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The anti-CD19 antibody-drug conjugate SAR3419 prevents hematolymphoid relapse post induction therapy in preclinical models of pediatric acute lymphoblastic leukemia.

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Translational Relevance

Antibodies and their drug conjugates (ADCs) are being used increasingly for cancer treatment. However, limited preclinical information is available on how best to combine ADCs with conventional chemotherapy. SAR3419 is a maytansinoid DM4/anti-CD19 ADC currently in clinical trials for the treatment of CD19-positive adult malignancies. Here, we report the results of preclinical studies in which SAR3419 was combined with a 3-drug regimen (VXL) that mimics the induction treatment of pediatric acute lymphoblastic leukemia (ALL) patients, using well characterized xenograft models of aggressive and chemoresistant ALL. SAR3419 alone significantly delayed the progression of xenografts and elicited Objective Responses modeled after the clinical setting, the extent of which correlated with cell surface CD19 expression. However, the most profound effects were observed following remission induction with VXL, where SAR3419 prevented disease recurrence into hematolymphoid and other major organs. These results have significant implications for the combined use of established chemotherapy and SAR3419 in the treatment of relapsed/refractory ALL.
Abstract

**Purpose:** Relapsed or refractory pediatric acute lymphoblastic leukemia (ALL) remains a major cause of death from cancer in children. In this study we evaluated the efficacy of SAR3419, an antibody-drug conjugate of the maytansinoid DM4 and a humanized anti-CD19 antibody, against B cell precursor (BCP)-ALL and infant mixed lineage leukemia (MLL) xenografts.

**Experimental Design:** ALL xenografts were established as systemic disease in immune-deficient (NOD/SCID) mice from direct patient explants. SAR3419 was administered as a single agent and in combination with an induction-type regimen of vincristine/dexamethasone/L-asparaginase (VXL). Leukemia progression and response to treatment were assessed in real-time, and responses were evaluated using strict criteria modeled after the clinical setting.

**Results:** SAR3419 significantly delayed the progression of four out of four CD19⁺ BCP-ALL and three out of three MLL-ALL xenografts, induced objective responses in all but one xenograft, but was ineffective against T-lineage ALL xenografts. Relative surface CD19 expression across the xenograft panel significantly correlated with leukemia progression delay and objective response measure scores. SAR3419 also exerted significant efficacy against chemoresistant BCP-ALL xenografts over a large (10-fold) dose range, and significantly enhanced VXL-induced leukemia progression delay in two highly chemoresistant xenografts by up to 82 days. When administered as protracted therapy following remission induction with VXL, SAR3419 prevented disease recurrence into hematolymphoid and other major organs with the notable exception of central nervous system involvement.

**Conclusion:** These results suggest that incorporation of SAR3419 into remission induction protocols may improve the outcome for high-risk pediatric and adult CD19⁺ ALL.
Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, constituting approximately 80% of pediatric leukemias and nearly one-third of all childhood cancers (1). Over the past 50 years advances in the treatment of pediatric ALL have resulted in cure rates increasing from <10% to around 90% (2, 3). This improvement in patient outcome has principally resulted from the development of combination chemotherapy protocols, intensification of treatment for high-risk patients, and improvements in supportive care, rather than through the introduction of new drugs. Current induction treatment protocols, which utilize vincristine (VCR), a glucocorticoid and L-asparaginase (L-ASNase) with or without an anthracycline, result in >97% complete remission (CR) rates. Patients who relapse often exhibit multi-drug resistance, which limits subsequent treatment options and contributes to poor outcome. In addition, ALL subtypes associated with specific chromosomal translocations, such as Philadelphia chromosome positive (Ph+) ALL (t9;22) or those involving the Mixed Lineage Leukemia (MLL) oncogene, remain particularly difficult to cure (4). While the incorporation of tyrosine kinase inhibitors into therapy for Ph+ ALL patients has resulted in improved outcomes, the prognosis remains dismal for the majority of relapsed/refractory patients (2).

Monoclonal antibodies (MAbs), and their toxin or drug conjugates, constitute a unique class of therapeutics that are particularly attractive as cancer treatments due to their antigen specificity and the effector/cytotoxic functions they can trigger. Advances in recombinant DNA technology led to the development of chimeric or humanized MAbs with reduced immunogenicity, thus overcoming a major complication associated with their clinical application. A growing number of antibody-based treatments are being approved for use by the FDA, many of these for cancer indications, including hematological malignancies (reviewed in (5)(6)).

CD19 is a B-cell restricted co-receptor molecule expressed at high levels at all stages of B-cell differentiation except for mature plasma cells. Since CD19 constitutes an important component of the
B-cell receptor complex involved in signaling and antigen processing (7), and is expressed in the majority of B-cell precursor (BCP)-ALLs and B-cell non-Hodgkin’s lymphomas (8), it has long been recognized as a possible target in these malignancies. In the early 1990s anti-CD19 antibodies conjugated to the blocked ricin toxin (9), pokeweed antiviral protein (10) and genistein (11) showed evidence of efficacy in preclinical studies. However, the initial clinical trials involving these agents were disappointing (12-14). Since then a new generation of anti-CD19 antibody-drug conjugates (ADCs) have been developed, which are currently at various stages of preclinical and clinical evaluation (15-17). Also, the bi-specific anti-CD19/anti-CD3 chimeric construct blinatumomab has shown promising results in a phase II clinical trial, further validating this target (18).

Maytansine is a potent anti-mitotic agent, which binds to tubulin and inhibits microtubule dynamics similar to *Vinca* alkaloids but with over 100-fold higher potency (19). Due to high toxicity and a small therapeutic index its clinical development was halted (20). Interest in maytansine, and the two derived maytansinoids DM1 and DM4, has recently been revived in the context of targeted delivery of drugs, which should minimize their toxicity (21, 22). Some of these agents such as trastuzumab emtansine (T-DM1), a DM1-ADC targeting the Her2 receptor in breast cancer, are advanced in their clinical development (23, 24). Other DM1 or DM4 ADCs are currently in preclinical and clinical development (25-28).

The principal objective of the Pediatric Preclinical Testing Program (PPTP) is to identify novel agents with significant antitumor activity against preclinical xenograft models of childhood solid tumors and ALL in immune-deficient mice, and to prioritize the advancement of drugs into clinical trials. SAR3419 is an anti-CD19 humanized monoclonal antibody (huB4) conjugated to DM4 (17, 29) currently tested in phase I/II clinical trials in relapsed or refractory B-cell non-Hodgkin’s lymphoma (NCT00539682 and NCT00549185) and adult ALL (NCT01440197) (30, 31). In this study we describe PPTP preclinical evaluation of SAR3419 and show that it is effective as a single agent over a wide
range of doses against CD19⁺ BCP-ALL and MLL-ALL xenografts. More importantly, we show that when used as continuous treatment following remission induction with an induction-type regimen consisting of a combination of VCR, the glucocorticoid dexamethasone (DEX) and L-ASNase (VXL), SAR3419 effectively prevented hematolymphoid relapse with mice succumbing to morbidity associated with central nervous system (CNS) relapse. Overall our data suggest that SAR3419 is a highly effective novel therapeutic agent for ALL and its incorporation into treatment protocols may improve the outcome for high-risk pediatric and adult CD19⁺ ALL patients.
Material and Methods

Xenograft models of pediatric ALL

All experimental studies were approved by the Animal Care and Ethics Committee of the University of New South Wales. The establishment and characterization of a pediatric patient-derived ALL xenograft panel in NOD/SCID (NOD.CB17-Prkdc<sup>scid</sup>/J) mice has been previously described (32, 33) (details can also be obtained from http://pptp.nchresearch.org/documents.html) and that of an infant MLL-rearranged xenograft panel will be published elsewhere. Mice obtained from Australian BioResources (Moss Vale, NSW, Australia) were inoculated intravenously with 2.5 - 5 x 10<sup>6</sup> ALL or MLL-ALL xenograft cells purified from spleens of previously engrafted mice. Leukemia engraftment was monitored by flow cytometric quantification of the proportion of human CD45-positive (huCD45<sup>+</sup>) cells versus total CD45<sup>+</sup> leukocytes (human + murine) cells in the peripheral blood (PB) and tissues as described previously (32, 33). When the median %huCD45<sup>+</sup> cells in the PB was above 1% for each xenograft cohort, mice were randomized and allocated to groups (6-10 mice per group) and treatments initiated.

In vivo drug treatments

All drugs were administered by intraperitoneal injection (i.p.). Unless otherwise specified drug schedules were as follows: SAR3419 1-10 mg/kg and huB4 10 mg/kg (provided by sanofi, France, through the Cancer Therapy Evaluation Program, NCI) once a week for 3 weeks; VCR (Baxter, NSW, Australia) 0.15 mg/kg once a week for 2 weeks; DEX (Sigma-Aldrich, Australia) 5 mg/kg, and L-ASNase (Leunase®, sanofi, Australia) 1000 U/kg, Monday to Friday for 2 weeks. In some experiments, in order to prevent morbidity due to the immediate hypersensitivity reaction induced by MAb treatment, a pre-treatment similar to standard clinical practice was administered 30 min before SAR3419. This prophylaxis consisted of acetaminophen (34) (100 mg/kg, Bristol-Myers Squibb,
Victoria, Australia) and promethazine (35) (4 mg/kg, Hospira, Australia) in a 0.2 M NaCl/4 mg/mL glucose solution, i.p.

**Determination of in vivo treatment response**

An in-depth description of the analysis methods is included in the Summary Statistics and Analysis Methods section in Supplemental Material. Briefly, ALL xenograft responses to drug treatments were assessed using two activity measures, leukemia growth delay (LGD) and objective response measure (ORM) as previously described (36). Event-free survival (EFS) was calculated for each mouse as the number of days from treatment initiation until the %huCD45\(^+\) cells in the PB reached 25%, or until mice reached a humane end-point with evidence of leukemia-related morbidity. EFS values for the different treatments were compared by Kaplan-Meier survival curves and logrank test. The LGD was calculated for each group as the difference in median EFS between treated and control mice.

Each mouse was assigned an ORM score from 0 (progressive disease type 1, PD1) to 10 (maintained complete response, MCR), depending on its response profile (37) from which an overall response for the cohort was determined as the median ORM. According to this model the following responses were defined during the 42 day monitoring period: progressive disease (PD) as a continuous increase in %huCD45\(^+\) in the PB that reached event; stable disease (SD) as %huCD45\(^+\) in the PB that did not decrease below 1% and did not reach event; partial response (PR) as a decrease of %huCD45\(^+\) in the PB to under 1% at only one time point; complete response (CR) when the %huCD45\(^+\) in the PB remained under 1% for two consecutive measures; and MCR when the %huCD45\(^+\) in the PB stayed under 1% for the last three weeks of the monitoring period, or at 6 weeks from treatment initiation if monitoring extended beyond this time point. PD was further subdivided into the classifications PD1 or PD2 based on whether the EFS of the treated mouse was \(\leq 1.5\)- or \(> 1.5\)-fold, respectively.
median EFS of the control group. Median ORMs of PR, CR and MCR were considered to be Objective Responses.

Responses were also expressed in a “COMPARE-like” format, which combines EFS and median ORMs around the midpoint (0) representative of SD. Bars to the right or left of the midpoint represent Objective Responses or non-Objective Responses, respectively. Significant and non-significant differences in EFS distribution between control and treated cohorts are represented by red or blue color, respectively. Xenografts were excluded from analysis if >25% of mice within any one cohort experienced non-leukemia-related toxicity or morbidity.

**CD19 expression analysis**

RNA was extracted from cryopreserved xenograft cells harvested from spleens of highly engrafted animals (enriched by differential density centrifugation on Ficoll) using trizol (Sigma) and/or affinity purification spin columns (RNeasy, Qiagen). High quality RNA (500 ng, verified using RNA 6000 LabChip kits and an Agilent 2100 Bioanalyzer) was amplified and cRNA was biotinylated using the Illumina® TotalPrep RNA Amplification kit (Ambion, Foster City, CA) as recommended by the manufacturer. Gene expression profiling was performed using the Illumina Human Ref-12 Expression BeadChip (Illumina Inc., San Diego, CA). Data from individual arrays were subjected to quantile normalization, and heatmaps were generated using the Heatmap Viewer module of GenePattern (38). Expression levels were normalized for each gene, where the mean is 0. Expression levels greater than the mean are shown in red and less than the mean are in blue as a logarithmic scale. Increasing distance from the mean is represented by increasing color intensity.

Relative surface expression of CD19 was evaluated by flow cytometry of xenograft cells obtained from both spleens and bone marrows of highly engrafted mice. For each xenograft three independent samples were assessed. Samples were co-stained for huCD45⁺ (allophycocyanin, APC-
conjugated anti-human CD45, Biolegend, San Diego, CA) and CD19+ (phycoerythrin, PE-conjugated anti-human CD19, BD Biosciences, San Jose, CA) and the mononuclear cell population within the leukocyte region (forward vs. side scatter plot) gated. For comparison purposes, unstained and PE-stained beads were included in each sample and the geometric mean of the fluorescence intensity of the human population was normalized to both the corresponding signals of the unstained (RFI =1) and stained beads (RFI =100). Results are expressed as relative fluorescence intensity (RFI).

**Histological detection of leukemia infiltration**

Tissues were fixed in buffered formalin, dehydrated, cleared and paraffin embedded as per standard protocols. Sections (5 μm) were re-hydrated and stained with hematoxylin and eosin (H&E) and images obtained with bright field ScanScope slide scanning equipment and analyzed with ImageScope software (Aperio, Vista, CA).

**Statistical methods**

The exact logrank test as implemented using Proc StatXact for SAS® was used to compare EFS distributions between treatment and control groups (two tailed), with $P$ values $\leq 0.05$ considered significant. Correlations between CD19 mRNA or surface expression levels and LGD or ORM were analyzed using Spearman’s correlation test. For comparison of relative CD19 surface expression between spleen and bone marrow samples a paired two tailed non-parametric test was applied.
Results

**SAR3419 is highly effective as a single agent against CD19⁺ ALL xenografts in vivo**

The efficacy of SAR3419 as a single agent was first examined against a panel of 10 xenografts *in vivo* (5 BCP-ALL, 2 T-ALL and 3 infant MLL-ALL). Treatments consisted of 3 injections administered once a week i.p. at a dose of 10 mg/kg. Compared to vehicle controls, SAR3419 significantly prolonged the EFS of all evaluable BCP-ALL and MLL-ALL xenografts (Figure 1A-D, Table 1). For these xenografts the LGDs ranged from 16.8 days for ALL-2 to 60.9 days for ALL-19 (Table 1). As expected, SAR3419 was ineffective in significantly delaying the progression of the 2 T-ALL xenografts (Figure 1E; Table 1, and Supplementary Figure S1A), which lack CD19 expression (32). SAR3419 elicited Objective Responses in 3 out of 4 evaluable BCP-ALL xenografts (1 PR, 2CRs) and two out of two evaluable MLL-ALL xenografts (1 PR, 1 CR) (Table 1 and Figure 2A). In contrast, one of the most chemosensitive BCP-ALL xenografts in the panel (ALL-3) (33) only achieved a PD2, and both T-ALL xenografts were scored as PD (1 PD1, 1 PD2). Xenograft responses to SAR3419 are also summarized in a “COMPARE-like” plot (Figure 2B). A complete summary of results is provided in Supplementary Table S1, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to events, LGD values, as well as numbers of responses and T/C values. One BCP-ALL xenograft (ALL-17) was excluded from analysis due to toxicity; of the remaining 5 mice in the ALL-17 SAR3419-treated cohort 3 achieved CR, 1 PR and 1 PD1 (Supplementary Figure S1B). The xenograft MLL-14 was not evaluable in terms of ORM since due to its engraftment kinetics treatments commenced before the median leukemia load in PB was over 1% (Supplementary Figure S1C).

*In vivo* efficacy of SAR3419 correlates with CD19 mRNA and cell surface expression
Microarray analysis of gene expression confirmed relatively high CD19 mRNA expression in BCP-ALL and MLL-ALL xenografts compared to T-lineage xenografts (Figure 3A). These differences in relative expression were corroborated by flow cytometric analysis of cell surface CD19 expression (Figure 3B and Supplementary Table S2). CD19 mRNA and cell surface protein expression in spleen-derived cells significantly correlated across 6 xenografts analyzed \( (P < 0.001, R^2 = 0.98, \text{data not shown}) \). Furthermore, CD19 mRNA expression correlated with \textit{in vivo} responses to SAR3419 assessed by either median ORMs or LGD values of the xenografts tested (Figure 3C). Overall, surface CD19 expression was significantly higher in xenograft cells collected from the bone marrow than in cells collected from the spleen \( (P = 0.004, \text{Figure 3C; Supplementary Table S2}) \). Nevertheless, relative CD19 surface expression on cells collected from either anatomical site showed positive correlations with both the median ORMs and LGD values elicited by SAR3419 treatment (Figure 3C).

**SAR3419 exerts single-agent efficacy over a broad range of doses, while the unconjugated antibody is relatively ineffective**

In order to further characterize the efficacy of SAR3419, a dose response study was conducted against two CD19+, chemoresistant xenografts, ALL-4 (Ph+ ALL) and ALL-19, derived from patients who succumbed early to their disease (33). SAR3419 was highly efficacious against ALL-4, resulting in CRs for all doses tested (2.5 to 10 mg/kg, Figure 2C and D, Table 2, Supplementary Figure 2A). A dose-response relationship was evident when the efficacy of SAR3419 was quantified by LGDs, which ranged from 22.3 to 36.8 days for 2.5 and 10 mg/kg doses, respectively (Supplementary Figure 2A and Table 2). In order to further delineate the therapeutic window of SAR3419 we tested its efficacy at only 1 mg/kg against ALL-19. Even at this much reduced dose SAR3419 treatment elicited an ORM of SD in ALL-19, resulting in an LGD of 18.1 days (Figure 2C and D, Table 2, Supplementary Figure 2B).
We also assessed the contribution of the DM4 drug component to the efficacy of this ADC, by comparing the effect of SAR3419 to its unconjugated humanized MAb counterpart, huB4. At the same dose (10 mg/kg), huB4 only moderately (albeit significantly) delayed the progression of ALL-4 (Figure 2C and D, Table 2, Supplementary Figure 2A) or ALL-19 (data not shown), but did not elicit an Objective Response, indicating that DM4 is critical for the high efficacy of SAR3419 against ALL in this xenograft experimental model.

**SAR3419 prevents hematolymphoid relapse post remission induction**

Our next aim was to assess whether SAR3419 could delay leukemia relapse post induction therapy with established drugs. We have previously optimized, based on pharmacokinetic and tolerability parameters, an experimental induction-type protocol in NOD/SCID mice consisting of a VXL treatment platform (39). Mice engrafted with ALL-4 or ALL-19 were treated with VXL for two weeks to induce remission, followed immediately by one 3-week cycle of SAR3419 treatment with or without an ensuing 10-week block of weekly SAR3419 treatments at 10 mg/kg. VXL alone delayed the progression of both xenografts, by 18.0 days for ALL-4 (Figure 4A and B, Table 2) and by 25.1 days for ALL-19 (Figure 4C and D, Table 2), and resulted in Objective Responses (CRs) for both xenografts (Figure 2C and D, and Table 2).

The additional treatment with a three week cycle of SAR3419 following VXL significantly extended disease remission by 46.7 days for ALL-4 ($P=0.0003$, Figure 4A and B, Table 2) and by 82.3 days for ALL-19 ($P=0.005$, Figure 4C and D, Table 2) compared with VXL alone, and resulted in therapeutic enhancement for both xenografts (Figure 2C and D, and Table 2). The effects of the VXL/SAR3419 combination were greater than additive for ALL-4, by 9.9 days, and for ALL-19, by 21.4 days. All mice treated with one 3-week cycle of SAR3419 post VXL eventually reached
leukemia-related events (as defined by 25% huCD45\(^+\) in PB) and exhibited high leukemic infiltration of all major organs (Figure 4E and F).

Cohorts of mice engrafted with ALL-4 or ALL-19 also received an additional 10 weekly doses of SAR3419 treatment following the “VXL-SAR3419 x 3” regimen. This protracted SAR3419 treatment (VXL-SAR3419 x 13) also extended the disease remission induced by VXL alone for both xenografts, and further extended the remission for ALL-4 in comparison with that obtained by the 3-week cycle of SAR3419 post VXL schedule (Figure 4, Table 2). However, all mice in the extended treatment cohorts eventually became morbid and were euthanized with undetectable levels of leukemic cells in the PB (Figure 4E and F). Examination of tissues at necropsy revealed negligible leukemic infiltration of all major organs except for the brain and spinal fluid, as assessed by flow cytometry (Figure 4E and F). The disparity in leukemic infiltration of spleen, bone marrow and brain, detected by flow cytometry, following either 3-week or prolonged SAR3419 administration post VXL treatment was confirmed by histology (Supplementary Figure S3).

To exclude the possibility that the morbidity observed in this experiment was due to cumulative toxicity caused by prolonged exposure to SAR3419, we performed a long-term toxicity study consisting of 13 weekly injections of 10 mg/kg SAR3419 or huB4 (10 in addition to the original three week cycle) using non-engrafted NOD/SCID mice. No clear evidence of toxicity was recorded. Throughout the study all mice appeared healthy and asymptomatic, with no appreciable weight loss, while hematological and biochemical analyses of PB samples, as well as histological examination of tissues, revealed no relevant abnormalities (data not shown).
Discussion

This study is the first to provide preclinical evidence of high-level \textit{in vivo} efficacy of the anti-CD19 DM4-ADC, SAR3419, against BCP-ALL and infant MLL-ALL xenografts. SAR3419 exerted potent single agent activity against highly chemoresistant leukemia xenografts, which include ALL subtypes that remain difficult to treat such as Ph$^+$ ALL (ALL-4) and infant MLL-ALL. SAR3419 ranks among the four most efficacious single agents tested to date by the PPTP against the ALL xenograft panel, out of over 25 novel drugs tested, together with PR-104, MLN8237 and topotecan (37). The efficacy of SAR3419 against ALL xenografts was directly related to CD19 expression levels, with negligible activity towards T-ALL xenografts, and there was no nonspecific cytotoxicity against CD19$^+$ cells \textit{in vivo}. These data indicate that SAR3419 is highly specific in targeting CD19$^+$ BCP-ALL xenografts. The higher density of CD19 expression in the bone marrow, together with the density-dependent efficacy of SAR3419, raises the possibility that SAR3419 may target ALL xenograft cells residing in the bone marrow more effectively than those in the spleen or PB. Consistent with results from a lymphoma model study (17, 29) the unconjugated MAb had only minimal effect on the target cells indicating that DM4 is an essential component of this ADC responsible for its cytotoxic effect. Target bound anti-CD19 Abs are reported to undergo fast internalization making them particularly suitable for targeted delivery of cytotoxic molecules to malignant cells while reducing off-target toxicity (29). Upon internalization the ADC is degraded, releasing DM4 intracellularly, which binds to tubulin and disrupts microtubule assembly leading to apoptosis (29).

In a recent study using preclinical models of B-cell lymphoma \textit{in vivo}, the efficacy of SAR3419 was demonstrated to be comparable with that of chemotherapy and superior to that of rituximab (17). Although rituximab is capable of inducing apoptosis directly, its clinical efficacy appears largely dependent on complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC), recruitment of effector cells and up-regulation of cytokine secretion (40). The
contributions of CDC and ADCC cannot be adequately assessed in the immune-deficient NOD/SCID mouse host used in this study, thereby precluding direct comparisons between SAR3419 and rituximab.

The relevance of CD19 as a target in B lineage malignancies is highlighted by encouraging clinical trial results with blinatumomab (18). Historical preclinical studies using other anti-CD19 MAbs and their conjugates were encouraging, showing efficacy in preclinical models of B cell leukemias and lymphomas (9, 10, 41). However the ensuing clinical trials produced disappointing results (12, 42, 43). The reasons for these failures are likely to be multifactorial, including limited access of the ADCs to certain disease sites, excessive non-specific toxicity associated with the conjugated toxin component, resistance to the drug used in the conjugate, and development of immunity against mouse Abs (HAMA). The humanized anti-CD19 Ab component of SAR3419 is expected to elicit minimal immune response, which should increase its therapeutic potential and allow for repeated use. Recent data from phase I/II clinical trials of SAR3419 in relapsed or refractory B-cell NHL were very encouraging (30, 31). SAR3419 is relatively safe with few toxic effects mainly associated with prolonged treatment, however, no significant myelosuppression was observed. There was strong evidence of single agent activity; objective responses (CRs or PRs) were observed in 33% of patients with stage III or IV NHL treated with SAR3419 at its MTD. Approximately 50% of patients exhibited more than 20% reduction in their tumor burden in response to SAR3419 at any dose (it is worth noting that approximately half of these patients had rituximab resistant disease). These early results suggest that SAR3419 is a safe and effective agent against B-cell NHL with a large therapeutic window. These findings are consistent with a previously reported study using lymphoma models (29) and data observed in this study where we demonstrate a large therapeutic window of efficacy. Of note, our mouse xenograft model is unable to assess the impact that this treatment may have on the normal human B lymphocyte compartment.

We have previously reported that ALL-4 and ALL-19 are relatively chemoresistant xenografts, exhibiting resistance to a broad range of therapeutic agents including DEX, L-ASNase, rapamycin,
sunitinib and vorinostat (33, 37, 44). A critical finding of the current study is that a 3-week course of SAR3419 following VXL “induction” treatment significantly extended the length of remission and ORMs, while protracted SAR3419 post-VXL prevented relapse of leukemia into hematolymphoid tissues and other peripheral organs, except for the CNS. This observation may not be surprising since in our experimental model treatment begins when systemic disease is already well established, and MAbs are known to be inefficient in penetrating the blood-brain barrier. Therefore, any residual disease within the CNS not eradicated with the VXL therapy is likely to cause relapse. In the long term treatment experiments mice became morbid with no evidence of leukemia in the PB, and since the SAR3419 preparation contains <1% of free DM4 (17, 29) it was conceivable that continuous exposure to low doses of DM4 may still induce toxicity. The long term toxicity study carried out in non-engrafted mice revealed there were no obvious side effects caused by SAR3419 exposure, suggesting that the observed morbidity in leukemia-bearing mice was likely due to the invasion of the CNS by the leukemia. Although a study of lymphoma infiltration by tomography monitoring in a mouse model suggests that there may be a reduction in the lymphoma burden of the spinal canal following SAR3419 treatment (29), this may be insufficient to prevent CNS relapse in the long term (17). Our results suggest that SAR3419 may be particularly effective in eliminating residual disease following remission induction with standard therapy that includes intrathecal methotrexate to prevent CNS relapse.

In conclusion, this study has demonstrated the following: (1) SAR3419 is a highly potent and selective cytotoxic agent targeting BCP-ALL and infant MLL-ALL xenografts expressing cell surface CD19; (2) SAR3419 has a large therapeutic window and minimal toxicity in a mouse xenograft model; (3) when administered following an induction-type VXL regimen SAR3419 induces durable remissions in highly chemoresistant ALL xenografts; and (4) when administered as maintenance therapy following VXL treatment, SAR3419 effectively prevents leukemia relapse in hematolymphoid and other major peripheral organs except for the CNS. Therefore, we provide strong preclinical evidence for the
efficacy of SAR3419 against broadly chemoresistant pediatric BCP-ALL and infant MLL-ALL xenografts, which argues for its incorporation into existing therapies that could result in improved outcomes for pediatric and adult patients with otherwise poor prognosis.
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Authorship

Contributions: H.C., B.S., P.J.H., M.A.S., and R.B.L. designed the study; H.C., B.S., K.E., and I.B. performed the experiments; H.C., B.S., P.J.H., M.A.S. and R.B.L. interpreted the data and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no actual or perceived conflicts of interest.

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## Tables

**Table 1. Summary of xenografts and their \textit{in vivo} responses to single-agent SAR3419\(^1\)**

<table>
<thead>
<tr>
<th>Xenograft</th>
<th>Median EFS (days)</th>
<th>LGD (days)</th>
<th>(P) value*</th>
<th>Median ORM</th>
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<tbody>
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<td>Control</td>
<td>SAR3419</td>
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<td><strong>BCP-ALL</strong></td>
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</tr>
<tr>
<td>ALL-8</td>
<td>3.4</td>
<td>3.5</td>
<td>0.1</td>
<td>0.466 PD1</td>
</tr>
<tr>
<td>ALL-16</td>
<td>11.6</td>
<td>21.0</td>
<td>9.4</td>
<td>0.580 PD2</td>
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<tr>
<td><strong>Infant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MLL-3</td>
<td>10.5</td>
<td>47</td>
<td>36.5</td>
<td>(&lt; 0.001) CR</td>
</tr>
<tr>
<td>MLL-8</td>
<td>7.0</td>
<td>28.2</td>
<td>21.2</td>
<td>(&lt; 0.001) PR</td>
</tr>
<tr>
<td>MLL-14</td>
<td>7.0</td>
<td>37.6</td>
<td>30.6</td>
<td>(&lt; 0.001) n.e.(^3)</td>
</tr>
</tbody>
</table>

\*Statistically significant differences are in bold; \(^1\) of the ten xenografts tested ALL-17 is not included in this table due to toxicity greater than 25%; \(^2\) ALL-4 is a Ph+ xenograft; \(^3\) not evaluable for ORM response measure.
Table 2. Summary of *in vivo* ALL-4 and ALL-19 responses to a broad range of SAR3419 doses, unconjugated huB4 MAb, and SAR3419 following VXL treatment

<table>
<thead>
<tr>
<th>Xenograft</th>
<th>Treatment</th>
<th>Median EFS (days)</th>
<th>LGD (days)</th>
<th>P value</th>
<th>Median ORM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL-4</td>
<td>Control (Vehicle)</td>
<td>8.9</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>SAR3419 10 mg/kg</td>
<td>45.7</td>
<td>36.8</td>
<td>0.00016</td>
<td>CR</td>
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<tr>
<td></td>
<td>SAR3419 7.5 mg/kg</td>
<td>39.2</td>
<td>30.3</td>
<td>&lt;0.0001</td>
<td>CR</td>
</tr>
<tr>
<td></td>
<td>SAR3419 5 mg/kg</td>
<td>34.4</td>
<td>25.5</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>SAR3419 2.5 mg/kg</td>
<td>31.2</td>
<td>22.3</td>
<td>0.0050</td>
<td>CR</td>
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<tr>
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<td>huB4 10 mg/kg</td>
<td>13</td>
<td>4.1</td>
<td>0.00226</td>
<td>PD1</td>
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<td>Control (Vehicle)</td>
<td>10.1</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>VXL</td>
<td>28.1</td>
<td>18</td>
<td>&lt;0.0001</td>
<td>CR</td>
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<td>VXL-SAR3419 x 3</td>
<td>74.8</td>
<td>64.7</td>
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<td>VXL-SAR3419 x 13</td>
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<td>0.0014</td>
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<tr>
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<td>SAR3419 10 mg/kg</td>
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<td>60.9</td>
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<td>MCR</td>
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<tr>
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<td>SAR3419 1 mg/kg</td>
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<td>18.1</td>
<td>0.00117</td>
<td>SD</td>
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<tr>
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<td>Control (Vehicle)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>VXL</td>
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<td>25.1</td>
<td>0.06913</td>
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<td>96.7</td>
<td>0.00833</td>
<td>MCR</td>
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</tbody>
</table>

* Assessed at 6 weeks from treatment initiation.
Figure legends

Figure 1. SAR3419 exerts significant \textit{in vivo} single-agent efficacy against B-lineage pediatric ALL xenografts. NOD/SCID mice were inoculated with cells from BCP-ALL xenografts ALL-2 (A) and ALL-3 (B), infant MLL-ALL MLL-3 (C) and MLL-8 (D) or T-ALL ALL-8 (E). Following establishment of disease, mice were randomized and treated with vehicle (dashed lines) or 10 mg/kg SAR3419 (solid lines) once a week for 3 weeks. Response to treatment was monitored by weekly enumeration of the \%huCD45$^+$ cells in the PB of individual mice (left panels); corresponding Kaplan-Meier curves (based on EFS) are shown on the right. Gray arrows indicate treatment times.

Figure 2. Summary of SAR3419 single-agent efficacy, dose response and VXL combination efficacy studies against pediatric ALL xenografts. (A) Distribution of ORMs of individual mice engrafted with BCP-ALL or infant MLL-ALL xenografts and treated with 10 mg/kg SAR3419 as described in the legend to Figure 1. (B) “COMPARE-like” plot of the midpoint difference representing the median ORM of xenografts shown in A. (C) Distribution of median ORMs of individual mice engrafted with ALL-4 or ALL-19 and treated with SAR3419 at different doses, MAb huB4, VXL or VXL followed by SAR3419. (D) “COMPARE-like” plot of the midpoint difference representing the median ORM of xenografts of xenografts shown in C. For the “COMPARE-like” plots in B and D a score of -5 to 0 indicates that an Objective Response was not achieved for a particular xenograft, whereas a score of >0 to 5 indicates an Objective Response. Red bars indicate that the EFS was significantly different between control and treated mice. Blue bars indicate no significant difference.
Figure 3. **CD19 expression correlates with in vivo response to SAR3419.** (A) CD19 gene expression expressed in heat map format (normalized to the mean, with relative expression depicted in logarithmic scale). (B) Representation of CD19 surface expression on three different xenografts (gray, isotype control; green, ALL-16; blue, ALL-3; red, ALL-19) as detected by flow cytometry. (C) Regression curves and 95% confidence intervals of CD19 mRNA in spleen-derived cells (top panels), and protein expression on cells derived from spleen (middle panels) or BM (bottom panels) correlated with the median ORMs (left panels) and LGDs (right panels) of the corresponding xenografts.

Figure 4.

**Efficacy of SAR3419 against BCP-ALL xenografts following remission induction with VXL therapy.** Mice engrafted with ALL-4 (A, B, E), or ALL-19 (C, D, F), were treated with vehicle (black dotted lines); VXL (gray solid lines); VXL followed by 3 weekly doses of SAR3419 (black solid lines) or VXL followed by up to 13 weekly doses of SAR3419 (gray dashed lines). For additional details see Materials and Methods. The VXL treatment period is indicated by the gray shading, and SAR3419 treatment time points are marked by black (x 3) and gray arrows (x 10). Left panels represent the %huCD45⁺ cells in PB of individual mice, and the right panels depict Kaplan-Meier plots of EFS. Infiltration of %huCD45⁺ cells (E and F) in various organs or tissues at necropsy: VXL/SAR3419 times 3 weeks (black bars), VXL/SAR3419 extended treatment (white bars). PB, peripheral blood; Spl, spleen; BM, bone marrow; LN, lymph node; Liv, liver; Lu, lung; Kid, kidney; Br, brain; SF, spinal fluid.
Figure 1

(A) % huCD45+ cells in PB vs. days post-treatment initiation for ALL-2.

(B) % huCD45+ cells in PB vs. days post-treatment initiation for ALL-3.

(C) % huCD45+ cells in PB vs. days post-treatment initiation for MLL-3.

(D) % huCD45+ cells in PB vs. days post-treatment initiation for MLL-8.

(E) % huCD45+ cells in PB vs. days post-treatment initiation for ALL-8.
Figure 2
Figure 3
Figure 4