EpCAM⁺ DiFi tumor cells, DuoBody-EpCAMx4-1BB increased activation of polyclonally stimulated T cells (**Fig 5B**), and enhanced proinflammatory cytokine secretion (**Fig 5C**).
- DuoBody-EpCAMx4-1BB also enhanced T-cell activation and granzyme B secretion in co-cultures of PBMCs from cancer patients with EpCAM⁺ DiFi tumor cells (**Fig 5D-E**).
- Enhanced T-cell activation and cytokine secretion were dependent on target cross-linking, as monovalent EpCAM-specific or 4-1BB-specific antibodies had no effect (**Fig 5B-E**).

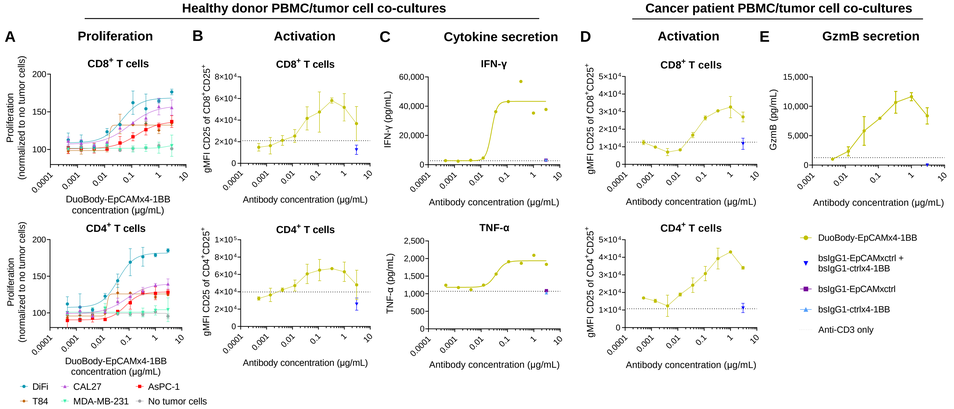

Figure 5: PBMCs were co-cultured with EpCAM⁺ tumor cells and stimulated with an anti-CD3 antibody in the presence of DuoBody-EpCAMx4-1BB or control antibodies for 4 days. **A.** Co-cultures of different tumor cell lines with healthy donor PBMCs: T-cell proliferation, as determined by CFSE dye dilution **B-C.** Co-cultures of DiFi tumor cells with healthy donor PBMCs: Cell-surface CD25 expression levels (C) and supernatant cytokine concentrations (D). **D-E.** Co-cultures of DiFi tumor cells with PBMCs of colorectal cancer patients: Cell-surface CD25 expression levels (D) and supernatant GzmB concentrations (E). Data shown are mean ± SD of duplicate wells (A, B, D, E) or single measurements (C) from one representative out of three to four donors.

CD8⁺ T-cell mediated tumor cell killing *in vitro*

- Cytotoxic function of antigen-specific CD8⁺ T cells was assessed in co-culture with EpCAM-transduced MDA-MB-231 tumor cells expressing the model antigen claudin-6 (**Fig 6A**).
- DuoBody-EpCAMx4-1BB increased the percentage of activated CD8⁺ T cells showing cytotoxic markers (**Fig 6B**) and enhanced CD8⁺ T-cell mediated tumor-cell killing (**Fig 6C-D**).
- Enhanced cytotoxic function was dependent on target cross-linking, as monovalent EpCAM-specific or 4-1BB-specific antibodies had no effect (**Fig 6B-D**).

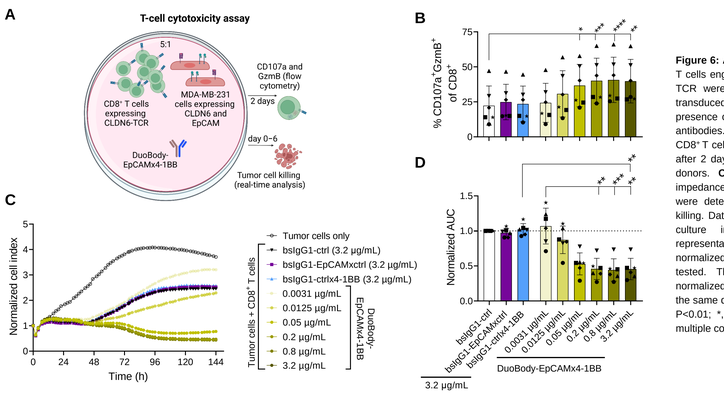

Figure 6: A. Healthy donor PBMC-derived CD8⁺ T cells engineered to express a CLDN6-specific TCR were co-cultured with CLDN6⁺ EpCAM-transduced MDA-MB-231 tumor cells in the presence of DuoBody-EpCAMx4-1BB or control antibodies. **B.** Percentages of CD107a⁺GzmB⁺ CD8⁺ T cells were determined by flow cytometry after 2 days. Data represent mean ± SD of six donors. **C.** Cell index values derived from impedance measurements during the co-culture were determined as a measure of tumor-cell killing. Data normalized to the time point of co-culture initiation are shown from one representative donor. **D.** AUC analysis of normalized cell-index curves of all six donors tested. The AUC of each treatment was normalized to bsIgG1-ctrl-treated cultures from the same donor. ****, P<0.0001; ***, P<0.001; **, P<0.01; *, P<0.05; Friedman test with Dunn's multiple comparisons test. CLDN6, claudin-6.

Expansion of human tumor-infiltrating lymphocytes *ex vivo*

- DuoBody-EpCAMx4-1BB enhanced the expansion of NK cells and CD8⁺ T cells from cultured human colorectal cancer tissue fragments, compared to no antibody control (**Fig 7**); observed in cultures from four of five patients tested.
- CD4⁺ T-cell expansion was enhanced in two of five patients, whereas T_{reg}-cell expansion was not enhanced in the presence of DuoBody-EpCAMx4-1BB.

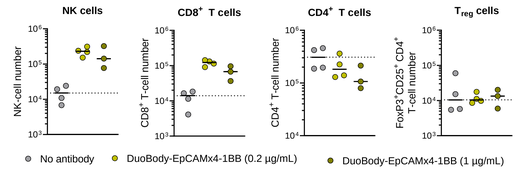

Figure 7: Tumor fragments derived from resected human colorectal cancer tissue were cultured in the presence of low-dose IL-2 (≤50 U/mL) for approximately 2 weeks. The cultures were treated on Days 0 and 3 with DuoBody-EpCAMx4-1BB. Cell numbers were determined by flow cytometry at the end of the culture. Data shown are cell numbers of three to four replicate wells and their median, from one patient of five tested.

Antitumor activity *in vivo*

- In human EpCAM-transgenic mice bearing human EpCAM-expressing MC38 tumors, an Fc-inert mouse IgG2a bsAb targeting human EpCAM and mouse 4-1BB significantly inhibited tumor outgrowth (**Fig 8A**) and significantly prolonged survival (**Fig 8B**).

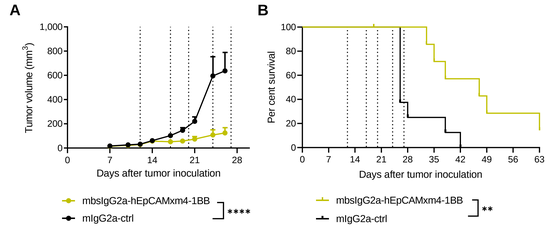

Figure 8: Human EpCAM-transgenic mice (C57BL/6 background; n=8 per group) were inoculated subcutaneously with 5 × 10^5 human EpCAM-expressing MC38 tumor cells. Starting on Day 12 after inoculation (mean tumor volume of 31 mm^3), the mice were treated intraperitoneally with 100 µg mbsIgG2a-hEpCAMxm4-1BB or control antibody twice weekly for 2.5 weeks. **A.** Tumor volumes over time (group mean ± SEM). ****, P<0.0001; Two-Way ANOVA. **B.** Kaplan-Meier analysis of survival. **, P<0.01; Log-rank (Mantel-Cox) test. Dotted lines indicate treatment days.

Non-clinical safety in cynomolgus monkeys

- DuoBody-EpCAMx4-1BB was well-tolerated in cynomolgus monkeys at doses up to 50 mg/kg (the highest tested dose, QWx5) in a GLP-compliant repeat-dose toxicity study.
- Antibody exposure increased in a dose-proportional manner after the first dose (**Table 1**). Exposure was lower in most cases after the fourth dose, which was attributed to treatment-emergent anti-drug antibodies in all monkeys.

Table 1: Mean sex-combined toxicokinetic parameters

	Dose 1			Dose 4		
Dose (mg/kg)	**5**	**25**	**50**	**5**	**25**	**50**
C_{max} (mg/L)	148	810	1550	86.3	730	2010
$AUC_{0\text{-}tau}$ (mg*d/L)	321	2040	4460	24.0	414	4070

$AUC_{0\text{-}tau}$, area under the concentration-time curve during a single dosing interval; C_{max}, maximum observed concentration.

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