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ARTICLE

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Enhanced potency of immunotherapy against B-cell precursor acute lymphoblastic leukemia by combination of an Fc-engineered CD19 antibody and CD47 blockade

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Abstract

CD19-directed immunotherapy has become a cornerstone in the therapy of B-cell precursor acute lymphoblastic leukemia (BCP-ALL). CD19-directed cellular and antibody-based therapeutics have entered therapy of primary and relapsed disease and contributed to improved outcomes in relapsed disease and lower therapy toxicity. However, efficacy remains limited in many cases due to a lack of therapy response, short remission phases, or antigen escape. Here, BCP-ALL cell lines, patient-derived xenograft (PDX) samples, human macrophages, and an in vivo transplantation model in NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice were used to examine the therapeutic potency of a CD19 antibody Fc-engineered for improved effector cell recruitment (CD19-DE) and antibody-dependent cellular phagocytosis (ADCP), in combination with a novel modified CD47 antibody (Hu5F9-IgG2σ). For the in vivo model, only samples refractory to CD19-DE monotherapy were chosen. Hu5F9-IgG2σ enhanced ADCP by CD19-DE in various BCP-ALL cell line models with varying CD19 surface expression and cytogenetic backgrounds, two of which contained the *KMT2A-AFF1* fusion. Also, the antibody combination was efficient in inducing ADCP by human macrophages in pediatric PDX samples with and adult samples with and without *KMT2A*-rearrangement in vitro. In a randomized phase 2-like PDX trial using seven *KMT2A*-rearranged BCP-ALL samples in NSG mice, the CD19/CD47 antibody combination proved highly efficient. Our findings support that the efficacy of Fc-engineered CD19 antibodies may be substantially enhanced by a combination with CD47 blockade. This suggests that the combination may be a promising therapy option for BCP-ALL, especially in relapsed patients and/or patients refractory to CD19-directed therapy.

INTRODUCTION

Interventions targeting CD19 have improved therapy in BCP-ALL, especially in relapsed disease. These include chimeric antigen receptor T-cells and the bispecific T-cell engager (BiTE) blinatumomab.[1] The antibody-drug conjugate loncastuximab tesirine and the Fc-engineered monoclonal antibody tafasitamab, approved for treatment of diffuse large B cell lymphoma, may provide further options.[2–5] Monoclonal antibodies have several advantages over BiTEs, because they are easier to apply, have longer serum half-lives, are broadly available and, most likely, have fewer side effects due to the absence of cytokine release syndrome. However, CD19-targeted therapy also has limitations, including a substantial amount of patients nonresponding despite CD19-positivity,[6] nonsustained

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remission phases,[7] and antigen escape leading to CD19-negative relapse.[8] There are specific patient subgroups particularly prone to these phenomena, for example, patients with *KMT2A*-rearranged leukemias, which may acquire resistance to CD19-targeted therapies by lineage switch.[9]

Some of these barriers can potentially be tackled by making CD19-directed antibody therapy more efficient and thereby causing deeper levels of remission in shorter amounts of time. We had proposed that an Fc-modified CD19 antibody, a surrogate for the approved antibody tafasitamab termed CD19-DE, is an efficient strategy to activate ADCC and ADCP in BCP-ALL therapy.[4,10] CD19-DE carries the variable domains from tafasitamab and the same two specific amino acid substitutions in its Fc domain (S239D/I332E) to improve Fcγ receptor (FcγR) engagement, but was produced in our laboratory. ADCP thereby led to a high anti-leukemic efficacy in preclinical models of highly aggressive *KMT2A*-rearranged infant leukemia.[10]

Activation of macrophages is increasingly recognized as an important effector mechanism of monoclonal antibodies in tumor therapy. Therefore, the macrophage immune checkpoint and "don't-eat-me" signal CD47 has gained attention as a target.[11] CD47 ligates the inhibitory receptor SIRPα, which upon engagement interferes with FcγR-mediated activation and impairs ADCP. For example, CD47 blockade can be highly efficient in combination with rituximab in B-cell lymphoma[12] or with demethylating agents in acute myeloid leukemia.[13] We demonstrated that the monoclonal antibody daratumumab, directed against CD38, can be highly efficient in xenograft models of T-ALL by activating ADCP.[14] This can be improved by combining daratumumab with Hu5F9-IgG2σ, a proprietary Fc-silent CD47 blocking monoclonal antibody, leading to long-lasting remissions in preclinical models of relapsed/refractory T-ALL.[15]

We therefore asked if the combination of CD19-DE with Hu5F9-IgG2σ may be able to achieve similar results in preclinical models of BCP-ALL. We demonstrate that CD19/CD47 co-targeting with two monoclonal antibodies can be highly efficient in vitro. This is shown in BCP-ALL cell lines, isolated leukemia cells from adult patients and PDX cells from pediatric patients carrying *KMT2A*-rearrangements. Importantly, CD19-DE in combination with Hu5F9-IgG2σ is able to induce an in vivo survival prolongation in NSG xenograft models of *KMT2A*-rearranged pediatric BCP-ALL, even in samples refractory to CD19-DE monotherapy.

MATERIALS AND METHODS

Cell lines and PDX samples

Nalm-6 (*t* [5;12]), RS4;11 (*t* [4;11]), REH (*t* [12;21]), and SEM (*t* [4;11]) cells were purchased from DSMZ. All pediatric patients carried *KMT2A*-rearrangements (*KMT2A*::*AFF1* [samples Ped ALL #5, Ped ALL #6, Ped ALL #7], *KMT2A*::*ENL* [samples Ped ALL #1, Ped ALL #2], *KMT2A*::*MLLT3* [sample Ped ALL #4] and *KMT2A*::*MLLT10* [sample Ped ALL #3]). Adult patients carried a *KMT2A*::*ENL* fusion (sample Ad ALL p#1), a translocation *t*(7;9) resulting in a *PAX5*-fusion (sample Ad ALL p#3) or no known cytogenetic aberrations (samples Ad ALL p#2 and Ad ALL p#4). Adult patients were treated according to institutional standards and pediatric patients according to the ALL-BFM 2000/2009 protocols. Informed consent was obtained in accordance with institutional regulations.

Antibodies

The Hu5F9-IgG2σ CD47 antibody, CD19-DE, CD19-IgG1, and the control antibodies HER2-DE and HER2-IgG2σ were generated as described.[10,15,16] Trastuzumab (Roche) was obtained from the institutional pharmacy. Murine CD47 and CD19 antibodies were purchased from Invitrogen and Biolegend, respectively.

Analysis of antigen expression levels by flow cytometry

Cell surface expression of CD47 and CD19 was analyzed by flow cytometry using the Qifikit to determine specific antibody binding capacities (SABC) per cell.[16]

Xenografts

NSG mice (Charles River Laboratories) were maintained in IVCs. BCP-ALL PDX cells of seven patients were injected into the tail vein (four mice/PDX) of 6–8 weeks-old female mice. Sample size was calculated assuming 20% survival at 8 weeks in control and 90% in treatment groups ($\alpha = 0.05$, $\beta = 0.2$, power = 0.8, one-sided *t*-test). Mice were allocated to treatments (one treatment/IVC, to minimize confounder risk) by simple randomization (one mouse/group) by a blinded researcher. Antibodies were applied by intraperitoneal injection of 1 mg/kg (CD19-DE) or 2 mg/kg (Hu5F9-IgG2σ) body weight on days 1, 3, 5, 7, 10, and 15, and every 3rd week thereafter,[10,15] in a specific-pathogen free facility by other researchers. The "one animal per model per treatment" approach used was previously published[15] and used to assess survival as primary outcome measure, with predefined humane endpoints regarding general condition upon daily monitoring. Blast cell counts as secondary outcome measures were determined by flow cytometry.[10,15] The ARRIVE guidelines[17] were used.

Phagocytosis assays

Macrophages were differentiated from monocytes with macrophage colony stimulating factor (Peprotech).[15,16] In vitro phagocytosis was measured by fluorescence microscopy or live cell imaging using the IncuCyte System (Sartorius).[15,16] Target cells were coincubated with macrophages at a 2:1 target cell to macrophage ratio. Target cells were labeled with CFSE (Biolegend, microscopy) or with pHrodo (Sartorius, IncuCyte). CD19-DE and Hu5F9-IgG2σ antibodies were applied to a concentration of 10 µg/mL. Engulfed cells were microscopically counted and a phagocytic index calculated (number of engulfed target cells per 100 macrophages), or displayed as relative red object counts per image (IncuCyte) after normalization. The largest value was defined as 100%. Experiments were performed with macrophages from different donors, as indicated.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.0.2. *p*-Values ≤ 0.05 were considered statistically significant.

RESULTS

CD47 blockade enhances ADCP by CD19-DE in BCP-ALL cell lines

To investigate a potential induction of ADCP by CD19-DE in combination with Hu5F9-IgG2σ, we first initiated in vitro microscopic phagocytosis assays using the BCP-ALL cell lines Nalm-6 (*ETV6*::*PDGFRB* fusion), RS4;11 (*KMT2A*::*AF4* fusion), REH (*TEL*::*AML1* fusion), and SEM

(*KMT2A*::*AF4* fusion) and human macrophages from healthy human donors (Figure 1A). CD19 expression measured by quantitative flow cytometry was highest in SEM, intermediate in Nalm-6 and RS4;11 and low in REH (Figure 1B). CD47 expression levels were similar in all cell lines (Figure 1B). Treatment with Hu5F9-IgG2σ alone had no effects on ADCP induction in any cell line. Treatment with CD19-DE raised ADCP levels in all cell lines, which is in line with our previous findings.[10] Importantly, combination treatment significantly enhanced ADCP levels in all cell lines, as compared to control and either monotherapy alone, and was independent of CD19 expression levels (Figure 1A,B). Treatment with a control IgG2σ antibody had no significant effects (Supporting Information S1: Figure S1A), nor did a therapy with a version of trastuzumab (HER2-DE) carrying the same Fc-modifications as CD19-DE (Supporting Information S1: Figure S1B).

Microscopy results were confirmed by automated phagocytosis assays using live-cell imaging in Nalm-6 and REH cells, showing that over time, ADCP was induced by CD19-DE and further enhanced by the addition of Hu5F9-IgG2σ (Figure 1C,D). Our results show enhanced potency of CD19-DE by combination with CD47 blockade in BCP-ALL cell lines with varying CD19 surface expression and cytogenetics.

Higher efficacy of CD19-DE vs. CD19-IgG1 in combination with CD47 blockade

To determine whether the high efficacy of the CD19/CD47 combination was due to the S239D/I332E mutation, we next tested the CD19-DE antibody in parallel to the parental CD19-IgG1 version.

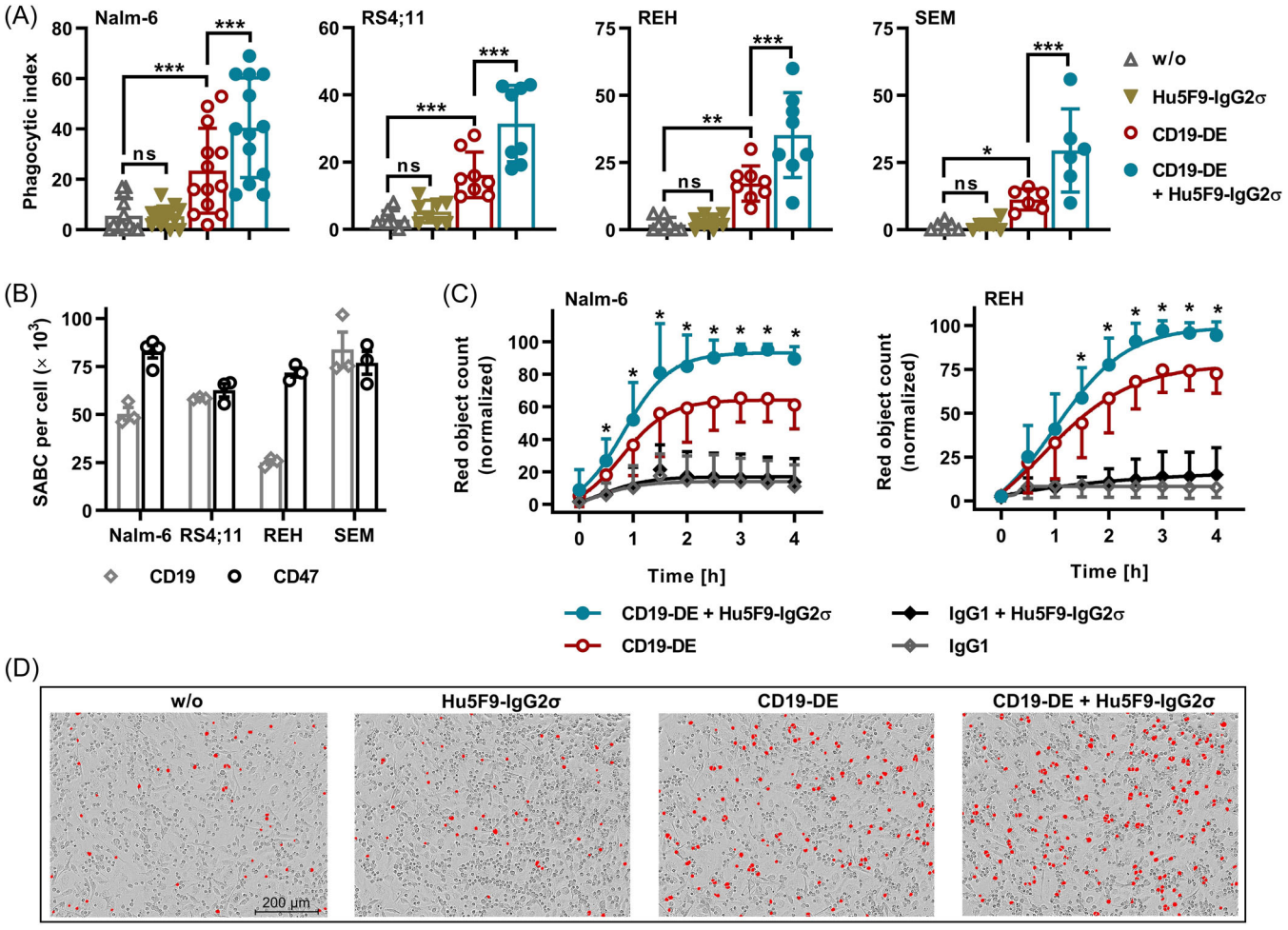

FIGURE 1 CD47 antibody blockade enhances antibody-dependent cellular phagocytosis (ADCP) by CD19-DE in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) cell lines. (A) Human macrophages were incubated with CFSE-labeled BCP ALL cell lines Nalm-6 (n = 13), RS4;11 (n = 8), REH (n = 8), and SEM (n = 6) in the absence (w/o) or in the presence of antibodies CD19-DE, Hu5F9-IgG2σ, or their combination. After 2 h, ADCP was analyzed by fluorescence microscopy. Data points indicate phagocytic index values for individual preparations of macrophages from different donors and bars represent mean values ± SD. Statistically significant differences between treatment groups are indicated (*p < 0.05; **p < 0.01; ***p < 0.001; ns, not statistically significant; one-way analysis of variance [ANOVA] and Holm-Šídák multiple corrections test). (B) BCP-ALL cells were stained with CD19 or CD47 specific antibodies and specific antibody binding capacities (SABC) were determined by calibrated flow cytometry. Bars indicate mean values ± SEM (n = 3). (C) Nalm-6 (n = 7) or REH cells (n = 5) were labeled with pHrodo and incubated with macrophages in the presence of antibodies CD19-DE, Hu5F9-IgG2σ, trastuzumab (IgG1), or their combinations as indicated. ADCP was analyzed by live cell imaging. The number of red fluorescent objects per image was determined and for each experiment the results were normalized to the highest value. Data points indicate mean values ± SD. Statistically significant differences between CD19-DE and the combination CD19-DE plus Hu5F9-IgG2σ are indicated (*<0.05 two-way ANOVA and Holm-Šídák multiple corrections test). (D) Representative images of engulfed Nalm-6 cells (indicated in red) that were incubated with macrophages for 2.5 h in the absence of an antibody (w/o) or in the presence of Hu5F9-IgG2σ, CD19-DE, or the combination CD19-DE plus Hu5F9-IgG2σ.

Analyzing in vitro phagocytosis in Nalm-6 cells by microscopy, CD19-IgG1 induced ADCP modestly as compared to CD19-DE (Figure 2A). Yet, combination of CD19-IgG1 with Hu5F9-IgG2σ raised ADCP to a level comparable to CD19-DE monotherapy, indicating that the impairment of CD47 inhibitory signaling compensates for sub-optimal FcγR activation. The maximum level of ADCP was detected by combining CD19-DE and Hu5F9-IgG2σ (Figure 2A). These results were confirmed in real-time imaging assays using the Nalm-6 and REH cell lines (Figure 2B). These data suggest that CD47 blockade is efficient with native and Fc-engineered antibodies, but the highest ADCP is achieved when an Fc-engineered CD19 antibody is used rather than an unmodified version.

CD47 blockade enhances ADCP by CD19-DE in BCP-ALL cells of adult and pediatric patients

In order to substantiate our cell line findings, we conducted in vitro phagocytosis experiments with primary BCP-ALL cells including four adult (Ad ALL #1–#4) and seven pediatric samples (Ped ALL #1–#7) amplified in and retrieved from NSG mice. Microscopic phagocytosis assays in the Ad ALL samples revealed that only CD19-DE was able to cause a significant increase in ADCP as compared to control and HER2-DE, while CD19-IgG1 was not (Figure 3A). Concomitant application of Hu5F9-IgG2σ raised phagocytosis levels in combination with both CD19-IgG1 and CD19-DE, however, levels were significantly higher when

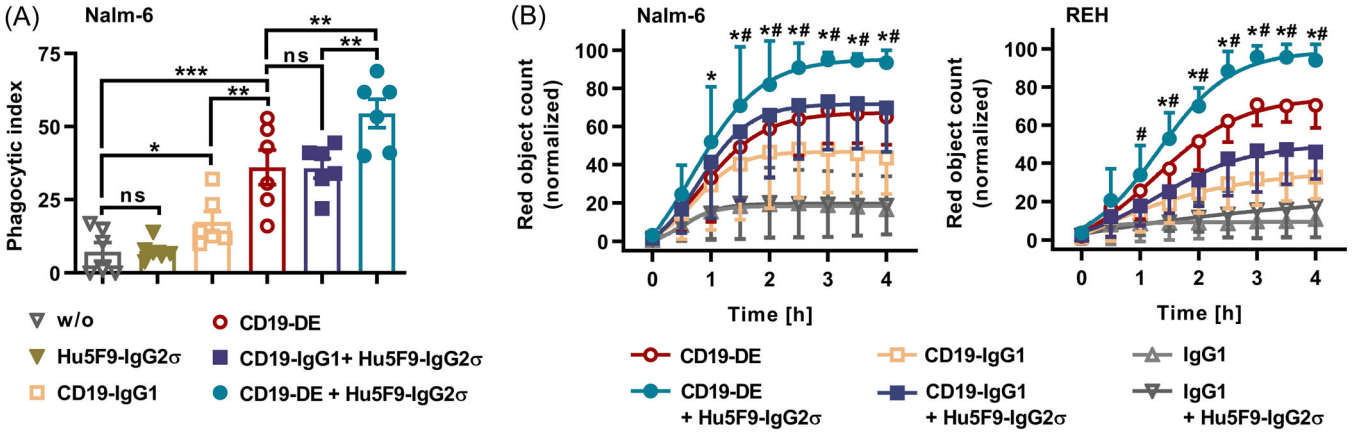

FIGURE 2 Higher efficacy of CD19-DE versus CD19-IgG1 in combination with CD47 blockade. (A) Induction of antibody-dependent cellular phagocytosis (ADCP) by CD19-DE and CD19-IgG1 antibodies with or without CD47 blockade was analyzed using CFSE-labeled Nalm-6 cells as target cells. ADCP by macrophages was determined by fluorescence microscopy and phagocytic index values were calculated (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not statistically significant; one-way analysis of variance [ANOVA] and Holm-Šídák multiple corrections test). (B) ADCP induction by CD19-DE and CD19-IgG1 alone or in combination with CD47 was analyzed with pHrodo labeled Nalm-6 (left graph; $n = 5$) or REH (right graph, $n = 4$) target cells using real-time imaging. Data points indicate mean values of normalized red object counts per image ± SD. Statistically significant differences between CD19-DE versus CD19-DE plus Hu5F9-IgG2σ (*$p < 0.05$) and CD19-DE plus Hu5F9-IgG2σ versus CD19-IgG1 plus Hu5F9-IgG2σ ($^{\#}$<0.05) are indicated (two-way ANOVA and Holm-Šídák multiple corrections test). Trastuzumab was employed as control (IgG1).

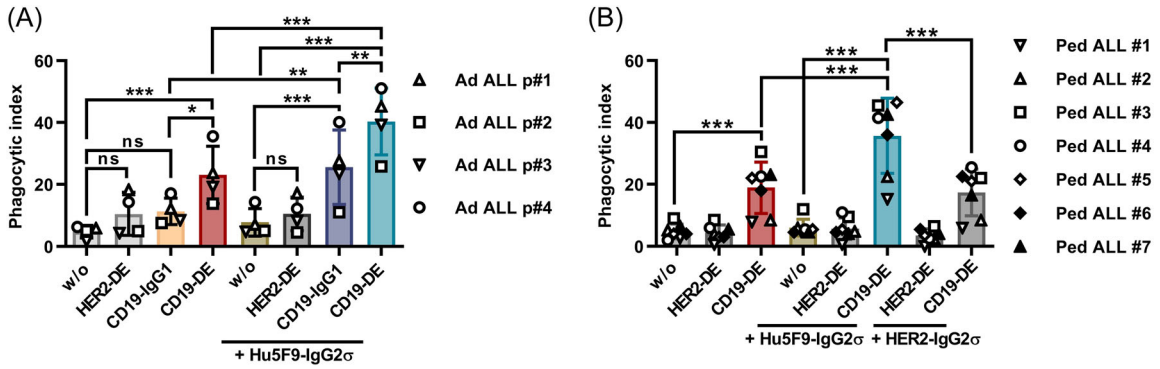

FIGURE 3 Enhanced antibody-dependent cellular phagocytosis (ADCP) of primary B-cell precursor acute lymphoblastic leukemia (BCP-ALL) cells by CD47 blockade. (A) CFSE-labeled ALL cells from four adult patients (Ad ALL # 1–4) were incubated with human macrophages without (w/o) or with the indicated antibodies or their combinations (each at a concentration of 10 μg/mL). After 2 h, ADCP was analyzed by fluorescence microscopy. Data points represent mean phagocytic index values for individual patient samples as determined in independent experiments using macrophages from different donors. Bars indicate overall mean phagocytic index values ± SD (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant; one-way analysis of variance [ANOVA] with Holm-Šídák multiple comparisons test). (B) Pediatric BCP-ALL samples (Ped ALL #1-#7) amplified in and retrieved from NSG mice were analyzed as target cells in ADCP assays using fluorescence microscopy as described in (A) for adult samples (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant; one-way ANOVA with Holm-Šídák multiple comparisons test). HER2-specific isotype antibodies (i.e., HER2-DE and HER2-IgG2σ) were used as control reagents.

CD47 blockade was applied with CD19-DE, in line with findings in cell lines (Figure 3A). Figure 3A depicts a mean of three independent experiments in each data point, individual results for each Ad ALL patient are shown in Supporting Information S1: Figure S2. We next tested CD19-DE in combination with Hu5F9-IgG2σ in the seven Ped ALL samples in vitro. All pediatric samples originated from *KMT2A*-rearranged infant BCP-ALL patients and previously shown to be refractory to CD19-DE in vivo.[10] CD19-DE was efficient in inducing ADCP in all Ped ALL samples in vitro compared to control and HER2-DE. Hu5F9-IgG2σ monotherapy was inefficient in all samples in vitro, replicating the cell line data. The combination of CD19-DE and Hu5F9-IgG2σ was

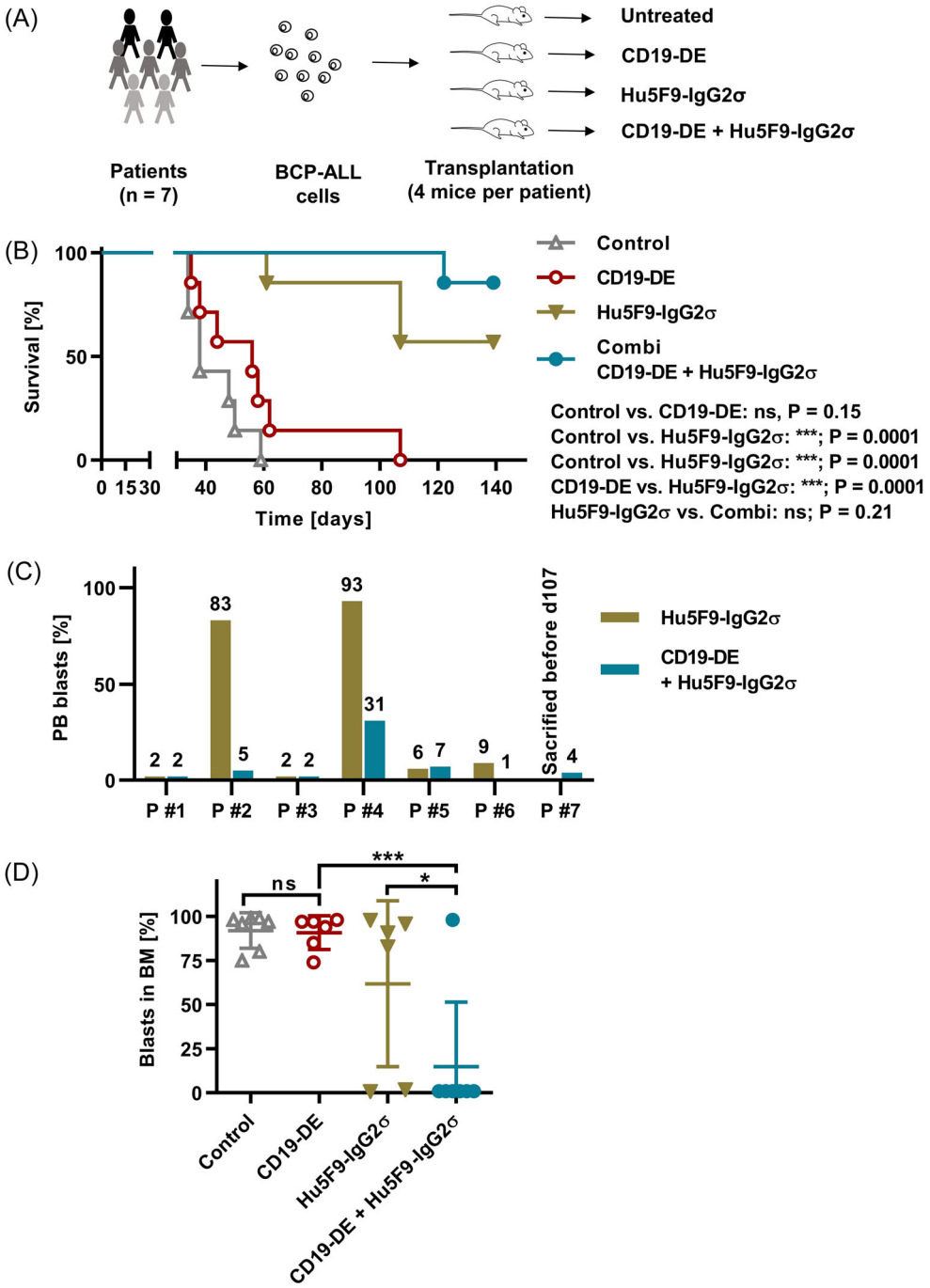

FIGURE 4 The combination of CD19-DE and CD47 blockade is highly efficient in vivo. (A) Experimental outline of phase II-like preclinical xenograft trial. (B) Survival analysis of phase II-like preclinical xenograft trials in the Ped ALL #1-#7 samples (***$p < 0.001$; Kaplan–Meier log-rank test). (C) Analysis of peripheral blood blast counts as percentage in the experimental animals from the Hu5F9-IgG2σ and CD19-DE plus Hu5F9-IgG2σ groups by flow cytometry. (D) Analysis of bone marrow blast counts as percentage *post mortem* in the experimental groups by flow cytometry (*$p < 0.05$; ***$p < 0.001$; ns, not significant; mixed-effects analysis with Holm-Šídák multiple comparisons test). Control ($n = 7$), CD19-DE ($n = 6$), Hu5F9-IgG2σ ($n = 6$) and CD19-DE plus Hu5F9-IgG2σ ($n = 7$). Please note that for animals that were not included in the analysis (i.e., PDX Ped ALL #5 and PDX Ped ALL #2 mice in CD19-DE and Hu-5F9-IgG2σ PDX treatment groups, respectively) the isolation of BM was not feasible for technical reasons.

highly efficient in all samples, compared to Hu5F9-IgG2σ monotherapy and to the combination of Hu5F9-IgG2σ and HER2-DE (Figure 3B). Figure 3B depicts a mean of at least three independent experiments in each data point, individual results for each Ped ALL patient are shown in Supporting Information S1: Figure S3. Our data show that in all BCP-ALL samples tested in vitro, the therapy combining CD19-DE and Hu5F9-IgG2σ is most efficient in inducing ADCP by macrophages of healthy human donors.

The combination of CD19-DE and CD47 blockade is highly efficient in vivo

We next assessed the in vivo efficacy of the CD19-DE/Hu5F9-IgG2σ combination. We therefore chose the same seven Ped ALL patients (Figure 3B) and injected each sample into four NSG mice each, in order to perform a phase II-like preclinical xenograft trial as previously published.[10,14,15] Mice were randomly assigned to four treatment groups and left untreated (control) or treated with CD19-DE, Hu5F9-IgG2σ, or the combination of both (Figure 4A). As expected, all seven Ped ALL patients were refractory to CD19-DE monotherapy in vivo, resulting in similar survival rates as in the control. Hu5F9-IgG2σ monotherapy was significantly more efficient in prolonging the survival of xenograft mice as compared to control and CD19-DE monotherapy. Importantly, the combination therapy reached the best survival results with only one mouse (bearing sample Ped ALL #4) succumbing to leukemic disease within the experimental window on day +122, which is a roughly twofold survival prolongation as compared to control or CD19-DE monotherapy (Figure 4B). On day +107, when the last CD19-DE treated mouse had to be sacrificed, an analysis of human blasts in the peripheral blood of the remaining living mice was performed. Four/seven patients (#2, #4, #6, and #7) showed clearly higher blast counts in the Hu5F9-IgG2σ monotherapy group as compared to the combination group. Only 1/7 mice (#5) showed a slightly higher blast count in the combination group as compared to the Hu5F9-IgG2σ monotherapy group (Figure 4C). On the day of sacrifice of a mouse (for surviving mice at the end of the experimental window of 140 days), bone marrow analysis for human blasts was performed. Bone marrow blast counts were significantly lower in the combination group (mean 14.9%), as compared to all other groups, including Hu5F9-IgG2σ monotherapy (mean 61.8%, Figure 4D). These results show a high in vivo efficacy of a CD19/CD47 combination antibody therapy in aggressive models of *KMT2A*-rearranged infant leukemia. Importantly, Hu5F9-IgG2σ in combination with CD19-DE causes a survival advantage even in samples refractory to the CD19-DE therapy alone.

DISCUSSION

We show that CD47 blockade employing Hu5F9-IgG2σ potentiates the efficacy of the CD19-directed, Fc-engineered monoclonal antibody CD19-DE by enhancing ADCP. This therapeutic combination is a promising alternative to other CD19-directed monotherapies.

The pediatric models were from infants with *KMT2A*-rearranged leukemia, a subgroup in particular need for more efficient agents. It was recently shown that this patient population benefits from blinatumomab in first-line therapy,[18] and the exploitation of maximum ADCP using CD47 blockade may be able to reduce chemotherapy toxicity in the future. Also, the combination of monoclonal antibodies alone or in combination instead of blinatumomab would avoid the blinatumomab side effects of T-cell activation such as cytokine release syndrome and encephalopathy. In addition, monoclonal antibodies are easier to apply than blinatumomab as continuous dosing is not required. CD47 blockade *per se* is tolerated with few side effects. Some are anemia, thrombocytopenia and gastrointestinal toxicity, which are likely due to unknown immunological mechanisms.[19] Our CD47 targeted antibody may have less side effects, because the CD47 binding domains of magrolimab, the most advanced CD47 targeting agent, were combined with a silent Fc-domain, which may provide pure CD47 blockade. In contrast, IgG4 antibodies are not fully silent in this aspect and activation of FcγR may contribute to the activity of magrolimab.[20,21]

It is important that combination with Hu5F9-IgG2σ also raised ADCP levels by the non-Fc-engineered CD19 antibody version CD19-IgG1. This suggests that CD47 blockade makes up for insufficient FcγR engagement by lowering the activation threshold of macrophages. Yet, even under CD47 blockade, improved FcγR binding was proven beneficial. Therefore, CD47 blockade may be a rational combination partner for a variety of native and Fc-engineered antibodies, also against other antigens. Our results were obtained using macrophages from human donors and we observed some variability in ADCP induction with different donors and the same leukemia and antibody combinations. This suggests that host factors contribute to the efficacy of CD47 blockade. This may for example be due to varying expression levels of SIRPα on donor macrophages, and measurements of SIRPα levels on patient macrophages may be important to predict therapeutic efficacy. Yet, the regulation of phagocytosis is complex and involves additional activating or inhibitory receptors such as low-density lipoprotein receptor-related protein 1, sialic acid binding Ig-like lectin 10 and leukocyte immunoglobulin-like receptor subfamily B member 1.[22] These may also contribute to the regulation of CD19 antibody mediated ADCP. We are currently investigating these issues.

It also remains unclear which temporal sequence is the best when our novel Hu5F9-IgG2σ/CD19-DE co-targeting approach is employed, as both antibodies were applied simultaneously. It is possible that a combination with elements of conventional chemotherapy affecting macrophage efficacy, for example, cyclophosphamide,[23] may contribute to an optimized regimen with even higher efficacy.

The preclinical phase II-like PDX trial recapitulates patient characteristics and heterogeneity. The recognition of human CD47 by murine SIRPα allows the evaluation of CD47 checkpoint inhibition in xenografts, although certain differences in the immune system between mice and humans exist, that may affect antibody effector functions. Intriguingly, Hu5F9-IgG2σ monotherapy was efficient in vivo, although the antibody lacked efficacy in in vitro ADCP assays when applied alone. However, the in vitro assays were not designed to uncover long-term effects. In addition, in the murine xenograft model the presence of prophagocytic factors that may emerge in the leukemia niche in vivo or the poor cross-species recognition of other "don't-eat-me" signal molecules on leukemia cells by murine macrophages may ease phagocytosis upon disruption of the CD47/SIRPα axis. Along this line, differences in the responsiveness of human and murine macrophages to CD47 blockade have recently been suggested. Thus, the comparison of CD47 F(ab')2 fragments with intact antibodies harboring a functional Fc domain revealed that CD47 blockade was sufficient to facilitate phagocytosis of human lymphoma cells by murine macrophages in vitro. This happened also

in the absence of FcγR engagement, whereas human macrophages required concomitant FcγR activation to efficiently engulf target cells.[24]

The fact that the Hu5F9-IgG2σ/CD19-DE combination works even in patient cells refractory to CD19-DE monotherapy in vivo, is most astonishing in our study. It is possible that Hu5F9-IgG2σ restores a great deal of ADCP induced by CD19-DE, even when CD19 is potentially alternatively spliced and rendered unrecognizable, retained in the endoplasmic reticulum by mutation or otherwise downregulated under therapeutic pressure, as has been observed with other CD19-directed immunotherapies.[25]

Our data show that ADCP is a relevant effector mechanism in immunotherapy of BCP-ALL in children and adults in our models. We advocate that CD47 blocking approaches be transferred into age-overriding clinical trials in combination with other monoclonal antibodies in ALL.

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AUTHORS CONTRIBUTIONS

Christian Kellner, Fotini Vogiatzi, Ira A. Münnich, Tobias Zeller, Kristina Müller, Roland Windisch, and Lennart Lenk performed the experiments. Denis M. Schewe, Christian Kellner, Heiko Bruns, and Matthias Peipp developed the methodology. Denis M. Schewe and Christian Kellner wrote the manuscript. Christian Kellner, Denis M. Schewe, Hilal Bhat, Christian Wichmann, Dimitrios Mougiakakos, Heiko Bruns, Thomas Valerius, Elisa Felix, Matthias Peipp, and Andreas Humpe provided research directions and regularly discussed the data. All authors discussed the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was approved by the ethics committees of the Christian-Albrechts University Kiel and the Ludwig-Maximilians University Munich. Animal experiments were authorized by the Ministry for Energy Transition, Climate Protection, Environment and Nature, State Government of Schleswig-Holstein.

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

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