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In Vitro Pharmacological Comparison of a Carcinoembryonic Antigen (CEA)/CD3 Bispecific Cynomolgus-Reactive Biosimilar BiTE Antibody (CyS111) with the Preclinical Candidate MEDI-565 (MT111)

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Introduction

Carcinoembryonic antigen (CEA; CD66e, CEACAM5) is a glycosylated human oncofetal antigen that belongs to the CEA-related cell adhesion molecules (CEACAM) family of the immunoglobulin gene superfamily. CEA expression occurs at low levels in several normal tissues of epithelial origin but is highly expressed in multiple tumor types. We have developed a novel bispecific single-chain antibody construct of the bispecific T cell engager (BiTE[®]) class, MEDI-565/MT111, designed to target CEA on tumor cells and the CD3 ϵ subunit of the T cell receptor (TCR) complex present on T cells (Figure 1). MEDI-565 transiently links T cells to CEA-expressing tumor cells, leading to simultaneous T cell activation and killing of CEA-expressing tumor cells by T cells. Other than the chimpanzee, no relevant animal model exists for toxicology testing of MEDI-565 since the BiTE antibody only binds to human and chimpanzee CD3. Because use of the chimpanzee is limited due to ethical considerations and the inability to conduct full GLP-compliant toxicological studies, we have developed a biosimilar BiTE molecule, cyS111, that cross-reacts with both human and cynomolgus (cyno) CEA and binds non-human primate CD3, making it a candidate for use in toxicology studies with cynomolgus monkeys.

Objective

The objective of this set of studies was to compare the in vitro biological activity of the cyno CD3-specific BiTE cyS111 with that of the preclinical candidate MEDI-565/MT111 and to evaluate its use in nonclinical safety profile studies in cyno monkeys.

Results

MEDI-565 bound to human CEA with an 8-fold higher affinity than cyS111 bound to cyno CEA, whereas cyS111 bound with an 8-fold higher affinity to cyno CD3 than did MEDI-565 to human CD3 as determined by flow cytometry-based cell binding assays (Table 1). Despite these moderate differences in binding affinity to their respective antigens, MEDI-565 and cyS111 redirected T cells to kill human CEA-expressing (CHO/huCEA) and cyno CEA-expressing CHO cells (CHO/cyno CEA), respectively, with similar EC₅₀ values (Figure 2). In addition, MEDI-565 and cyS111 displayed similar kinetics of target cell lysis and T cell activation and cytokine release (Figure 3).

Interestingly, MEDI-565 induced proliferation of CD8 T cells substantially more than CD4 T cells (Figure 4A). In contrast, cyS111 mediated robust proliferation of both CD4 and CD8 T cell subpopulations indicating a different quality of T cell response (Figure 4B).

MEDI-565 demonstrated specific target cell lysis, T cell activation, and cytokine release only in the presence of CHO/huCEA cells (Figure 5). In contrast, whilst cyS111 specifically killed only CHO/cyno CEA cells, it also non-specifically activated T cells and induced the release of cyno cytokines when T cells were incubated with CEA-negative CHO cells or in the absence of appropriate target cells entirely (Figure 5).

Self-association of BiTE antibodies over time may increase the potency of their biological activity through an increased ability to bind and independently signal through CD3. Samples with various aggregate content of cyS111 or MEDI-565 were compared for potency of T cell killing and activation (Figure 6). MEDI-565 with high aggregate content enhanced cytotoxicity; however, high aggregate content cyS111 did not enhance cyS111-mediated cell killing. Similar results were found for T cell activation and cytokine release (data not shown).

Mechanism of Action of MEDI-565 and cyS111

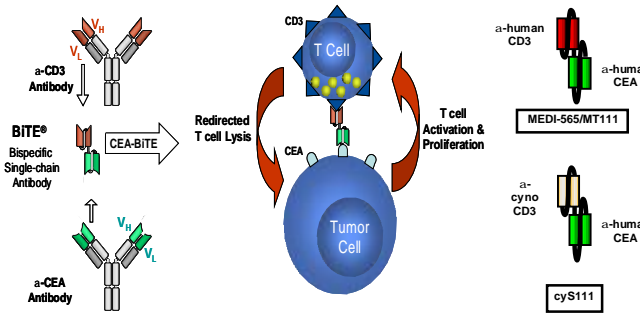


Figure 1. MEDI-565 and cyS111 BiTE molecules (right) were created by fusion of single-chain variable fragments (scFvs) from an antibody that recognizes human and cyno CEA with scFvs from antibodies that recognize either human or cyno CD3, respectively (left). BiTE antibodies engage both CD3 T cells and CEA-expressing target cells to specifically activate T cells for the redirected lysis of CEA-expressing cancer cells (Middle).

Binding Affinity of MEDI-565 and cyS111 for CEA and CD3

Antigen	Human CEA	Cyno CEA	Human CD3	Cyno CD3
BiTE	MEDI-565	cyS111	MEDI-565	cyS111
K _D ± SD (nM)	5.6 ± 1.0	43 ± 5.7	310 ± 67	38 ± 15

Table 1. Equilibrium dissociation binding constant (K_D) values were determined by flow cytometry-based saturation binding experiments using MEDI-565 or cyS111 with CHO/huCEA or CHO/cyno CEA or with freshly isolated human or cyno CD3 T cells.

MEDI-565 and cyS111-Mediated Cytotoxicity

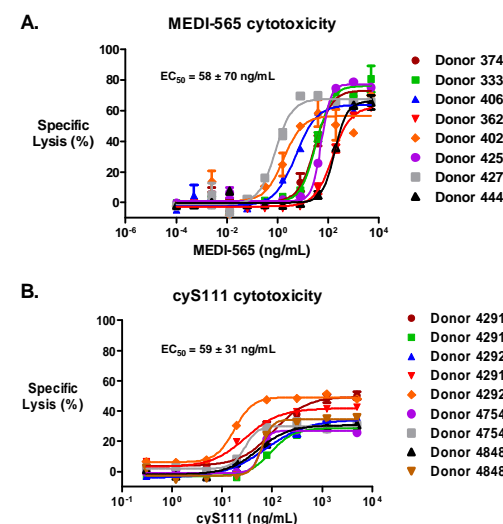


Figure 2. CHO/huCEA cells were incubated with human CD3 enriched T cells and MEDI-565 (A) or CHO/cyno CEA were incubated with cynomolgus peripheral blood mononuclear cells (PBMCs) and cyS111 (B), at an effector-to-target (E:T) ratio of 10:1 for 72 hours. Subsequently, lysis of target cells was determined using a flow cytometry-based viability assay where propidium iodide uptake by Vybrant[®] DIO (Invitrogen) dye-labeled target cells was monitored. Different dose response curves represent unique T cell or PBMC donors as indicated. Symbols, mean of duplicate measurements. Error bars, SEM. Mean EC₅₀ values ± standard deviation of lysis are shown at top of graph, where EC₅₀ = concentration of BiTE required for 50% of maximal lysis.

Kinetics of T Cell Killing, Activation and IFN γ Release by MEDI-565 and cyS111

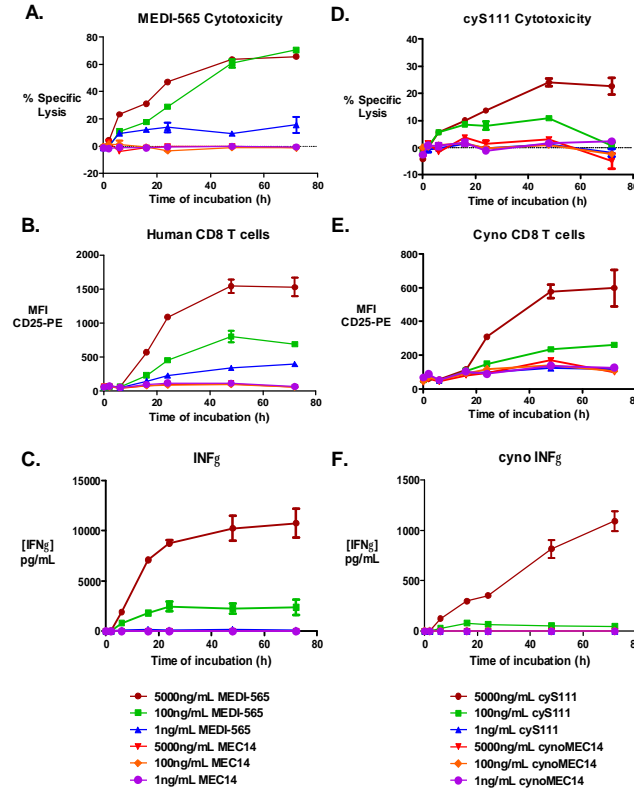


Figure 3. Human T cells and CHO/huCEA cells were incubated with MEDI-565 or the control BiTE MEC14 as indicated (left) and cytotoxicity (A), CD25/IL-2R up-regulation (B), and IFN γ release (C) were measured by flow cytometry. For comparison, cyno PBMC and CHO/cyno CEA cells were incubated with cyS111 or the control BiTE cynoMEC14 (right) and cytotoxicity (D), CD25/IL-2R up-regulation (E), and cyno IFN γ cytokine release (F) were measured. Error bars, SEM; MFI CD25-PE, mean fluorescence intensity of PE-conjugated anti-human/cyno CD25 antibody, a measure of T cell activation.

Induction of CD4 and CD8 T Cell Proliferation by MEDI-565 and cyS111

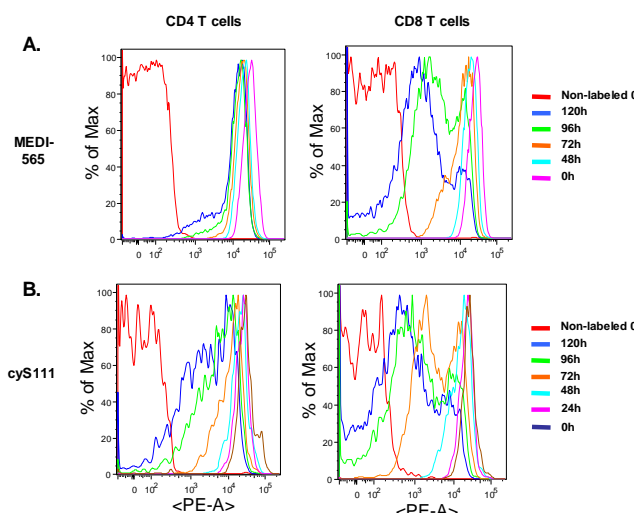


Figure 4. Induction of CD4 and CD8 T cell proliferation by MEDI-565 or cyS111. Human (A) or cyno (B) CD4 and CD8 T cells were labeled with PKH26 dye (Sigma-Aldrich) and incubated with 200 ng/mL BiTE and CHO/huCEA or CHO/cyno CEA target cells, respectively, in cellular cytotoxicity assays for the indicated times at right. Dye dilution occurs during cell division and is seen as a leftward shift in PKH26 (PE-A channel) intensity towards that of non-labeled cells (non-labeled 0h).

Specificity of T Cell Killing, Activation and IFN γ Release by MEDI-565 and cyS111

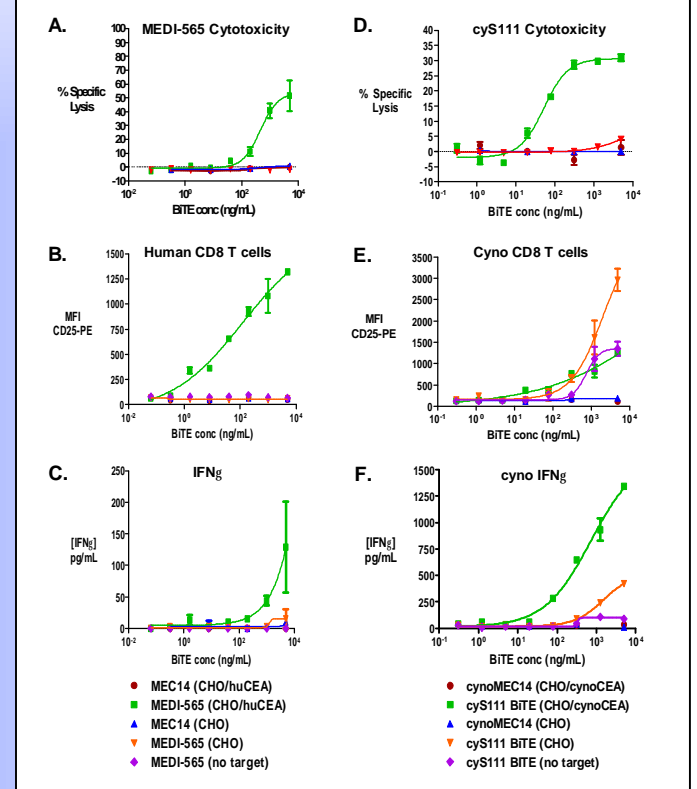


Figure 5. MEDI-565-induced cytotoxicity (A), CD8 T cell activation (B), and IFN γ release (C) was measured by flow cytometry and compared to cyS111-mediated cytotoxicity (D), cyno CD8 T cell activation (E), and cyno IFN γ release (F). MFI CD25-PE, mean fluorescence intensity of antibody-bound CD25 (IL-2R) on the surface of CD8 T cells, a measure of T cell activation. CHO, CEA-negative parental CHO cells. Error bars, SEM.

Potency of MEDI-565 and cyS111 Aggregates

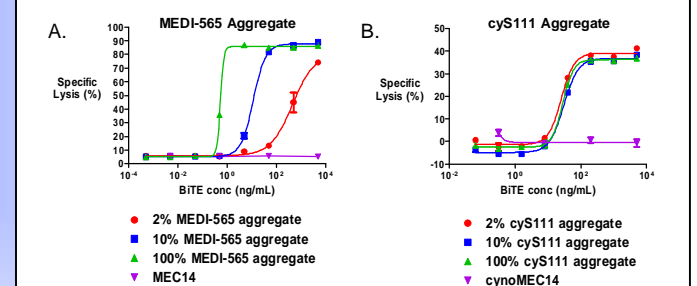


Figure 6. Specific activity of MEDI-565 and cyS111 of various antibody aggregate contents. Human T cells were incubated with CHO/huCEA cells and MEDI-565 of various aggregate contents, as indicated, and specific lysis was measured by flow cytometry (A). Similarly, cyno PBMCs were incubated with CHO/cyno CEA target cells and cyS111 of various aggregate contents and specific lysis measured (B).

Conclusions

CyS111 and the preclinical candidate MEDI-565 demonstrated similar potency of CEA-specific target cell killing, but differed in the specificity with which they induced T cell activation and in the proliferation of T cell subpopulations, and in the activity of their aggregated forms. Due to these differences, cyS111 may have limited utility in toxicity studies in cynomolgus monkeys in predicting the safety profile of MEDI-565 in humans.