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A Novel Fc-optimized Antibody Drug Conjugate Targeting CD7 as a Therapeutic Strategy in T-Cell Acute Lymphoblastic Leukemia

Carina Lynn Gehlert[1], Denis Martin Schewe[2,9], Katja Klausz[1,9], Steffen Krohn[1], Dorothee Winterberg[1], Fotini Vogiatzi[3], Ammelie Svea Boje[1], Anja Lux[4], Falk Nimmerjahn[4], Regina Scherließ[5], Andreas Humpe[6], Martin Schrappe[3,9], Gunnar Cario[3,9], Thomas Valerius[7,9], Lars Fransecky[8,9], Monika Brüggemann[8,9], Claudia Dorothea Baldus[8,9], Martin Gramatzki[1], Lennart Lenk[3,9], Christian Kellner[6], Matthias Peipp[1,9]

[1]Division of Antibody-Based Immunotherapy, Department of Hematology and Oncology, Kiel University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany; [2]Medical Faculty, Otto-von-Guericke University Magdeburg, Magdeburg Germany; [3]Department of Pediatrics I, University Hospital Schleswig-Holstein, Kiel, Germany; [4]Division of Genetics, Department of Biology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; [5]Department of Pharmaceutics and Biopharmaceutics, Kiel University, Kiel, Germany; [6]Division of Transfusion Medicine, Cell Therapeutics and Haemostaseology, University Hospital, LMU Munich, Munich, Germany; [7]Division of Stem Cell Transplantation and Immunotherapy, Department of Hematology and Oncology, University Hospital Schleswig-Holstein, Campus Kiel, Germany; [8]University Hospital Schleswig-Holstein, Department of Hematology and Oncology, Campus Kiel, Kiel, Germany; [9]Clinical Research Unit CATCH ALL (KFO 5010/1) funded by the Deutsche Forschungsgemeinschaft, Kiel, Germany

Abstract

Despite progress in improving treatment regimens for patients with T-cell acute lymphoblastic leukemia (T-ALL), the therapeutic options are still limited, and especially antibody-based immunotherapy is not established. The CD7 antigen represents a promising target structure in T-ALL since it is strongly expressed in different T-ALL subtypes including early T-cell precursor (ETP)-ALL. Therefore, different approaches are currently pursued for targeting CD7, including CAR-T cell therapy. Due to its high internalization capacity CD7 also represents an ideal target structure for antibody drug conjugates (ADC).

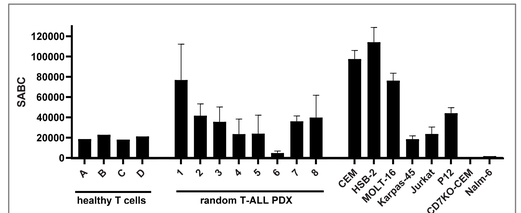
Here, a novel antibody engineering approach for CD7-targeting was evaluated in vitro and in xenograft mouse models of T-ALL. A CD7 antibody was optimized for its ability to trigger antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) by introducing two amino acid substitutions (S239D/I332E; DE-variant) in the Fc-domain. In addition, the Fc-engineered antibody was conjugated to the microtubule-disrupting agent monomethyl auristatin E (MMAE) via an enzymatic-cleavable linker (mc-vc-PABC).

The Fc-optimized ADC, CD7-DE-vcMMAE, as well as the unconjugated antibody, CD7-DE, showed improved binding to activating Fcγ receptors (FcγRIIa, FcγRIIIa) compared to a CD7 antibody lacking the DE-modification. This resulted in potent ADCC- and ADCP-activity against different T-ALL cell lines mediated by NK-cells or macrophages as effector cells. In contrast to the CD7-DE antibody, CD7-DE-vcMMAE showed strong cytotoxic effects independently of immune effector cell engagement by releasing its cytotoxic compound into T-ALL cells in an antigen-restricted manner, thereby inducing G2/M cell cycle arrest and apoptosis. CD7-DE-vcMMAE was active at subnanomolar concentrations demonstrating dose-dependent cytotoxic effects in six T-ALL cell lines. The extent of maximum growth inhibition ranged between 54-98 % and correlated with CD7 antigen density. Yet, although CD7-DE-vcMMAE did not kill CD7-negative cells directly, the linker design facilitated bystander killing activity. Thus, in co-culture experiments using CEM cells and CEM-CD7-knockout cells mimicking CD7-antigen escape, the ADC demonstrated significant killing of neighboring CD7-negative cells, thereby extending its mode of action.

The antitumor activity of the CD7-ADC was further investigated in xenograft mouse models of T-ALL. In a first model, CEM cells were injected subcutaneously into NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice and CD7-DE-vcMMAE treatment was evaluated in comparison to the unconjugated antibody CD7-DE. Treatment with CD7-DE-vcMMAE led to a significantly reduced tumor growth in comparison to CD7-DE or untreated animals. A preclinical phase II-like patient-derived xenograft (PDX) study employing eight randomly selected T-ALL-PDX samples from pediatric and adult patients was conducted. PDX-cells were injected intravenously into NSG mice and treatment was started when the leukemia load reached 1 % human blasts in the peripheral blood, reflecting an overt leukemia situation. Animals receiving therapy with CD7-DE-vcMMAE showed significant prolongation of median survival in comparison to animals treated with a similarly designed control ADC targeting an irrelevant antigen (control-DE-vcMMAE) or which were left untreated. Importantly, no leukemic blasts were found in the peripheral blood, spleen or bone marrow in animals treated with CD7-DE-vcMMAE and surviving the experimental period of 150 days.

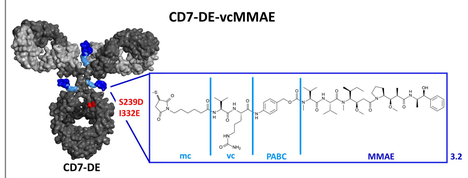
Together, the novel ADC CD7-DE-vcMMAE showed a unique set of Fc effector functions, potent direct growth inhibitory effects, bystander killing activity and efficacy in xenograft models of T-ALL. These results exhibit CD7-DE-vcMMAE as a promising therapeutic strategy and form the basis for new approaches in the treatment of T-ALL.

CD7 Cell Surface Expression in T-ALL

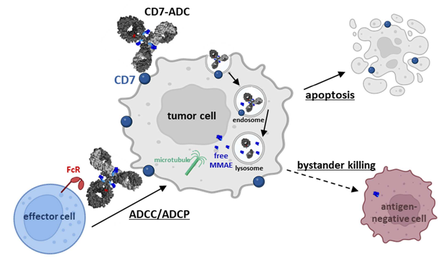


CD7 quantification on T cells from healthy donors (A-D), T-ALL patient-derived xenograft (PDX) samples (1-8) and T-ALL cell lines (CEM, HSB-2, MOLT-16, Karpas-45, Jurkat, P12) in comparison to CD7-knockout CEM cell line (CD7KO-CEM) and CD7-negative BCP-ALL cell line Nalm-6 were was performed by quantitative flow cytometry analysis to determine the Specific Antibody Binding Capacity (SABC).

Design and Mode of Action of the Novel Fc-Optimized CD7 Antibody Drug Conjugate



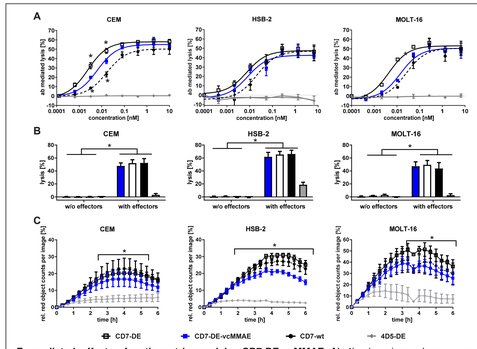
Design of CD7-DE-vcMMAE. The CD7-DE antibody is optimized for enhanced Fcγ receptor (FcγRIIa and FcγRIIIa) binding and its ability to trigger ADCC and ADCP by introducing two amino acid substitutions (S239D/I332E). CD7-DE was conjugated to the microtubule-disrupting agent monomethyl auristatin E (MMAE) via an enzymatic-cleavable linker (mc-vc-PABC), resulting in the ADC CD7-DE-vcMMAE with a drug to antibody ratio (DAR) of 3.2 MMAE-molecules per antibody.



Proposed mode of action:

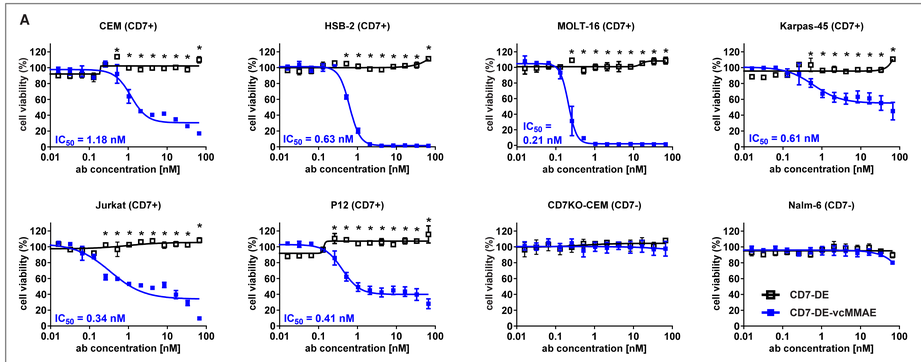
CD7-DE-vcMMAE is internalized after antigen specific binding and the valine-citruline linker is cleaved due to cathepsin B in the lysosome. The free MMAE leads to inhibition of the tubulin polymerization and results in **cell cycle arrest** and subsequent **apoptosis.** The free MMAE is permeable to cross cell membranes and is able to diffuse into antigen-negative neighboring cells and may mediate killing, called **bystander killing** / **activity.** Besides the direct cytotoxic activity of the conjugated payload, the **Fc-mediated effector functions** of the unconjugated antibody are maintained and can thus mediate antitumor effect also by engagement of effector cells.

CD7-DE-vcMMAE Triggers Fc-Mediated Effector Functions



Fc-mediated effector functions triggered by CD7-DE-vcMMAE A) 4h chromium release assays were performed to analyze ADCC. CD7-positive T-ALL cell lines (CEM, HSB-2, MOLT-16) were used as target cells and peripheral blood mononuclear cells (PBMC) of healthy donors at an Effector:Target (E:T) ratio of 40:1 were applied as effectors. Lysis triggered by CD7-DE-vcMMAE was compared to CD7-wt, CD7-DE or the 4D5-DE control antibody. * p<0.05 CD7-DE-vcMMAE vs. CD7-DE/wt **B)** ADCC of T-ALL cell lines was analyzed in presence or absence of effector cells at an antibody concentration of 6.67 nM. **C)** Phagocytosis of pHrodo-labelled T-ALL cell lines was measured for 6h by live cell imaging as relative red object counts per image in percent (%) representing phagocytosed cells. * p<0.05 CD7-DE-vcMMAE, -DE, -wt vs. 4D5-DE

CD7-DE-vcMMAE Triggers Significant Growth Inhibition in T-ALL Cell Lines



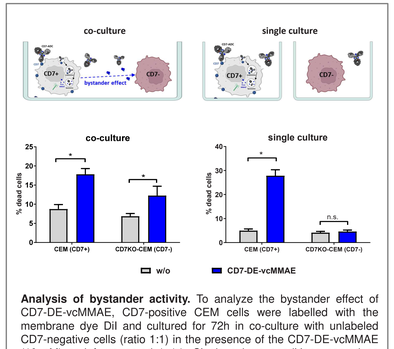
Growth inhibitory activity mediated by CD7-DE-vcMMAE. A) Cell viability of CD7-positive cell lines CEM, HSB-2, MOLT-16, Karpas-45, Jurkat, P12 and CD7-negative cell line Nalm-6 and CD7-knockout CEM cells (CD7KO-CEM) was tested by MTT-assay after 96h treatment with increasing concentrations of CD7-DE-vcMMAE or CD7-DE. CD7-DE-vcMMAE was active at subnanomolar concentrations demonstrating dose-dependent cytotoxic effects in six T-ALL cell lines (IC_{50} = 0.2 - 1 nM). * p<0.05 CD7-DE-vcMMAE vs. CD7-DE **B)** Linear correlation between the CD7 Specific Antibody Binding Capacity (SABC) of the depicted cell lines and the maximal inhibition of the cell viability in percent after CD7-DE-vcMMAE treatment.

Induction of cell cycle arrest and apoptosis. To analyze induction of apoptosis and G2/M cell cycle arrest triggered by CD7-DE-vcMMAE, CEM cells were cultured for 72h in presence of CD7-DE-vcMMAE, CD7-DE (9nM) or left untreated. For target antigen blocking, CEM cells were preincubated for 30 minutes with the parental murine antibody (30nM).

C) Annexin V and PI staining of early and late apoptotic cells. **D)** Representative histograms of hypotonic PI staining of the cell nucleus and cell cycle analysis.

Together these data show that CD7-DE-vcMMAE has antigen specific direct cytotoxic activity against T-ALL cell lines by cell cycle arrest and apoptosis induction.

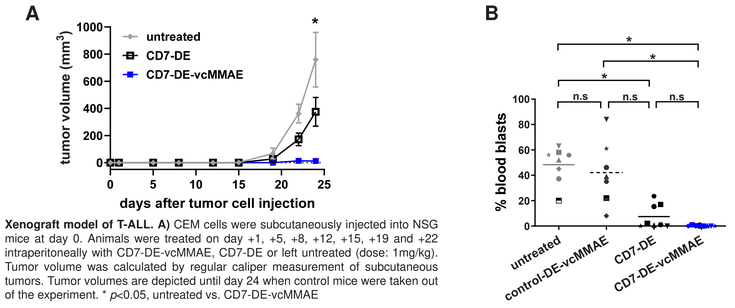
CD7-DE-vcMMAE Triggers Bystander Killing



Analysis of bystander activity. To analyze the bystander effect of CD7-DE-vcMMAE, CD7-positive CEM cells were labelled with the membrane dye DiI and cultured for 72h in co-culture with unlabeled CD7-negative cells (ratio 1:1) in the presence of the CD7-DE-vcMMAE (10 nM) or left untreated (w/o). Single culture conditions served as control. Apoptotic/necrotic cells (dead cells) were determined after 72h with Annexin V-APC staining and cell populations were separated via gating DiI-positive and DiI-negative cells. * p<0.05, n.s. not significant.

A significant number of apoptotic CD7-negative T-ALL cells (CD7KO-CEM), were detected only in co-culture with CD7-positive cells (CEM), but not in single culture.

In Vivo Anti-Tumor Efficacy of CD7-DE-vcMMAE in Xenograft Models of T-ALL



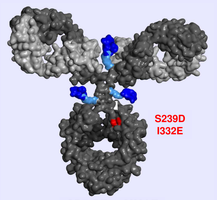
Xenograft model of T-ALL. A) CEM cells were subcutaneously injected into NSG mice at day 0. Animals were treated on day +1, +5, +8, +12, +15, +19 and +22 intraperitoneally with CD7-DE-vcMMAE, CD7-DE or left untreated (dose: 1mg/kg). Tumor volume was calculated by regular caliper measurement of subcutaneous tumors. Tumor volumes are depicted until day 24 when control mice were taken out of the experiment. * p<0.05, untreated vs. CD7-DE-vcMMAE

Kaplan-Meier log-rank test:

untreated vs. control-DE-vcMMAE	p= n.s.
untreated vs. CD7-DE	p= 0.0364
untreated vs. CD7-DE-vcMMAE	p= 0.0241
CD7-DE vs. CD7-DE-vcMMAE	p= n.s.

Efficacy of CD7-DE-vcMMAE in a preclinical phase 2-like PDX study. Eight random T-ALL PDX samples (one r/r and seven *de novo* patients) from pediatric and adult patients were intravenously injected into NSG mice and antibody therapy was started when 1 % human blasts were detected in the peripheral blood (overt leukemia model). Animals receiving therapy with CD7-DE-vcMMAE, CD7-DE, control-DE-vcMMAE or left untreated on day +1, +3, +6, +10, +13 and every 7 days thereafter (dose: 1mg/kg). **B)** Blood blasts were analyzed using flow cytometry at time points when control animals had a mean blast load of 48 %. * p<0.05, n.s. not significant **C)** Survival was analyzed by using Kaplan-Meier method and log-rank statistics.

Summary and Conclusion



- The novel antibody drug conjugate CD7-DE-vcMMAE showed significant cytotoxic activity against T-ALL cell lines
- CD7-DE-vcMMAE was capable in activating immune effector cells and triggered Fc-mediated effector functions ADCC & ADCP
- CD7-DE-vcMMAE triggered significant bystander killing of antigen negative cells which extends its mode of action
- CD7-DE-vcMMAE showed significant antitumor activity and prolonged survival in two *in vivo* models of T-ALL

➢ **With its unique set of effector functions CD7-DE-vcMMAE may represent a novel therapeutic avenue for patients with T-ALL**

Correspondence to m.peipp@med2.uni-kiel.de

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