EUR Research Information Portal

Pediatric Leukemia

Publication status and date:
Published: 12/09/2023

Document Version
Publisher's PDF, also known as Version of record

Citation for the published version (APA):
Brivio, E. (2023). *Pediatric Leukemia: new treatment opportunities*. [Doctoral Thesis, Erasmus University Rotterdam]. Erasmus University Rotterdam (EUR).

Link to publication on the EUR Research Information Portal

Terms and Conditions of Use
Except as permitted by the applicable copyright law, you may not reproduce or make this material available to any third party without the prior written permission from the copyright holder(s). Copyright law allows the following uses of this material without prior permission:

- you may download, save and print a copy of this material for your personal use only;
- you may share the EUR portal link to this material.

In case the material is published with an open access license (e.g. a Creative Commons (CC) license), other uses may be allowed. Please check the terms and conditions of the specific license.

Take-down policy
If you believe that this material infringes your copyright and/or any other intellectual property rights, you may request its removal by contacting us at the following email address: openaccess.library@eur.nl. Please provide us with all the relevant information, including the reasons why you believe any of your rights have been infringed. In case of a legitimate complaint, we will make the material inaccessible and/or remove it from the website.

Erasmus University Rotterdam
Making Minds Matter

Pediatric Leukemia: new treatment opportunities

Erica Brivio

ISBN: 978-94-6483-194-8
Cover design: adapted from The Tudor pattern book MS. Ashmole
Lay-out: Publiss | www.publiss.nl
Print: Ridderprint | www.ridderprint.nl
© Copyright 2023, Erica Brivio, The Netherlands

All rights reserved. No part of this publication may be
reproduced or transmitted in any form by any means, without prior permission of the author.

Pediatric Leukemia: New treatment opportunities

Leukemie bij kinderen: nieuwe behandelopties

Thesis

to obtain the degree of Doctor from the
Erasmus University Rotterdam
by command of the
rector magnificus

Prof.dr. A.L. Bredenoord
and in accordance with the decision of the Doctorate Board.
The public defence shall be held on

Tuesday 12 September 2023 at 13.00 hrs
by

Erica Brivio
born in Monza (Italy)

Erasmus University Rotterdam



Doctoral Committee:

Promotor: Prof.dr. C.M. Zwaan
Prof.dr. M.L. den Boer

Other members: Prof.dr. A.A. Van de Loosdrecht
Prof.dr. S.N. de Wildt
Dr. A.W. Rijneveld

Copromotor: Dr. V.H.J. van der Velden

Ad Alessandro,
Anita e Vittorio

Pediatric leukemia: New treatment opportunities

Contents

Chapter 1	General introduction	**9**
Chapter 2	Targeted inhibitors and antibody immunotherapies: novel therapies for pediatric leukemia and lymphoma	31
Chapter 3	A phase I study of inotuzumab ozogamicin in pediatric relapsed/ refractory acute lymphoblastic leukemia (ITCC-059)	65
Chapter 4	Inotuzumab ozogamicin as single agent in pediatric patients with relapsed and refractory acute lymphoblastic leukemia: results from a phase ii trial	97
Chapter 5	Inotuzumab ozogamicin in infants and young children with relapsed or refractory acute lymphoblastic leukaemia: a case series	147
Chapter 6	Bosutinib in resistant and intolerant pediatric patients with chronic phase chronic myeloid leukemia: results from the phase i part of study ITCC054/COG AAML1921	161
Chapter 7	General discussion	205
Chapter 8	Summary/ Samenvatting	227

Appendices

List of affiliations of co-authors	**238**
List of publications	**242**
About the author	**245**
PhD portfolio	**246**
Acknowledgments	**247**

Chapter 1

General introduction

Pediatric Leukemia: new treatment opportunities

1. Pediatric leukemias

Hematological cancers in children are heterogeneous classes of diseases and comprise more than one third of pediatric cancer cases. Leukemias represents the most common form of childhood cancer. Of these, acute lymphoblastic leukemia (ALL) accounts for about 75% of cases, while acute myeloid leukemia (AML) for about 15-20%. Chronic leukemia is rarely seen in children, accounting for less than 4% of cases, mainly chronic myeloid leukemia (CML) and even more rarely juvenile myelomonocytic leukemia (JMML).(1) Chronic Lymphocytic leukemia does not occur in childhood.

Acute leukemias are characterized by clonal expansion of malignant immature hematopoietic or lymphoid precursors with a differentiation arrest, while in chronic leukemias the expansion of more mature marrow elements occurs. Acute leukemia is characterized by replacement of normal marrow elements with malignant blast cells, resulting in anemia, thrombocytopenia and neutropenia, explaining the origin of the clinical manifestations at diagnosis. Common presenting symptoms include fever, pallor due to anemia, bleeding manifestations (from petechiae to life-threatening organ bleeding), bone pain (presumably secondary to stretching of the periosteum by leukemic infiltration) and limp (as a result of bone-pain). Although leukemia is primarily a disease of the bone marrow and peripheral blood, any organ or tissue may be infiltrated by the abnormal cells. Common clinical signs of leukemic infiltration are enlargement of the liver or spleen and lymphadenopathy. Extramedullary infiltration of the testes (more common in ALL) and of the central nervous system (CNS) can also be documented. More typical extramedullary manifestations of AML may include chloromas (tumor nodules), skin infiltrations and gingival infiltration. When acute leukemia presents with hyperleukocytosis, the risk of mortality can be increased due to leukostasis, affecting mainly the lungs and the brain.

Chronic myelogenous leukemia, and in general myeloproliferative disorders, are characterized by multilineage clonal proliferation involving the entire spectrum of myeloid cells, suggesting involvement of a pluripotent stem cell as the origin of the disorder. A translocation resulting in the constitutive activation of a receptor tyrosine kinase, t(9;22)(q34;q11) or the Philadelphia chromosome for CML resulting in the *BCR-ABL* fusion gene, explains the increased hematopoietic stem and progenitor cellular proliferation that characterizes these disorders. Most CML patients are diagnosed in a chronic phase and may be asymptomatic. When present, symptoms are generally mild and aspecific, such as fatigue, malaise, weakness, fever, abdominal

pain or weight loss. Typical clinical sign at physical examination is organomegaly, hepatomegaly and more specifically splenomegaly. In the laboratory analysis a high leukocyte count with eosinophilia and basophilia is typical, as well as high platelets count and variable grade of anemia, which make the clinical presentation of chronic myelogenous leukemia in chronic phase different from acute leukemia. Symptoms of leukostasis are very rare compared to acute leukemias, even with very high white blood cell count (WBC). Blast percentages at diagnosis, both in bone marrow and peripheral blood, are typically low (less than 10%), while in acute leukemias there is generally a massive infiltration of blasts accounting for 80-90% of cells in bone marrow. An increase in the percentage of blasts may indicate the transition to the accelerated or blast crisis phase, when a chronic leukemia is transforming to an acute form of leukemia. Accelerated or blast crisis CML is very rare in children and has more acute leukemia features, although definitions are variable among collaborative groups. In Europe mostly the ELN classification is used (Table 1).(2) CML in children often presents with more aggressive clinical and biological features such as a higher proportion of patients exhibiting a larger spleen size and higher leukocyte and platelets counts, indicating a more aggressive natural course of the disease than in adults. (3, 4)

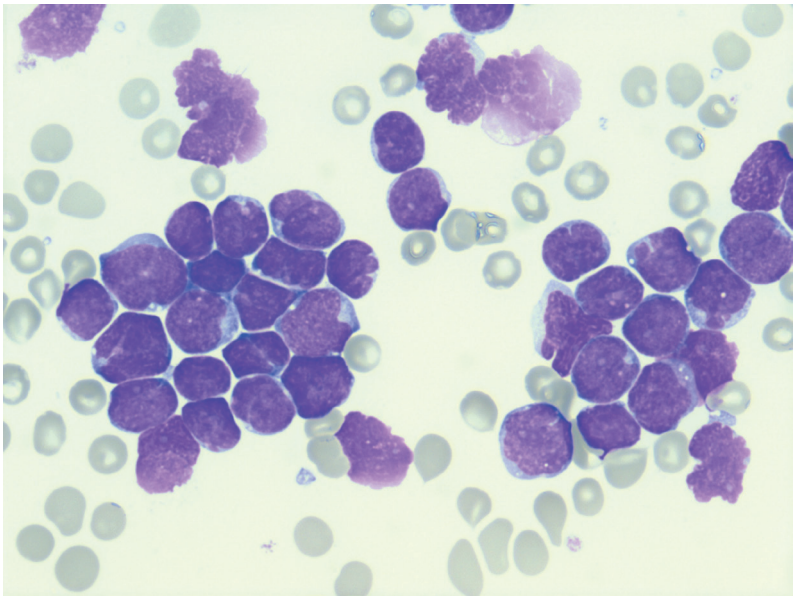


Figure 1A. Bone marrow aspirate smear: precursor B-cell ALL. (courtesy of diagnostic laboratory Princess Máxima Center for pediatric Oncology)

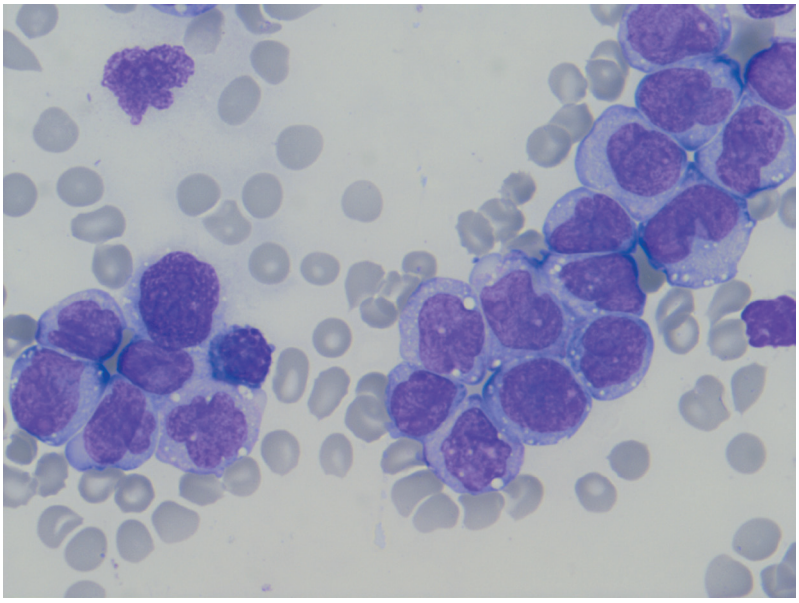


Figure 1B. Bone marrow aspirate smear: AML (courtesy of diagnostic laboratory Princess Máxima Center for pediatric Oncology)

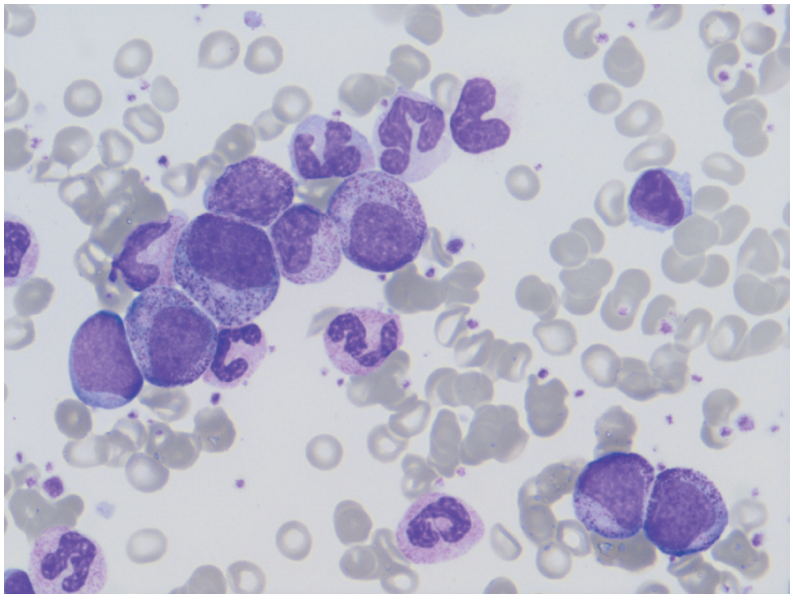


Figure 2. Peripheral blood smear: CML (courtesy of diagnostic laboratory Princess Máxima Center for pediatric Oncology)

Table 1. Criteria to identify the various phases of CML(2)

Phase	Peripheral Blood Findings	Marrow Findings
Blast Phase	≥30% Blasts in blood or bone marrow Extramedullary Blasts other than in spleen If either of these 2 criteria is present, the patient is classified as Blast Phase regardless of other criteria.	
Accelerated Phase	15-29% Blasts or >30% Blasts + Promyelocytes with Blasts <30% ≥20% Basophils Platelets <100 x 10^9/L (not related to therapy) Clonal chromosomal abnormalities in Ph+ cells (CCA/Ph +), major route, on treatment	15-29% Blasts or >30 % Blasts + Promyelocytes with Blasts <30%
Chronic Phase	<15% Blasts <20% Basophils ≤30% Blasts + Promyelocytes with Blasts <15% Platelets ≥100 x 10^9/L (unless related to therapy)	<15% Blasts ≤30% Blasts + Promyelocytes with Blasts <15% No Extramedullary Blasts (except spleen)

Different prognostic factors in acute leukemia have been identified, including biologic and clinical characteristics at diagnosis. In ALL these includes immunophenotype, age, presenting leukocyte count, chromosomal abnormalities and CNS involvement. At the present time, risk-stratified treatment is based mainly on biological characteristics of the disease and response to the initial treatment, measured as minimal residual disease by flow cytometry or quantitative reverse transcription PCR (RT-qPCR). For CML in chronic phase, although characterized by a more indolent clinical course, the response to TKI treatment and/or the occurrence of secondary resistance mutations are the factors impacting the need of a more aggressive therapy with hematopoietic stem cell transplantation (HSCT) and the outcome.(1) Nevertheless, the number of children undergoing HSCT for CML has drastically diminished after the introduction of TKIs.(5, 6)

1.1 ALL

Acute lymphoblastic leukemia is the most frequent malignant disease in childhood with an incidence of about 4/100,000 children per year. In the past decades, significant improvement in outcome has been made in the treatment of childhood ALL, with current rates of overall survival (OS) in first complete remission (CR) of 85-90% with the use of multi-drug front-line protocols.(7, 8) However, the

prognosis for those children who experience a relapse remains poor, especially in case of early relapse or high risk genetics.(9) With the recent improvements in front-line treatments the incidence of relapse, however, is significantly decreased. As an example, the Nordic Society of Pediatric Hematology and Oncology has recently published the results of three consecutive front-line trials for ALL: the 5-year cumulative incidence of relapse was 17.3% (95% CI 15.4–19.2%) and 16.5% (95% CI 14.3–18.8%) for patients in the ALL1992 and ALL2000 trials, respectively, but decreased to 8.4% (95% CI 7.0–10.1%) for patients in the ALL2008 trial.(9) Figure 3 shows improvement in ALL outcome from 1979 to 2019 in the Netherlands, registered by Dutch Childhood Oncology Group (DCOG). In the last 5 years the OS is increased up to above 90%.

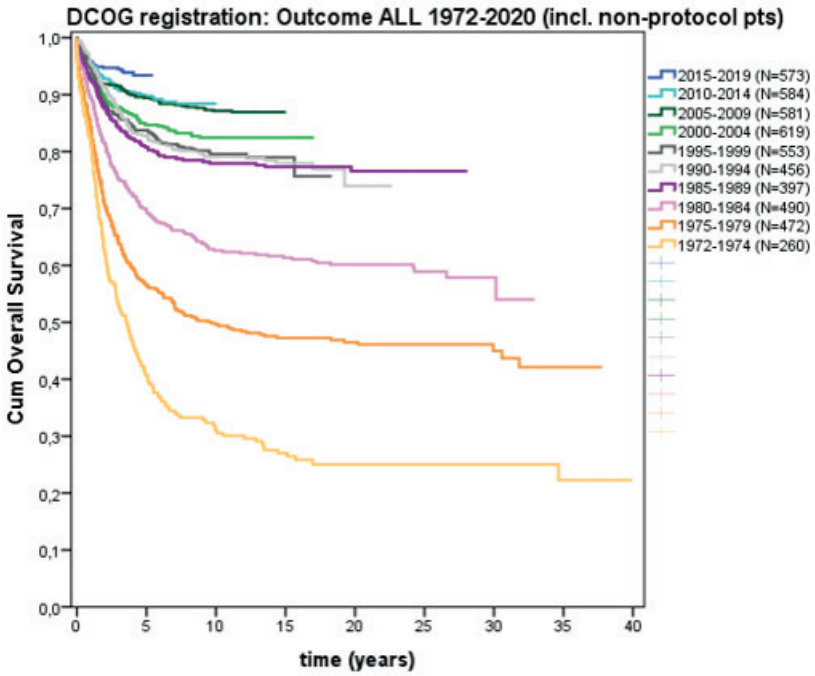


Figure 3. Survival of childhood ALL in the Netherlands: 1972-2019

Following a relapse, about 50-60% of children can be successfully salvaged with intensive chemotherapy treatment, followed by allo-HSCT in high-risk cases.(10, 11) The results of recent trials for relapsed ALL (and other hematologic malignancies) are

summarized in Table 1 of *chapter 2*. The overall survival (OS) may differ according to the risk factors at the time of first relapse. Main determinants of outcome at relapse are duration of first complete remission and immunophenotype. BCP-ALL with bone marrow relapses occurring later than 6 months after stopping therapy has survival rates of about 80%.(12, 13) In contrast, patients with relapses within 18 months of first diagnosis or with isolated medullary relapses occurring within 6 months of stopping therapy, and T-cell isolated or combined medullary relapses at any time, have survival rates between 15% and 30%.(14, 15)

Since 1995, the ALL-REZ BFM group has used the S1-4 (defined below) stratification of patients to treat relapsed ALL patients. The results of the ALL-REZ BFM 2002 protocol showed a significant improvement of EFS and OS compared to preceding trials, particularly in patients in the intermediate risk group. The improvement in survival can be attributed particularly to the improved selection of patients eligible for HSCT and to the standardization of transplant procedure and management, leading to lower rates of treatment-related mortality. S1 patients (late isolated extramedullary relapse) had acceptable EFS rates with chemo/radiotherapy of 70%, whereas S3 (early isolated bone marrow relapse of BCP immunophenotype) and S4 (very early bone marrow relapse and any T-lineage bone marrow relapse) patients achieved EFS rates below 5% with chemotherapy alone and were assigned to allo-HSCT since then. The large and heterogeneous S2 groups (very early/early isolated extramedullary relapse, early/late combined bone marrow and late isolated bone marrow relapse of BCP immunophenotype) achieved intermediate EFS rates of about 60% (Figure 4).

A different treatment strategy was used in the UKALL-R3 trial for ALL in 1st relapse and consisted of a continuous schedule of repetitive chemotherapy elements, avoiding longer treatment-free intervals compared to the BFM strategy. The main question of UKALL-R3 was to evaluate the benefit of replacing idarubicin in a randomized manner with mitoxantrone during reinduction therapy. The mitoxantrone arm showed a significantly superior EFS, leading to early cessation of the trial (Figure 5).(11) In a subgroup analysis, the improved outcome in the mitoxantrone arm was determined to be restricted to standard risk and intermediate risk patients, and to patients undergoing HSCT. In contrast, the results in HR patients remained unsatisfactory. The two strategies (ALL-REZ BFM 2002 vs UKALL-R3) were compared in the IntReALL standard risk (SR) 2010 trial. SR relapse criteria were late ($\geq$ 6 months after end of initial therapy) isolated bone marrow (BM) relapse, late or early ($<$ 6 months after end of initial therapy and $\geq$ 18 months after initial diagnosis) combined BCP-ALL BM relapse, any late/early isolated extramedullary relapse. The BFM approach resulted in to superior outcome

of isolated CNS relapse (5-years EFS 81.6%±6.3, n=40 vs 43.3%±8.3, n=45, p=0.001), while UKALL-R3 induction showed a better MRD response rate and a lower relapse rate in patients with BM relapse.(16) Therefore these different backbones will be implemented for future trials in patients with CNS relapse or BM involvement.

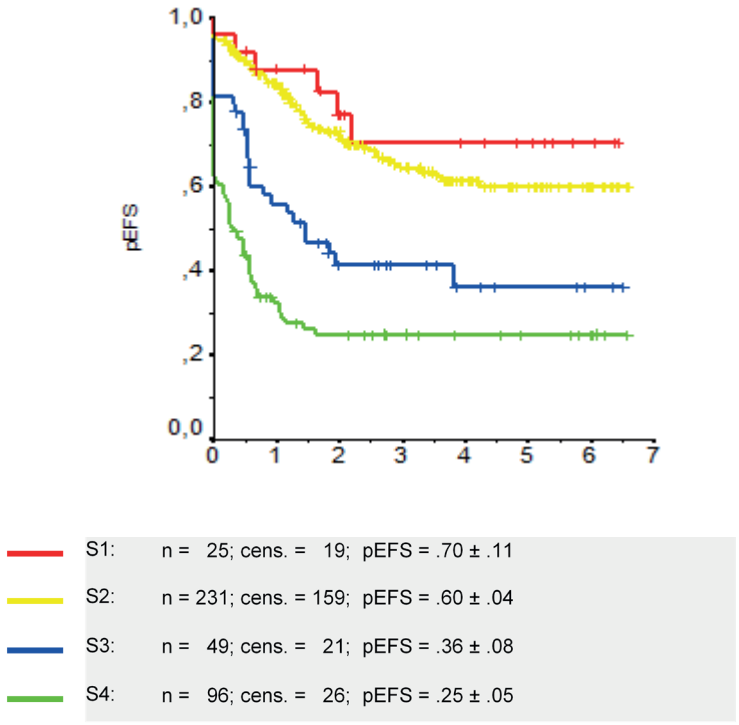


Figure 4. EFS probability for risk group S1-4 within the ALL-REZ BFM 2002 (personal communication A. Von Stackelberg).

ALL at 2nd relapse has poor outcome rates, and remissions are often short-lived. In a report of 2010, intensive salvage regimens only result in 5-year disease-free survival of 27% and 15% for children in second and third remission respectively.(17)
At the present time, the overall survival for relapsed ALL, including patients with a second relapse or further, is improving with the use of novel therapeutic agents.
Two randomized trials have highlighted the superiority of blinatumomab as post-reinduction consolidation treatment compared to chemotherapy in first relapse high risk BCP-ALL. The improved results consisted of both enhanced anti-leukemic activity as well as less hematologic toxicity and infections. These remarkable results are of primary

importance considering that treatment-related mortality (TRM) does occur during ALL relapse therapy, and is mainly related to infection. In the UKALL-R3 protocol, TRM rates, throughout study treatment in the idarubicin and mitoxantrone arm, were 16.5% and 9.7%, respectively.(11) In the phase III trial in intermediate and high-risk first relapse of BCP-ALL NCT02101853, two cycles of blinatumomab as post-reinduction consolidation treatment demonstrated higher disease-free survival (DFS) at 2 years (54.4% vs 39.0%) compared to conventional chemotherapy.(18) In the same trial there were 5 toxic deaths during chemotherapy (all infections) versus none during blinatumomab.

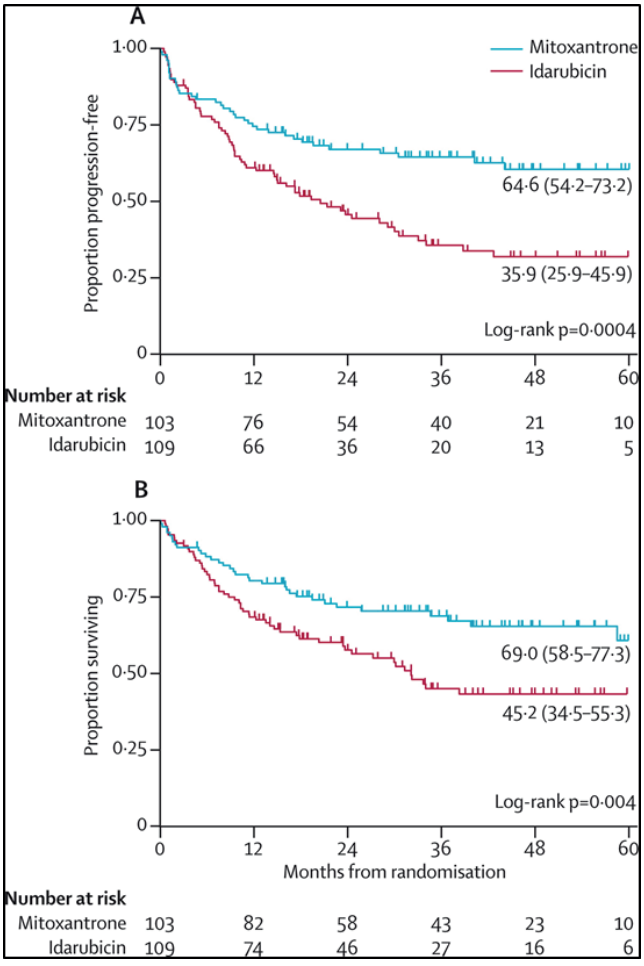


Figure 5. Kaplan Meier estimates of progression-free survival (A) and overall (B) survival by randomized treatment 3-year estimated survival percentages within the UKALLR3. Figure reproduced with permission. (11)

These data document the need to develop novel therapeutic strategies for high risk patients and patients with multiple relapsed ALL who do not adequately benefit from current chemotherapy and allo-HSCT treatments. Recent reinduction regimens have relied on further intensification of standard chemotherapeutic agents with higher doses and/or compacted drug schedules. However, these strategies fail to address one of the major challenges present in many high-risk ALL patients at relapse: intrinsic chemoresistance. Most current strategies have not only failed to improve remission rates, but have also reached tolerability limits, with toxic death rates generally ranging from 3%-8%.(19) Therefore, as further dose intensification is not tolerable, new therapeutic approaches which incorporate novel agents into conventional therapeutic strategies are urgently needed to overcome chemotherapy resistance and improve outcome in high risk patients.

In a series of phase I and phase II studies conducted during the last 15 years, the safety, tolerability and activities of new agents such as the antibody-drug conjugate epratuzumab (COG ADVL04P2; NCT00098839) and the proteosome inhibitor bortezomib (COG AALL07P1; NCT00873093) were explored, paving the way for future integration of new agents into relapsed ALL protocols in an international scenario. (20, 21)

Exciting results have been achieved with the use of immunotherapy and specifically by Tisagenlecleucel, a CD19 chimeric antigen receptors (CAR) T-cell therapy.(22) Tisagenlecleucel is manufactured from autologous T cells transduced to express a costimulatory and a T-cell activation signaling domain and have produced remarkably high CR rates of 70%-97%. Nevertheless relapse remains a significant challenge (2-years relapse-free survival (RFS) rate 62% (95% CI, 47 to 75)).(22, 23).

Blinatumomab and tisagenlecleucel were both approved for children shortly before or during the running period of the research presented in this thesis and the long-term outcomes of these novel approaches are awaited.

Part of our research consisted of conducting a phase I-II study on the use of inotuzumab ozogamicin (InO), an anti-CD22 antibody linked to calicheamicin, a potent cytotoxic antitumor antibiotic, in multiple R/R BCP-ALL.

Antibody-targeted chemotherapy enables a cytotoxic agent to be delivered specifically to tumor cells by conjugating the cytotoxic agent with a monoclonal antibody that binds to a tumor-associated antigen. Antibody-targeted chemotherapy relies on the specific binding of the target: once the monoclonal antibody-drug conjugates to the tumor antigen, the antigen-antibody complex is internalized and the cytotoxic agent is delivered inside the targeted tumor cells. This

strategy maximizes antitumor efficacy as it delivers the cytotoxic agent to targeted tumor cells, reducing exposure of calicheamicin to normal non-malignant cells which lack the targeted antigen, resulting in a significantly improved therapeutic index and less toxicity to the normal cell compartment.(24) Calicheamicins are DNA minor-groove-binding cytotoxic natural products that are significantly more potent than conventional cytotoxic chemotherapeutic agents *in vitro* and cause cell death by inducing double-strand DNA breaks.(24, 25) The first antibody-targeted chemotherapy introduced into practice was gemtuzumab ozogamicin (Mylotarg), a CD33-targeted immunoconjugate of calicheamicin. The interest for a calicheamicin-conjugated antibody in pediatric BCP-ALL was raised about 20 years ago, after *in vitro* evidence of extreme calicheamicin-sensitivity of ALL blasts provided by a study from Zwaan et al.(26) These findings were further supported some years later by *in vitro* data, published by de Vries et al (27), on mechanism of action of InO in BCP-ALL blasts compared to Mylotarg in AML blasts. InO seemed potentially more effective in BCP-ALL than Mylotarg in AML, due to the intrinsic higher sensitivity for calicheamicin of ALL blasts, but also due to a different interaction with the target antigen CD22, compared to CD33 in AML. Although CD22 expression was essential for InO binding, a repetitive loop of CD22 saturation, internalization and renewed CD22 expression was not required to achieve intracellular threshold levels of calicheamicin sufficient for efficient apoptosis in BCP-ALL cells, as instead needed for effective treatment of AML with Mylotarg.(27) Nevertheless, InO was first studied in phase I-III clinical trials in adult B-NHL, also in combination with the anti-CD20 antibody rituximab and conventional chemotherapy, failing to provide evidence of superiority to standard of care. When studied as single agent for adult patients with relapsed or refractory BCP-ALL, InO demonstrated clearly superiority compared to standard of care chemotherapy.(28) The INO-VATE adult phase 3 study (NCT01564784) in relapsed BCP-ALL, showing a complete remission rate of 80.7% in the InO arm as compared to 29.4% in the standard intensive chemotherapy control arm, led to the approval of InO for adults with R/R CD22-positive BCP-ALL.(28) InO was approved by the European Medicines Agency (EMA) and by the Food and Drug Administration (FDA) for the treatment of adults with relapsed/refractory (R/R) CD22+ BCP ALL in 2017. The activity of InO in adult ALL and the medical need in pediatric relapsed/refractory ALL drove the design of the InO ITCC-059 study presented in *chapter 3 and 4.*

With the therapeutic options introduced in the last years, BCP-ALL treatment is undergoing major changes, showing the efficacy and often lower toxicity of immunotherapy and cellular therapy options.

1.2 CML

Chronic myeloid leukemia (CML), although a rare disease in children accounting for fewer than 3% of all pediatric leukemias, with an approximate annual incidence of 1 per million children, was one of the first benefitting from targeted therapy.(29) As in adults, over 90% of children with the clinical features of CML carry the characteristic t(9;22)(q34;q11) translocation resulting in the *BCR-ABL1* fusion oncogene.(30) The advent of therapies targeting the BCR-ABL fusion protein, such as imatinib, has improved the prognosis for many patients with CML and avoided the use of HSCT as standard treatment.(6) Before the advent of the TKI era, treatment such as hydroxyurea and interferon alpha, with or without cytarabine, have been attempt to induce responses. Nevertheless none of them were curative. Allo-HSCT, introduced around 1975-80, was the only proven curative treatment for CML.(5) Unfortunately, high rates of transplant-related mortality (approximately 20%) and post-transplantation recurrence (17%) remained major problems.(5) The use of TKIs significantly improved long-term survival in adult, as well as in children with CML, as shown in Figure 6.

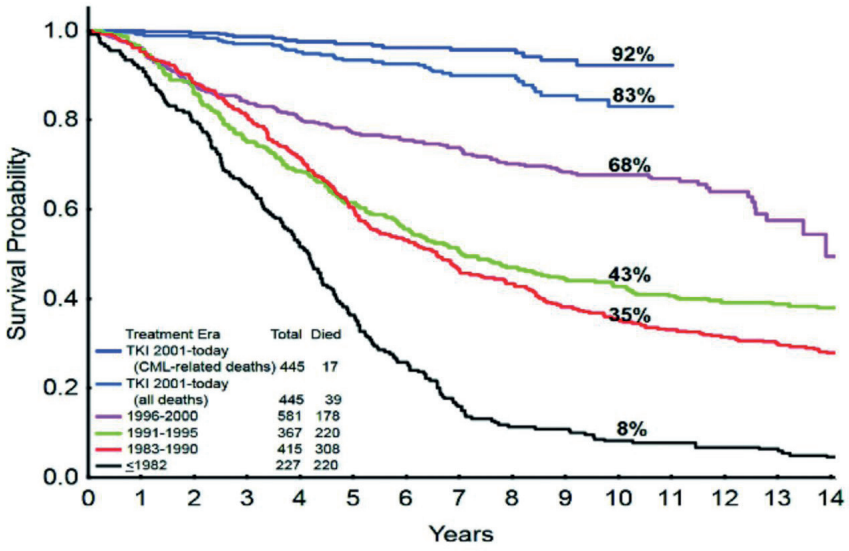


Figure 6. Survival with chronic myeloid leukemia over time (1993-2013): the German CML-Study Group experience. Figure reproduced with permission (31)

Imatinib, the first selective inhibitor of the abnormal BCR-ABL protein tyrosine kinase, has dramatically changed the treatment of CML and was approved for adult with CML

in 2001. Approval in children was achieved in 2003. Favorable results from Phase 1 and Phase 2 studies with imatinib in pediatric patient populations supported its use as a first-line therapy for pediatric CML, with 96% of children achieving complete hematologic response (CHR) and 69% achieving CCyR after 1 year.(32, 33) During, or shortly before, the research period leading to this thesis, the second-generation TKIs dasatinib and nilotinib were approved as first-line treatment in children, which has expanded treatment options and made allogeneic stem cell transplantation (allo-SCT) a third-line treatment. Dasatinib is the best studied second generation TKI, approved for pediatric indication in November 2017.(34) In March 2018 nilotinib was also approved by FDA and EMA for pediatric patients, but differently from other TKIs requires twice daily dosing on an empty stomach, which is often not practical for children.(35)

Side effects of imatinib in children include myelosuppression, nausea, fluid retention, muscle cramps, bone pain, skin rash, diarrhea, lethargy and weight gain. A similar tolerability profile was observed with dasatinib, which showed an higher incidence of pleural effusion (especially in adults); while nilotinib was often associated with liver function abnormalities, nausea, rash, headache, fatigue, pruritus, vomiting, diarrhea, and cough, but less frequently with musculoskeletal pain.(34, 35) Of note, there is preclinical and clinical evidence that long-term exposure to imatinib (and other TKI as dasatinib) results in growth impairment and dysregulation of bone metabolism. Prepubertal children seem to be at greatest risk, with impaired bone remodelling resulting in growth retardation or arrest and a significant reduction in height.(36) A major issue is represented by the fact that about 30% of the patients have to discontinue imatinib due to insufficient response or to intolerance to the drug. Currently, no pediatric-specific response criteria are available, but ELN criteria may be used to assess the milestones of cytogenetic and molecular response, to be achieved especially in the first year of treatment.(37) If a patient fails imatinib, second generation TKIs may be a good treatment option. In adults, it was demonstrated that 43% of patients resistant to imatinib obtained a major molecular response when switched to dasatinib.(38) The phase 2 trial of dasatinib in pediatric patients showed complete cytogenetic response and major molecular response by 12 months in 76% and 41% in imatinib-resistant/intolerant (R/I) group (n=14).(34) In the phase 1 trial of dasatinib in children with imatinib-R/I CML-CP, 14/17 patients (82%) achieved CCyR and 8 (47%) achieved major molecular response (MMR); while in the phase 2 trial in the imatinib-R/I group (n=14), CCyR and MMR by 12 months, respectively, were 76% and 41%.(34, 39) The phase 2 trial of nilotinib including 34 patients in the R/I

cohort showed that 60.6% of the patients could reach MMR at least once by the end of study treatment.(35) In a retrospective data analysis of 27 children who were switched from imatinib to the second generation TKIs dasatinib or nilotinib for poor response or intolerance, deeper molecular responses were observed in 63% and maintained in 37% of patients.(40)

Bosutinib is a second generation multi-targeted tyrosine kinase inhibitor, that has been approved in 2017 for adult patients with newly diagnosed (or resistant/ intolerant) chronic, accelerated, or blast phase Ph+ CML. In imatinib-resistant adult patients treated with bosutinib, 48.4% of the subjects attained a CCyR and 32.1% of the subjects attained a MMR when bosutinib was used as a third line TKI.(41) In the phase 3 trial comparing bosutinib vs imatinib to treat newly-diagnosed CML-CP adult patients, the MMR rate at 12 months was significantly higher with bosutinib versus imatinib (47.2% v 36.9%), as was the CCyR rate by 12 months (77.2% v 66.4%), granting the approval of bosutinib also for newly diagnosed CML in adults.(42) Second generation TKIs dasatinib, nilotinib and bosutinib have been tested against imatinib in company-sponsored randomized trials, but they have never been tested against each other. The results of these trials have provided the basis for approval of these TKIs in the first-line setting, but there is no a clear benefit of one TKI over another as first line-treatment, as stated in the ENL guidelines 2020.(43)

For its cost-effectiveness the generic imatinib is the initial preferred treatment strategy for adults with CML-CP, as indicated by the ENL guidelines. Different aspects should be considered in newly diagnosed pediatric patients to choose the best initial treatment among the three approved TKIs (imatinib, dasatinib, nilotinib). In pediatrics, there is more experience and published data on the efficacy, toxicity profiles, and comorbidities of imatinib than with the other TKIs. Additionally drug availability , ease of administration, and financial can play a role. Imatinib and dasatinib are given once daily and can be given with food, whereas nilotinib is given twice daily and in a fasted state, which can be an issue for compliance in pediatric patients. On the other hand, molecular responses at 6 and 12 months are deeper in pediatric patients treated with second generation TKIs. Experts opinion suggests that if higher cost is not an issue, second generation TKIs might be a good option for first-line therapy in children, considering that TKI discontinuation needs to be considered and preferred above life-long treatment. To achieve this goal, deep prolonged remission is needed and might be achieved by a higher proportion of patients with second generation TKIs.(3)

As one third of the pediatric patients do not achieve a deep response or are intolerant to first generation TKI imatinib, second generation TKIs including bosutinib can be

a valuable option to be explored to improve efficacy and/or have improved safety profile. Differently from the other TKIs, bosutinib has more gastrointestinal and less musculo-skeletal side effects and perhaps affects growth to a lesser extent.(41)
The ITCC-054 study, presented in *chapter 6*, was designed to cover this unmet medical need, and explore the possible different safety profile of bosutinib, including a potential lower toxic effect observed on bone growth in animal models, which may represent a true benefit to treatment-naive newly diagnosed pediatric patients with CML when growth is less impaired than with the other TKIs.(44)
If a patient fails treatment with second generation TKIs, allo-SCT could be considered as a valuable option. Nevertheless new options are now available and could further reduce the number of patients in need of an all-SCT, such as the third generation TKI ponatinib and the the ABL Myristoyl Pocket (STAMP) inhibitor asciminib. The third generation TKI ponatinib is under investigation in children and is demonstrating potent ABL kinase inhibition, including activity against the T315I mutation, which leads to resistance to first- and second-generation TKIs (NCT03934372). In addition it inhibits other tyrosine kinases, including FLT3, KIT, RET, AKT, ERK1/2, FGFRs and others, implicated in a variety of myeloproliferative disorders and solid tumors. It is currently approved for second-line treatment in adult CML and Ph+ ALL, or first line in case of T315I mutation.(45) Asciminib is a BCR-ABL inhibitor specifically targeting the ABL Myristoyl Pocket (STAMP) recently approved for the treatment of adult patients with CML, previously treated with two or more TKIs or carrying the T315I mutation. In a phase 3 trial in adults R/I to 2 or more previous TKIs, asciminib has shown superior efficacy compared with that of bosutinib, together with a favorable safety profile.(42) Table 2 provides an overview of completed or ongoing clinical trials of TKIs for CML (and other malignancies) in the pediatric population.

Table 2. Overview of completed or ongoing pediatric clinical trials of TKIs (BCR-ABL inhibition)

Compound	Phase	Disease	Trial Number and Reference, if applicable
Completed			
Imatinib	Phase I	CML/ ALL	Champagne et al.(32)
Imatinib	Phase II	CML	Millot et al. (33)
Imatinib	Phase III	CML	Suttorp et al. (46)
Imatinib	Phase IV	CML	Millot et al. (47)
Dasatinib	Phase I	ALL/CML/AML/ solid tumors	Aplenc et al. (48)
Dasatinib	Phase I	ALL/CML/AML	Zwaan et al. (39)
Dasatinib	Phase II	CML	Gore et al.(34)
Nilotinib	Phase I	CML/ ALL	Hijiya et al.(49)
Nilotinib	Phase II	CML	Hijiya et al. (35)
Ongoing			
Bosutinib	Phase I-II	CML	ITCC-054/COG AAML1921 (EudraCT 2015-002916-34)
Ponatinib	Phase I-II	ALL/AML/CML/ solid tumors	NCT03934372
Asciminib	Phase I-II	CML	NCT04925479

2. General overview of the thesis

Development and approval of new agents for pediatric cancers lags behind adult development. Clinical trials in children, especially early phase 1-2 studies, requires international effort and collaboration, due to the small number of patients with cancer and relapsed or refractory disease. ***Chapter 2*** provides an overview of the current state of the art in targeted therapies for children with leukemia and lymphoma, including clinical trials available for the pediatric population.

The subsequent chapters present the results of phase 1-2 trials developed within the ITCC consortium and sponsored by Erasmus MC and performed in close collaboration with the Princess Máxima Center since its opening in 2018.

In ***chapter 3*** **and** ***4*** the results of the international phase 1 and phase2 clinical trial of single-agent inotuzumab ozogamicin (InO) (ITCC-059) in relapsed and refractory ALL patients are discussed. At first, we established the recommended phase 2 dose (RP2D) as single agent (in a fractionated schedule), followed by a phase 2 cohort to determine the ORR of single-agent InO and to collect additional safety and pharmacokinetic data at the RP2D. Blocks of single agent InO are being subsequently studied incorporated into existing ALL regimens. The trial was designed in the context of a Pediatric Investigational Plan (PIP) for the approval of InO for pediatric use by the regulatory agencies.

When specific information could not be retrieved through clinical trials, we performed retrospective data collection to fill a knowledge gap, as shown in ***Chapter 5*** for the use of InO in infants. Infants (children < 1 year of age) are generally not eligible for treatment in clinical trials, as well as in the ITCC-059 for InO. Specific questions were considered relevant in these very young patients: the management of drug administration in this category of patients with very low body weight and low Body Surface Area (BSA) is always challenging, especially in case of new agents with potential unknown toxicities. Additionally, when experiencing relapse these patients face a very poor prognosis with event free survival rates as low as 20%.(50, 51) We aimed to answer whether InO could be a valid treatment option in this setting.

In ***Chapter 6,*** the preliminary results of the ITCC-054 trial in children with CML treated with bosutinib are presented. In this study, bosutinib is evaluated in pediatric patients with CML who are resistant or intolerant to other TKIs, as these patients have an unmet medical need and require new treatment options that improve efficacy and provide a better tolerability profile compared to currently available TKIs. The preliminary results presented in this thesis concern the phase 1 part of the study focused on pharmacokinetics (PK) and safety in the subgroup of resistant and intolerant (R/I) patients. In the Phase 2 part of the study, still ongoing, newly diagnosed CML pediatric patients are included, together with additional R/I patients, each group treated at the specific RP2D established during the phase 1 part of the study, and based on safety results as well as PK profile of the drug. The phase 2 part of the study might provide important additional safety data relevant for the pediatric population, as bosutinib showed a potential lower toxic effect observed on bone growth in animal models, which may represent a true benefit to treatment-naive newly diagnosed pediatric patients.(52)

The overall focus of this thesis was to improve the treatment of pediatric leukemia patients, through novel therapeutic options, that could be more effective and possibly less toxic, in the context of early phase clinical trials.

References

1. Orkin S H FDE, Ginsburg D, Look A T, Lux S E, Nathan D G Nathan and Oski's Hematology and Oncology of Infancy and Childhood, 8th Edition 2014;2.
2. Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872-84.
3. Hijiya N, Suttorp M. How I treat chronic myeloid leukemia in children and adolescents. Blood. 2019;133(22):2374-84.
4. Suttorp M, Millot F, Sembill S, Deutsch H, Metzler M. Definition, Epidemiology, Pathophysiology, and Essential Criteria for Diagnosis of Pediatric Chronic Myeloid Leukemia. Cancers (Basel). 2021;13(4).
5. Cwynarski K, Roberts IA, Iacobelli S, van Biezen A, Brand R, Devergie A, et al. Stem cell transplantation for chronic myeloid leukemia in children. Blood. 2003;102(4):1224-31.
6. Suttorp M, Millot F. Treatment of pediatric chronic myeloid leukemia in the year 2010: use of tyrosine kinase inhibitors and stem-cell transplantation. Hematology Am Soc Hematol Educ Program. 2010;2010:368-76.
7. Moricke A, Zimmermann M, Reiter A, Henze G, Schrauder A, Gadner H, et al. Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia. 2010;24(2):265-84.
8. Pui CH, Yang JJ, Hunger SP, Pieters R, Schrappe M, Biondi A, et al. Childhood Acute Lymphoblastic Leukemia: Progress Through Collaboration. J Clin Oncol. 2015;33(27):2938-48.
9. Jensen KS, Oskarsson T, Lähteenmäki PM, Flaegstad T, Jónsson ÓG, Svenberg P, et al. Temporal changes in incidence of relapse and outcome after relapse of childhood acute lymphoblastic leukemia over three decades; a Nordic population-based cohort study. Leukemia. 2022.
10. Oskarsson T, Söderhäll S, Arvidson J, Forestier E, Montgomery S, Bottai M, et al. Relapsed childhood acute lymphoblastic leukemia in the Nordic countries: prognostic factors, treatment and outcome. Haematologica. 2016;101(1):68-76.
11. Parker C, Waters R, Leighton C, Hancock J, Sutton R, Moorman AV, et al. Effect of mitoxantrone on outcome of children with first relapse of acute lymphoblastic leukaemia (ALL R3): an open-label randomised trial. Lancet. 2010;376(9757):2009-17.
12. Parker C, Krishnan S, Hamadeh L, Irving JAE, Kuiper RP, Révész T, et al. Outcomes of patients with childhood B-cell precursor acute lymphoblastic leukaemia with late bone marrow relapses: long-term follow-up of the ALLR3 open-label randomised trial. The Lancet Haematology. 2019;6(4):e204-e16.
13. Eckert C, Groeneveld-Krentz S, Kirschner-Schwabe R, Hagedorn N, Chen-Santel C, Bader P, et al. Improving Stratification for Children With Late Bone Marrow B-Cell Acute Lymphoblastic Leukemia Relapses With Refined Response Classification and Integration of Genetics. Journal of Clinical Oncology. 2019;37(36):3493-506.
14. Eckert C, Parker C, Moorman AV, Irving JAE, Kirschner-Schwabe R, Groeneveld-Krentz S, et al. Risk factors and outcomes in children with high-risk B-cell precursor and T-cell relapsed acute lymphoblastic leukaemia: combined analysis of ALLR3 and ALL-REZ BFM 2002 clinical trials. European Journal of Cancer. 2021;151:175-89.

15. Nguyen K, Devidas M, Cheng SC, La M, Raetz EA, Carroll WL, et al. Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's Oncology Group study. Leukemia. 2008;22(12):2142-50.

16. von Stackelberg A, Bourquin J-P, Zimmermann M, Revesz T, Attarbaschi A, Ferster A, et al. ALL-REZ BFM 2002 Is Associated with Improved Outcome as Compared to ALL-R3 Strategy in Children with Standard Risk Isolated CNS Relapse of Acute Lymphoblastic Leukemia, while Maintaining Comparable Efficacy in Patients with Bone Marrow Relapse. Results of the Multi-National, Multi-Center Trial IntReALL SR 2010. Blood. 2022;140(Supplement 1):3247-9.

17. Ko RH, Ji L, Barnette P, Bostrom B, Hutchinson R, Raetz E, et al. Outcome of patients treated for relapsed or refractory acute lymphoblastic leukemia: a Therapeutic Advances in Childhood Leukemia Consortium study. J Clin Oncol. 2010;28(4):648-54.

18. Brown PA, Ji L, Xu X, Devidas M, Hogan LE, Borowitz MJ, et al. Effect of Postreinduction Therapy Consolidation With Blinatumomab vs Chemotherapy on Disease-Free Survival in Children, Adolescents, and Young Adults With First Relapse of B-Cell Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. JAMA. 2021;325(9):833-42.

19. Sun W, Orgel E, Malvar J, Sposto R, Wilkes JJ, Gardner R, et al. Treatment-related adverse events associated with a modified UK ALLR3 induction chemotherapy backbone for childhood relapsed/refractory acute lymphoblastic leukemia. Pediatr Blood Cancer. 2016;63(11):1943-8.

20. Raetz EA, Cairo MS, Borowitz MJ, Blaney SM, Krailo MD, Leil TA, et al. Chemoimmunotherapy reinduction with epratuzumab in children with acute lymphoblastic leukemia in marrow relapse: a Children's Oncology Group Pilot Study. J Clin Oncol. 2008;26(22):3756-62.

21. Messinger YH, Gaynon PS, Sposto R, van der Giessen J, Eckroth E, Malvar J, et al. Bortezomib with chemotherapy is highly active in advanced B-precursor acute lymphoblastic leukemia: Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) Study. Blood. 2012;120(2):285-90.

22. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018;378(5):439-48.

23. Gardner RA, Finney O, Annesley C, Brakke H, Summers C, Leger K, et al. Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. Blood. 2017;129(25):3322-31.

24. Lee MD, Manning JK, Williams DR, Kuck NA, Testa RT, Borders DB. Calicheamicins, a novel family of antitumor antibiotics. 3. Isolation, purification and characterization of calicheamicins beta 1Br, gamma 1Br, alpha 2I, alpha 3I, beta 1I, gamma 1I and delta 1I. The Journal of antibiotics. 1989;42(7):1070-87.

25. Zein N, Sinha AM, McGahren WJ, Ellestad GA. Calicheamicin gamma 1I: an antitumor antibiotic that cleaves double-stranded DNA site specifically. Science. 1988;240(4856):1198-201.

26. Zwaan C, Reinhardt D, Jürgens H, Huismans DR, Hählen K, Smith OP, et al. Gemtuzumab ozogamicin in pediatric CD33-positive acute lymphoblastic leukemia: first clinical experiences and relation with cellular sensitivity to single agent calicheamicin. Leukemia. 2003;17(2):468-70.

27. de Vries JF, Zwaan CM, De Bie M, Voerman JS, den Boer ML, van Dongen JJ, et al. The novel calicheamicin-conjugated CD22 antibody inotuzumab ozogamicin (CMC-544) effectively kills primary pediatric acute lymphoblastic leukemia cells. Leukemia. 2012;26(2):255-64.

28. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab Ozogamicin versus Standard Therapy for Acute Lymphoblastic Leukemia. N Engl J Med. 2016;375(8):740-53.

29. Faderl S, Talpaz M, Estrov Z, O'Brien S, Kurzrock R, Kantarjian HM. The Biology of Chronic Myeloid Leukemia. New England Journal of Medicine. 1999;341(3):164-72.

30. Millot F, Traore P, Guilhot J, Nelken B, Leblanc T, Leverger G, et al. Clinical and biological features at diagnosis in 40 children with chronic myeloid leukemia. Pediatrics. 2005;116(1):140-3.

31. Tariq IM, Jerald PR, Michael WD, Jane FA, Timothy PH, Christine JH, et al. Chronic myeloid leukemia: reminiscences and dreams. Haematologica. 2016;101(5):541-58.

32. Champagne MA, Capdeville R, Krailo M, Qu W, Peng B, Rosamilia M, et al. Imatinib mesylate (STI571) for treatment of children with Philadelphia chromosome-positive leukemia: results from a Children's Oncology Group phase 1 study. Blood. 2004;104(9):2655-60.

33. Millot F, Guilhot J, Nelken B, Leblanc T, De Bont ES, Békassy AN, et al. Imatinib mesylate is effective in children with chronic myelogenous leukemia in late chronic and advanced phase and in relapse after stem cell transplantation. Leukemia. 2006;20(2):187-92.

34. Gore L, Kearns PR, de Martino ML, Lee, De Souza CA, Bertrand Y, et al. Dasatinib in Pediatric Patients With Chronic Myeloid Leukemia in Chronic Phase: Results From a Phase II Trial. J Clin Oncol. 2018;36(13):1330-8.

35. Hijiya N, Maschan A, Rizzari C, Shimada H, Dufour C, Goto H, et al. Phase 2 study of nilotinib in pediatric patients with Philadelphia chromosome-positive chronic myeloid leukemia. Blood. 2019;134(23):2036-45.

36. Samis J, Lee P, Zimmerman D, Suttorp M, Hijiya N. The complexity of growth failure in children receiving tyrosine kinase inhibitor therapy for chronic myelogenous leukemia. Pediatr Blood Cancer. 2017;64(12).

37. Baccarani M, Castagnetti F, Gugliotta G, Rosti G. A review of the European LeukemiaNet recommendations for the management of CML. Ann Hematol. 2015;94 Suppl 2:S141-7.

38. Shah NP, Rousselot P, Schiffer C, Rea D, Cortes JE, Milone J, et al. Dasatinib in imatinib-resistant or -intolerant chronic-phase, chronic myeloid leukemia patients: 7-year follow-up of study CA180-034. Am J Hematol. 2016;91(9):869-74.

39. Zwaan CM, Rizzari C, Mechinaud F, Lancaster DL, Lehrnbecher T, Velden VHJvd, et al. Dasatinib in Children and Adolescents With Relapsed or Refractory Leukemia: Results of the CA180-018 Phase I Dose-Escalation Study of the Innovative Therapies for Children With Cancer Consortium. Journal of Clinical Oncology. 2013;31(19):2460-8.

40. Kurosawa H, Tanizawa A, Muramatsu H, Tono C, Watanabe A, Shima H, et al. Sequential use of second-generation tyrosine kinase inhibitors following imatinib therapy in pediatric chronic myeloid leukemia: A report from the Japanese Pediatric Leukemia/Lymphoma Study Group. Pediatr Blood Cancer. 2018;65(12):e27368.

41. Cortes JE, Kantarjian HM, Brümmendorf TH, Kim DW, Turkina AG, Shen ZX, et al. Safety and efficacy of bosutinib (SKI-606) in chronic phase Philadelphia chromosome-positive chronic myeloid leukemia patients with resistance or intolerance to imatinib. Blood. 2011;118(17):4567-76.

42. Cortes JE, Gambacorti-Passerini C, Deininger MW, Mauro MJ, Chuah C, Kim DW, et al. Bosutinib Versus Imatinib for Newly Diagnosed Chronic Myeloid Leukemia: Results From the Randomized BFORE Trial. J Clin Oncol. 2018;36(3):231-7.

43. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966-84.

44. Tauer JT, Hofbauer LC, Jung R, Erben RG, Suttorp M. Micro-osmotic pumps for continuous release of the tyrosine kinase inhibitor bosutinib in juvenile rats and its impact on bone growth. Med Sci Monit Basic Res. 2013;19:274-8.

45. Cortes JE, Kim DW, Pinilla-Ibarz J, le Coutre P, Paquette R, Chuah C, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. N Engl J Med. 2013;369(19):1783-96.

46. Suttorp M, Schulze P, Glauche I, Göhring G, von Neuhoff N, Metzler M, et al. Front-line imatinib treatment in children and adolescents with chronic myeloid leukemia: results from a phase III trial. Leukemia. 2018;32(7):1657-69.

47. Millot F, Baruchel A, Guilhot J, Petit A, Leblanc T, Bertrand Y, et al. Imatinib Is Effective in Children With Previously Untreated Chronic Myelogenous Leukemia in Early Chronic Phase: Results of the French National Phase IV Trial. Journal of Clinical Oncology. 2011;29(20):2827-32.

48. Aplenc R, Blaney SM, Strauss LC, Balis FM, Shusterman S, Ingle AM, et al. Pediatric Phase I Trial and Pharmacokinetic Study of Dasatinib: A Report From the Children's Oncology Group Phase I Consortium. Journal of Clinical Oncology. 2011;29(7):839-44.

49. Hijiya N, Zwaan CM, Rizzari C, Foà R, Abbink F, Lancaster D, et al. Pharmacokinetics of Nilotinib in Pediatric Patients with Philadelphia Chromosome-Positive Chronic Myeloid Leukemia or Acute Lymphoblastic Leukemia. Clinical Cancer Research. 2020;26(4):812-20.

50. Driessen EMC, de Lorenzo P, Campbell M, Felice M, Ferster A, Hann I, et al. Outcome of relapsed infant acute lymphoblastic leukemia treated on the interfant-99 protocol. Leukemia. 2017;31(12):2854.

51. Tomizawa D, Koh K, Hirayama M, Miyamura T, Hatanaka M, Saikawa Y, et al. Outcome of recurrent or refractory acute lymphoblastic leukemia in infants with MLL gene rearrangements: A report from the Japan Infant Leukemia Study Group. Pediatr Blood Cancer. 2009;52(7):808-13.

52. Samis J, Lee P, Zimmerman D, Arceci RJ, Suttorp M, Hijiya N. Recognizing Endocrinopathies Associated With Tyrosine Kinase Inhibitor Therapy in Children With Chronic Myelogenous Leukemia. Pediatr Blood Cancer. 2016;63(8):1332-8.

Chapter 2

Targeted Inhibitors and Antibody Immunotherapies: Novel Therapies for Pediatric Leukemia and Lymphoma

Brivio E, Baruchel A, Beishuizen A, Bourquin JP, Brown PA, Cooper T, Gore L, Kolb EA, Locatelli F, Maude SL, Mussai FJ, Vormoor-Bürger B, Vormoor J, von Stackelberg A, Zwaan CM

Eur J Cancer. 2022 Mar;164:1-17

Abstract

Despite improved outcomes achieved in the last decades for children with newly diagnosed leukemia and lymphoma, treatment of patients with refractory/relapsed disease remains a challenge. Cure rate is still unsatisfactory and often achieved at the cost of significant morbidity. Exploring treatment with novel agents should offer less toxic therapeutic options, without compromising efficacy. Bispecific and antibody-drug conjugates targeting CD19 and CD22 (blinatumomab and inotuzumab ozogamicin) play an important role in the treatment of relapsed and refractory B-cell acute lymphoblastic leukemia (BCP-ALL); antibodies targeting CD123 and CD38 are also under investigation for acute myeloid leukemia (AML) and T-ALL, respectively. Targeted therapy with small molecules is of primary importance for specific genetic subtypes, such as BCR-ABL positive ALL, *FLT3*-ITD AML, ALK-positive anaplastic large cell lymphoma. *KMT2A*-directed targeted therapy with menin inhibitors hold promise to be of relevance in *KMT2A*-rearranged leukemias, known to have dismal prognosis. Target inhibition in cellular pathways such as BCL-2, RAS, MEK, BTK, JAK-STAT or CDK4/CDK6 inhibition may be suitable for different diseases with common mutated pathways. Nevertheless, development and approval of new agents for pediatric cancers lags behind adult therapeutic options. New regulations were implemented to accelerate drug development for children. Considering the number of oncology medicinal products available for adults and the rarity of pediatric cancers, prioritization based on scientific evidence and medical need, as well as international collaboration, are critical. Herein we review the current status of drug development for children with leukemia and lymphoma, excluding cellular therapy despite its well-known significance.

Introduction

Leukemias and lymphomas in children are heterogeneous diseases comprising roughly one third of pediatric cancers. Although in acute lymphoblastic leukemia (ALL), mature B-cell ALL and lymphoblastic lymphoma outcome has increased to over 85% event-free survival (EFS), outcome in anaplastic large-cell lymphoma (ALCL) and acute myeloid leukemia (AML) still lag behind with EFS rates in the range of 55-75%.(1, 2) Salvage treatment options for B-cell precursor ALL (BCP-ALL) patients have increased significantly with the market authorization of blinatumomab and chimeric antigen receptor T cells (CAR T cells), changing the landscape for treatment options, and resulting in a new relapse category post-immunotherapy.(3, 4) For T-cell ALL, despite current high survival rates in CR1, first relapse salvage remains dismal and there is no unified approach to relapse.(5) In pediatric AML, first-relapse salvage rates have improved with re-treatment with intensive chemotherapy and stem-cell transplantation (4), with overall survival (OS) of about 40-50%, but there is a paucity of options for second or later relapse.(6) Moreover, liposomal daunorubicin is no longer available, that was considered standard of care for 1st relapse, at least in Europe. CAR T-cell therapy in AML is hampered by the problem of identifying antigens selective for the leukemia cell population while sparing hematopoietic stem cells, and thus avoiding the risks of prolonged myelosuppression. In pediatric non-Hodgkin Lymphomas (NHL), progress is highly dependent on the subtype of NHL. In mature B-cell NHL, prognosis after relapse remains dismal, similar to T-cell lymphoblastic lymphoma (T-LL). Progress has been made in ALCL with the availability of vinblastine, brentuximab vedotin and *ALK*-inhibitors, although crizotinib was approved only recently by the FDA and not yet in Europe.(7-9) Relapses of B-cell precursor NHL are approached in a similar way as in BCP-ALL.(1) Table 1 summarizes recent results of pediatric trials in relapsed/refractory hematological malignancies.

Improved outcomes have often been at the expense of significant acute morbidity and mortality as well as late effects.(10, 11) Replacing therapy elements with less toxic, but equally effective, drugs therefore remains an important goal. Hematopoietic stem cell transplantation (HSCT) is almost inevitably part of salvage treatment after relapse and is also associated with the risk of transplant-related mortality and long-term morbidity, and the long-term safety of CAR T-cell therapy as an alternative need to be awaited.(12) Development and approval of new agents for pediatric cancers lags behind adult development. New regulations in Europe and U.S. are enacted to ensure commitment of companies to develop drugs in children.(13) The new RACE (Research Acceleration for Cure and Equity) for Children Act requires pediatric investigation of new agents if directed at a relevant molecular target for pediatric cancer, even when the drug is intended for the treatment of an adult cancer.

Table 1. Recent results of pediatric trials in relapsed/refractory hematological malignancies

Disease category	Study Title	N. relapse	Era	Nr of patients	Overall Survival	Event-free Surival	Reference
Pediatric ALL							
B/T ALL	TACL retrospective data	2nd relapse	2005-2013	325	-	In CR3: 2 y: 41% +/- 6%	Sun et al, Leukemia 2018
	TACL retrospective data	1st relapse	1995-2004	225	-	2 y: 40% +/- 4% 5-y 27% +/- 4% In CR3: 2 y: 31% +/- 7%; 5y 15% +/- 7%	Ko et al. JCO 2010
	NOPHO retrospective data	1st relapse	2002-2011	239	5 y: 57.5% ± 3.4%	5 y: 51% +/- 3%	Oskarsson et al. Haematologica 2016
	ALLR3 MITOX arm only	1st relapse	2003-2009	103	3 y: 69.0% (95% CI 58.5–77.3)	3 y: 64.6% (95% CI 54.2–73.2)	Parker et al., Lancet 2010;376:1968-1970
B ALL	AALL1331 Blina arm only	1st relapse	2014-2019	105	2 y: 71.3%	2 y: 54.4%	[Brown et al. JAMA. 2021
	NCT02393859 Blina arm only	1st relapse	2015-2019	54	2y: 80%	2y: 66.2% (95%CI, 50.1%-78.2%)	Locatelli et al. JAMA 2021
B ALL	Austrian BFM	1st relapse	1981-1999	150	10 y: 37% ± 4%	10 y: 40% +/- 4%	Reismuller et al., 2009. Br J Haematol. 2009
T ALL	Austrian BFM	1st relapse	1981-1999	28	10 y: 21% ± 8%	10 y: 21% ± 8%	Reismuller et al., 2009. Br J Haematol. 2009

Table 1. Continued

Disease category	Study Title	N. relapse	Era	Nr of patients	Overall Survival	Event-free Surival	Reference
Pediatric AML							
	Relapsed AML 2001/01	1st relapse	2001-2009	394	4 y: 38% ± 3% Early 1st rel. 28% ± 3%; Late 1st rel. 48% ± 4%	-	Kaspers et al. JCO 2013
	TACL retrospective data	1st relapse	1995-2004	99	5 y: 29% ± 5%	5 y: 24 +/-5%	Gorman et al. Pediatr Blood Cancer.2010
	AAML1421	1st relapse	2016-2018	38	In CR2 2y 52.7% ± 21.1%	-	Cooper et al., JCO 2020
	BFM registry	1st relapse	2005-2010	155	5y: 45 ±4%	-	Rasche et al, Leukemia 2018
	ITCC-020/I-BFM 2009-02	Early 1st relapse or ≥2nd relapse	2010-2014	34	2 y: 32.4 ± 8.0%	2 y: 26.5% ± 7.6%	van Eijkelenburg et al. Haematologica 2018
Pediatric NHL							
ALCL	ALCL relapse study	-	2004-2014	118	3 y: 78% ± 4%	3 y: 59% +/- 5%	Ruf S. et al. BJH 2015 (Abstract)
	ALCL99-vinblastine (vinblastine arm only)	-	1999-2006	110	-	2 y: 73%	Le Deley et al. J Clin Oncol 2010
	ALCL-Relapse trial (NCT00317408)	Progression/ relapse	2005-2014	105	5y: 78% ± 4%.	5y: 53% ± 5%	Knörr et al, JCO 2020
B-NHL	CCLG retrospective data	-	2000-2010	33	4 y: 27.3%	-	Anoop et al. leukemia&Lymphoma 2012
	JPLSG retrospective data	-	2004-2011	33	5 y: 48.5%	-	Osumi et al. Pediatr Blood Cancer 2016
	SFCE retrospective data	-	1989-2007	67	5 y: 29.9%	-	Jourdain A et al. Haematologica 2015
T LL	BFM retrospective data	-	1990-2003	28	5 y:14% ±6%	-	Burkhardt B et al. JCO 2009
T/B LL	JPLSG retrospective data	-	1996-2004	48 (9 B LL, 32 TLL)	3 y 43.2± 7.4% T LL: 32.8± 8.6% B LL: 72.9±16.5%	3 y 37.0± 7.3%	Mitsui e al. Pediatr. Blood Cancer 2009

Considering the rarity of pediatric cancers, prioritization based on science and medical need are of primary importance, as discussed at Pediatric Forum meetings organized by ACCELERATE, an international multi-stakeholder network involving clinicians, regulators, industry and patients advocates (https://www.accelerate-platform.eu/). (14-16)

We aim to review the current status of drug development for leukemia and lymphoma in children, excluding Hodgkin lymphoma. Figures 1, 2 and 3 provide an overview of actionable targets with agents currently in development in pediatric settings, in AML, ALL and NHL respectively. Cellular therapies including CAR T-cells, while transformational for some patients, are outside the scope of this paper and reviewed elsewhere.

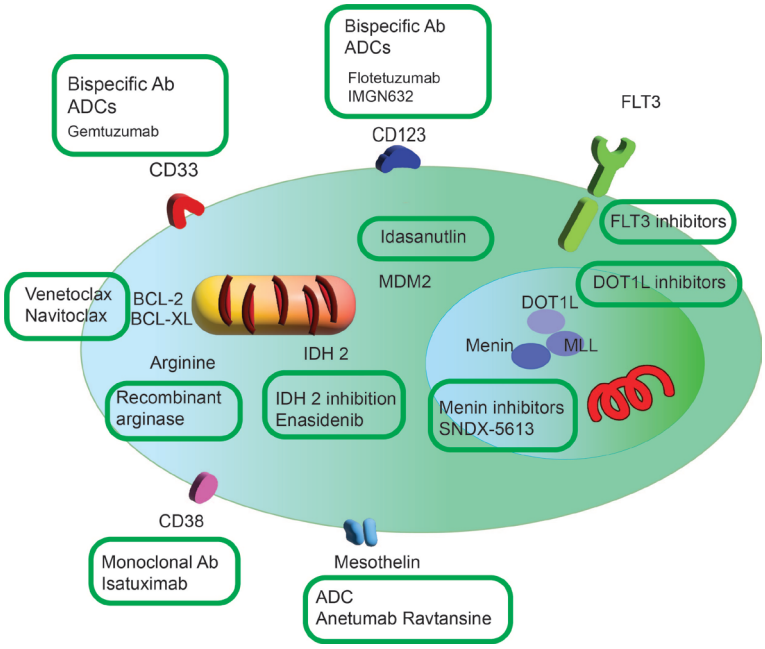


Figure 1. Overview of current targets in pediatric Acute Myeloid Leukemia
The figure shows actionable targets with agents currently in development in pediatric AML and discussed in this overview, excluding CAR T cells products.
Ab: antibody; ADC: Antibody-drug conjugate.

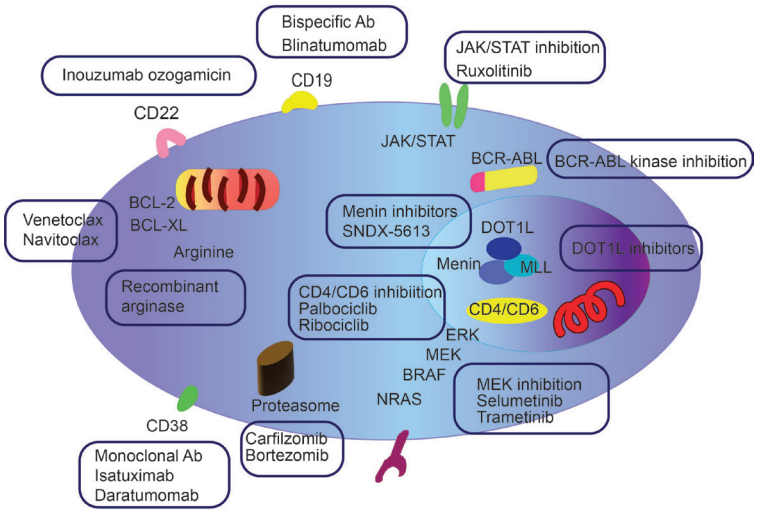


Figure 2. Overview of current targets in pediatric Acute Lymphoblastic Leukemia
The figure shows actionable targets with agents currently in development in pediatric ALL, excluding CAR T cells products.
BCR-ABL kinase inhibitors can target additional ABL-class fusions involving ABL1, ABL2, CSF1R, and PDGFRB (not shown in the figure).
Ab: antibody; ADC: Antibody-drug conjugate.

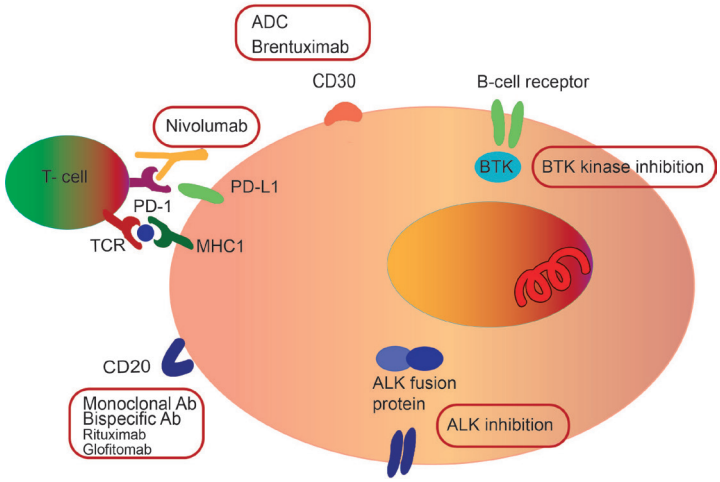


Figure 3. Overview of current targets in pediatric Non-Hodgkin Lymphoma
The figure shows actionable targets with agents currently in development in pediatric NHL, excluding CAR T cells products. The figure include actionable targets for ALCL as well as other types NHL. Red circles highlight targets actionable for ALCL, blue circles for Burkitt lymphoma, Diffuse large cell lymphoma or other NHL.
Ab: antibody; ADC: Antibody-drug conjugate

1. IMMUNOTHERAPY

1.1 Monoclonal antibodies:

1.1.1 Anti-CD38

Therapeutic CD38-targeting monoclonal antibodies (daratumumab; isatuximab) have recently been approved to treat adults with multiple myeloma (MM). Preclinical data show that CD38 is expressed in many pediatric hematological malignancies, with a strong expression in ALL, and more variable expression in AML.(17) Based on promising preclinical data and the high CD38 expression at diagnosis and relapse, targeting CD38 could have utility in ALL and AML.(17, 18) Daratumumab induces lysis through cytotoxic mechanisms (complement-dependent, cell-mediated and antibody-dependent cellular phagocytosis). A phase 1-2 trial of daratumumab with standard 4-drug re-induction in children and young adults with R/R BCP or T-cell ALL and LL is ongoing (NCT03384654). Isatuximab is an immunoglobulin G1 class monoclonal antibody, also binding CD38, that exerts its activity by the same mechanisms as daratumumab.(19) The NCT03860844 study ongoing for pediatric BCP/T-ALL and AML, observed CR+CRi in 3/7 (42.9%) in the B-ALL cohort, 2/6 (33.3%) in the T-ALL cohort, and 2/4 (50.0%) in the AML cohort . In this study, patients receive isatuximab in combination with chemotherapy (modified UKALL R3 for ALL and FLAG for AML). PK parameters in children (>2 y) are consistent with those observed in adult ALL.(20) Similar to other monoclonal antibodies, infusion reactions have been reported as the most common adverse event for both compounds.

1.1.2 Anti-CD20

Rituximab, a monoclonal antibody that binds to CD20, is currently the standard of care in addition to chemotherapy in mature B-cell non-Hodgkin lymphoma, and also as single-agent in post-transplant lymphoproliferative disease, especially in case of EBV-positive disease.(21) Its role in ALL is less established, with only 30 to 50% of BCP-ALL blasts expressing CD20. A French trial in adults (NCT00327678), adding rituximab to the ALL chemotherapy protocol, demonstrated an improved outcome for younger adults with CD20-positive, Ph-negative ALL.(22) CD20 expression has an adverse prognostic significance in adult ALL, while its impact in pediatric ALL is controversial.(23) Due to these characteristics, CD20 is a potential target that has not been widely used to treat R/R pediatric BCP-ALL. Novel anti-CD20 antibodies, such as obinutuzumab, an anti-CD20 monoclonal antibody with greater affinity for CD20, and the bispecific T-cell engagers glofitamab and mosunetuzumab, may be of interest for future studies in mature B-cell ALL/NHL, as well as in CD20-positive BCP-ALL.(24)

1.2 Bispecific antibodies

Bispecific antibodies bind two distinct antigens simultaneously, linking antigens on target cells to immune effector cells (i.e., T cells, NK cells, or macrophages).

1.2.1 Blinatumomab

Blinatumomab directs CD3-positive effector T cells to CD19-positive target cells. It is currently approved by the FDA as monotherapy for the treatment of children with relapsed or refractory (R/R) BCP-ALL, or in first or second complete remission (CR) with persisting positive minimal residual disease (MRD).(25) In Europe, blinatumomab is indicated for pediatric patients above the age of 1 year, with second or greater R/R BCP-ALL, or with high-risk first relapse BCP-ALL as part of the consolidation therapy. In the single-agent trial in overt relapse neurological toxicity and cytokine release syndrome (CRS) were noticed as a result of high tumor burden, which occur at a much lower frequency in minimal residual disease setting. (3)For example, the results of an expanded access trial of blinatumomab in R/R ALL showed a good safety profile and high MRD response rate (NCT02187354).(25) Two randomized trials have highlighted the superiority of blinatumomab as post-reinduction consolidation treatment compared to chemotherapy. This was due both to enhanced anti-leukemic activity as well as less hematologic toxicity and infections. In the phase III trial in intermediate and high-risk first relapse of BCP-ALL NCT02101853, two cycles of blinatumomab as post-reinduction consolidation treatment demonstrated higher disease-free survival (DFS) at 2 years (54.4% vs 39.0%) compared to conventional chemotherapy. (26) The NCT02393859 randomized trial demonstrated superior EFS for children with high-risk first relapse of BCP-ALL treated with blinatumomab as compared to those given a third block of consolidation chemotherapy before HSCT.(27) Based on these results, blinatumomab seems to be best positioned in MRD setting in post-induction therapy, rather than for remission re-induction. Novel strategies to overcome mechanisms of blinatumomab resistance are under investigation, including the combination with immune checkpoint inhibitors (NCT04546399/ AALL1821).(28) Currently, research is focused on moving blinatumomab to upfront treatment. Infants with ALL have a poor prognosis compared to older children, with a 4-year EFS rate of 47% and a survival rate after relapse of approximately 20%.(29) A pilot study adding blinatumomab to the Interfant-06 backbone for infants with *KMT2A*-rearranged (*KMT2A*-r) ALL (NCT03643276) is ongoing. Trials including newly diagnosed children above 1 year of age with BCP-ALL are also aiming to determine how to optimally incorporate blinatumomab. The Children's Oncology Group (COG)

NCT03914625/AALL1731 trial, and the BFM (Berlin-Frankfurt-Münster)/AIEOP (Associazione Italiana Emato-oncologia pediatrica) NCT03643276 trial include randomized questions of blinatumomab during consolidation. The ALLTogether1 trial (NCT03911128) evaluates blinatumomab as consolidation treatment in patients with Down syndrome, who have poor tolerance to chemotherapy. Nevertheless, a recent warning about increased risk of seizures during blinatumomab infusion in patients with Down syndrome >10 years has been raised by the COG group.(30)

1.2.2 Flotetuzumab

Flotetuzumab is a CD123xCD3 bispecific antibody. CD123 is overexpressed in AML and other disease subsets, such as BCP-ALL, ETP-ALL (early-T precursor ALL) and MPAL (mixed phenotype acute leukemia), as compared to normal hematopoietic stem cells, and has been associated with chemotherapy resistance and high-risk genetic alterations, including FLT3 internal tandem duplication (FLT3-ITD) in AML. (31, 32) A phase 1/2 study in adults with R/R AML (NCT02152956) established the maximum tolerated dose (MTD) at 500 ng/kg/day, the most frequent adverse events being infusion-related reactions and CRS. Complete response after flotetuzumab positively correlated with 'immune infiltration' profiles, that were mainly noted in patients with primary induction failure (PIF) or early relapse, leading to a clinical benefit for this specifc patient group (ORR 30% versus 20%).(33) A COG phase 1 trial of flotetuzumab in pediatric patients with refractory or second or later relapse AML is currently open for recruitment (NCT04158739).

Besides flotetuzumab, a variety of other CD123-targeted agents have been tested in preclinical models and translated into human clinical trials, including antibodies with enhanced antibody-dependent cellular cytotoxicity(34) and antibody-drug conjugates (ADC) such as IMGN632 (see section on ADCs).(35)

1.3. Antibody-drug conjugates

1.3.1 Inotuzumab ozogamicin

Inotuzumab ozogamicin (InO) is an anti-CD22 antibody linked to calicheamicin, a potent cytotoxic antitumor antibiotic. The INO-VATE adult phase 3 study (NCT01564784), with a complete remission rate of 80.7% in the InO arm as compared to 29.4% in the standard intensive chemotherapy control arm, led to the approval of InO for adults with R/R CD22-positive BCP-ALL.(36) In a compassionate use program in children with R/R BCP-ALL, treatment with single agent InO resulted in CR in 67% of patients.

(37) A phase 1/2 study is currently ongoing in children with R/R BCP ALL (study ITCC-059) within the European Innovative Therapies for Children with Cancer (ITCC) group. The phase 1-study established the recommended phase 2 dose (RP2D) at 1.8 mg/m^2, similar as in adults, with an ORR of 80%, which was confirmed in the phase 2 part of the study.(38, 39) An additional phase 1b cohort is ongoing to test the combination of InO with a modified R3 reinduction regimen. The study also includes a cohort of patients with very high-risk first relapse of CD22 positive ALL, who will receive single agent InO (NTR57360), followed by consolidation with SCT or CAR T-cells once in CR. The COG is performing two additional studies on InO in pediatric ALL, the AALL1621 phase 2 study, using the adult RP2D, and a phase 3 randomized trial of InO for newly diagnosed high-risk CD22-positive BCP-ALL, which is currently open for recruitment (AALL1732 study; NCT03959085). AALL1621 demonstrated an ORR of 58.6% in children and young adults with R/R CD22-positive BCP-ALL (40), and is proceeding with InO combined with chemotherapy in a manner to complement the ITCC-059 study. InO experience in infants is limited: a retrospective study showed a CR rate of 47% in R/R ALL.(41) InO is also being studied during consolidation in the ALLTogether1 protocol for newly diagnosed ALL patients with persistent high MRD (NCT03911128). A particular concern with the use of InO is sinusoidal obstruction syndrome (SOS), likely due to the calicheamicin component. Warnings were raised by studies in adults, showing a relatively high incidence in the transplant population (30% of transplanted patients post-InO), similar to GO. (36, 37, 39). Current recommendations, according to the summary of product characteristics, are to limit InO to 2-3 cycles if a patient is a candidate for HSCT, and to use non-alkylating chemotherapy.

The available data suggest that InO may be a very efficient drug for remission induction, and may be less toxic than conventional multi-agent (re-)induction chemotherapy. A comparative study between InO single-agent and chemotherapy as re-induction therapy will be performed by the IntReALL group, to further provide evidence for this.

1.3.2 IMGN632

As mentioned above, CD123 represents an attractive candidate for targeted therapy approach in different disease subsets and various CD123-targeted agents are in development. IMGN632 is a humanized anti-CD123 antibody linked to a novel DNA-alkylating payload of the novel IGN class, the indolinobenzodiazepine pseudodimer. (35) IMGN632 has demonstrated promising activity in a phase 1-2 trial in adults with R/R AML and blastic plasmacytoid dendritic cell neoplasms (BPDCN), which led to

FDA approval for the treatment of BPDCN at the recommended dose of 0,045 mg/kg once every 21 days (NCT03386513).(42) A phase 1-2 study in pediatric AML is in development in the international PedAL/EuPAL program (Pediatric Acute Leukemia/European Pediatric Acute Leukemia), which is a global precision medicine clinical trial with the aim to improve outcome in pediatric AML (see Future directions section below).(43) Another CD123-targeting compound, with a truncated diphtheria toxin payload, Tagraxofusp-erzs, was approved in December 2018 by the FDA for treatment of BPDCN in adult and pediatric patients.

1.3.3 Anetumab Ravtansine: a mesothelin-directed ADC

Anetumab Ravtansine (AR) is a mesothelin (MSLN)-directed antibody linked to the maytansinoid tubulin inhibitor DM4. MSLN is a cell surface glycoprotein normally expressed on mesothelial cells and it is overexpressed in several human cancers, including in 23-35% of paediatric AML samples, with a significantly higher expression in patients with inv(16), t(8;21) and *KMT2A* translocations. The phase I study in adults with advanced solid tumors showed a favourable safety profile.(44) Anetumab ravtansine showed a survival benefit and synergy with conventional chemotherapy in mouse models with disseminated MSLN+ leukemia.(45) A study in pediatric AML is in development.

1.3.4 Brentuximab vedotin

Brentuximab vedotin is a CD30-directed ADC linked to the cytotoxic payload monomethyl auristatin E (MMAE), approved for adult patients with Hodgkin Lymphoma (HL) and anaplastic large-cell lymphoma (ALCL). A pediatric phase 1-2 trial recently established the RP2D at 1·8 mg/kg once every 3 weeks, showing an ORR of 53% for R/R ALCL, and 47% for classical HL.(8) COG ANHL12P1 tested brentuximab vedotin with chemotherapy in children with newly diagnosed ALCL, reporting a two-year EFS of 79.1% (95% CI, 67.2% to 87.1%) and a two-year OS of 97.0% (95% CI, 88.1% to 99.2%), without significant additional toxicity compared to standard chemotherapy.(46)

1.3.5 Gemtuzumab ozogamicin

Gemtuzumab ozogamicin (GO) is a humanized anti-CD33 antibody conjugated to calicheamicin approved in combination with chemotherapy for the treatment of patients of 15 years and above with newly diagnosed AML by the EMA, and for all age

groups by the FDA. A large randomized COG trial in newly diagnosed AML showed that GO added to chemotherapy improved EFS through a reduction in relapse risk.(47)In Europe GO is studied in a fractionated schedule in newly diagnosed pediatric AML in a French-UK collaboration, still ongoing (NCT02724163). As most relevant toxicities veno-occlusive liver disease and prolonged cytopenia have been observed. During the past few years, several additional CD33-targeting antibody-based drugs were developed (i.e. the ADC IMGN779, the bispecific antibodies AMG330 and AMG673), but no pediatric trials are currently ongoing.

2. SMALL MOLECULE INHIBITORS

2.1 FLT3 inhibition

The incidence of *FLT3*-ITD in AML increases with age, occurring in approximately 10-15% of children and 20-30% of adults with *de novo* AML, conferring a poor prognosis in patients with AML harbouring this lesion.(48) To date, two FLT3 inhibitors have been approved for adult AML: midostaurin in combination with chemotherapy for newly diagnosed patients, and gilteritinib as monotherapy for R/R patients. No drugs are approved for pediatric AML as yet, but pediatric studies are ongoing.

2.1.1 Midostaurin

Midostaurin was initially developed as an inhibitor of protein kinase C, but was subsequently recognized to have strong inhibitory effects on FLT3, and various other targets as well. It was approved by FDA and EMA for use in adults with *de novo* *FLT3*-mutated AML in combination with chemotherapy, following a randomized study demonstrating improved median EFS and OS compared to patients treated with chemotherapy.(49) A phase 1 trial of midostaurin monotherapy was conducted in children with leukemia by ITCC, establishing the pediatric recommended dose at 30 mg/m^2 twice daily; 5 of 9 patients with AML and 3 of 13 patients with *KMT2A*-rearranged ALL had partial or complete responses.(50) A phase 2 study is open to evaluate midostaurin combined with standard chemotherapy, and as a single agent post-consolidation therapy in children with *FLT3*-mutated AML (NCT03591510).

2.1.2 Sorafenib

Sorafenib is a first-generation pan-kinase inhibitor with activity against RAF, c-KIT, PDGFR, VEGFR, and FLT3, and is FDA approved for the treatment of adults with

solid tumors. A COG phase 3 trial AAML1031 (NCT01371981) was conducted to assess the efficacy of sorafenib in addition to chemotherapy for children and young adults with de novo *FLT3*–ITD AML. Due to concerns for cardiac toxicity, the study was amended to start sorafenib only between cycles of chemotherapy rather than concurrently. In addition, one year of sorafenib maintenance therapy was added. This resulted in improved remission rates and an improved 3-year EFS, compared to historical controls (57.5% versus 34.3%, p = 0.007).(51) A randomized trial against a placebo arm in adults, showed that sorafenib maintenance therapy reduced the risk of relapse and death after HSCT for FLT3-ITD-positive AML.(52)

2.1.3 Quizartinib

Quizartinib is a second generation FLT-3 inhibitor and has 10–50 times greater *in vivo* potency than first-generation inhibitors against *FLT3*-ITDs, and demonstrates preclinical activity against overexpression of *FLT3*-WT and c-KIT (53). Recently, the QuANTUM-First trial in adults with newly diagnosed AML met its primary endpoint of increased overall survival (press release). Next to FLT3-mutated AML, in pediatric ALL, the highest levels of *FLT3*-WT expression occurs in *KMT2A*-r patients. Based on this observation, a phase 1 study tested quizartinib in combination with chemotherapy in 17 children with R/R AML or *KMT2A*-r ALL, and the optimal dose level was set at 60 mg/m^2 once daily.(54) Three of the seven patients with *FLT3*-ITD AML achieved complete response. Remissions were not achieved in wild-type *FLT3* AML or *KMT2A*-r ALL. An industry-sponsored phase 1/2 trial has opened in R/R AML combining quizartinib with chemotherapy, followed by consolidation therapy and/or HSCT, and maintenance treatment with quizartinib for one year (NCT03793478). *FLT3*-ITD+ cells with secondary *FLT3*- tyrosine kinase domain (TKD) mutations may become resistant to the first generation FLT3 inhibitors and to quizartinib, but may retain sensitivity to the other 2nd generation inhibitors crenolanib and gilteritinib.(55) When combined with chemotherapy developing resistance mutations may be less relevant, as known from Philadelphia-chromosome positive ALL and *BCR-ABL* inhibitors.

2.1.4 Crenolanib

Crenolanib was originally designed as a PDGFR inhibitor, although data suggest that this second-generation TKI has robust activity against both *FLT3*-ITD and *FLT3*-TKD mutations, including those that confer resistance to quizartinib. Tolerable pediatric dosing of crenolanib monotherapy was established at 170 mg/m^2 once daily in a

phase 1 trial conducted at St Jude Children's Research Hospital (SJCRH) in children with central nervous system gliomas, which have activated PDGFR signalling. The current SJCRH RELHEM2 phase 1 trial (NCT02270788) is assessing the safety of combined crenolanib and sorafenib in children with R/R hematologic malignancies.

2.1.5 Gilteritinib

Gilteritinib is a potent second generation FLT3 inhibitor, with moderate additional activity against AXL, and is also active against *FLT3*-TKD resistance mutations (96). Gilteritinib recently received FDA-approval as monotherapy for treatment of adult patients with R/R *FLT3 mutant* AML, based on improved OS for the Gilteritinib arm compared to standard chemotherapy in a phase 3 trial. This trial reported good tolerability and a 34% (versus 15.3%) CR/CRi rate in heavily pretreated patients.(56) In the COG AAML1831 study for newly diagnosed AML, patients with FLT3 mutations are treated with gilteritinib plus chemotherapy. An industry-sponsored international study is also open for pediatric patients with R/R AML (NCT04240002).

2.2 ALK-inhibition

Anaplastic lymphoma kinase (ALK) inhibitors are investigated in the treatment of malignancies that are *ALK* or *ROS* fusion gene driven, including ALCL. A variety of ALK inhibitors are available, including crizotinib, ceritinib, lorlatinib, alectinib, entrectinib and brigatinib. A Pediatric Strategy Forum meeting in 2017 and a follow-up meeting this year focused on ALK inhibition, highlighting its role in achieving remission in relapsed ALCL. Prospects for the future are establishing its role in curing newly diagnosed children, while reducing treatment toxicity, as well as combination approaches (i.e. brentuximab or vinblastine in combination with crizotinib or other ALK-inhibitors).(57)

A phase 1/2 study of crizotinib in 26 children with relapsed ALK-positive ALCL showed an ORR of 83% and 90% for patients treated at 165 mg/m^2 and 280 mg/m^2 twice daily.(7) In January 2021, the FDA approved crizotinib for pediatric patients (1 year of age and older) and young adults, with R/R ALK-positive ALCL at the 280 mg/m^2 oral dose twice daily. This dose is higher compared to the adult approved flat dose of 250 mg twice daily (500 mg daily). An ITCC phase 1/2 basket trial tested crizotinib at a fixed dose of 150 mg/m^2 twice daily in combination with a dose escalation of vinblastine in relapsed ALK-positive ALCL (ITCC053/CRISP); however, this combination raised tolerability issues, and hence the study will generate more data on single-

agent activity. The overall ALCL relapse strategy within the European Inter-Group for Childhood Non-Hodgkin Lymphoma (EICNHL) consists of the ITCC053/CRISP study in first relapse, and in the ALCL-Nivo study (NCT03703050) in second relapse, evaluating the response to nivolumab compared to allo-HSCT.(58) A recently published phase 1/2 study in 83 pediatric patients established the MTD of ceritinib to be 510 mg/m^2 once daily (fasted) and 500 mg/m^2 once daily (fed). and showed ORR of 75% for ALCL treated at MTD. The most commonly reported adverse event was transaminase elevation.(60) Although clinically active, crizotinib and ceritinib may not have durable efficacy, and secondary resistance mutations may occur. (7, 59) Moreover, these drugs do not penetrate in the central nervous system. In adults with non–small-cell lung cancer, brigatinib (dosed 180 mg once daily) achieved better intracranial response rates, more durable responses, and showed better tolerability and quality-of-life scores over crizotinib.(61) Moreover, brigatinib maintained activity against all 17 secondary ALK mutants tested in cellular assays. Brigatinib will be tested in a phase I-II trial for ALCL, IMT or other solid tumors (the ITCC-098 study).

2.3 BCR-ABL kinase inhibition

The chromosomal translocation t(9;22)(q34;q11), Philadelphia chromosome (Ph), is found in all patients with chronic myeloid leukemia (CML), and in 3-4% of pediatric patients with ALL. Its resulting product is the fusion protein BCR-ABL with aberrant kinase activity, in two major isoforms: p210, the hallmark of CML, and p190, which occurs in the majority of BCP-ALL cases. Ph-like ALL is an identified high-risk B-lineage ALL subtype. Its gene expression profile is similar to that of Ph+ ALL, with ABL-class fusions involving ABL1, ABL2, CSF1R, and PDGFRB, and other alterations (not targetable with ABL kinase inhibitors) including CRLF2, JAK2, EPOR that activate JAK/STAT signaling.(62)

Imatinib, dasatinib and nilotinib are first- and second-generation BCR-ABL inhibitors approved for pediatric patients with CML, while the second generation TKI bosutinib is currently under investigation (NCT04258943). Imatinib and dasatinib in combination with chemotherapy are also approved for pediatric patients with Ph+ ALL. The NCT02883049 COG trial is exploring the addition of dasatinib to backbone chemotherapy for ABL-class Ph-like ALL. Recent interesting results obtained in adults with Ph+ALL treated with a combination of dasatinib and blinatumomab as induction therapy may open the possibility of chemotherapy-free induction treatment for Ph+-ALL.(69)

Ponatinib is a third-generation kinase inhibitor demonstrating potent ABL kinase inhibition, including activity against the T315I mutation, which leads to resistance to

first- and second-generation TKIs. It is currently approved for second-line treatment in adult CML and Ph+ ALL, or first line in case of T315I mutation.(63) In addition, ponatinib has been shown to be a potent inhibitor of other tyrosine kinases, including FLT3, KIT, RET, AKT, ERK1/2, FGFRs and others, implicated in a variety of myeloproliferative disorders and solid tumors. A phase 1/2 study is evaluating ponatinib single-agent in pediatric patients with CML resistant or intolerant to a prior TKI-containing therapy, or Ph+ ALL with T315I mutation, or any other tumor in which standard therapy is not available or is not indicated (NCT04501614). An important consideration is the vascular toxicity reported at the approved dose of 45 mg ponatinib in adults. Studies with lower dosages of ponatinib for adult CML are ongoing, to see if they are associated with a better vascular safety signal.(65) Two reports on compassionate use of ponatinib in 11 and 21 pediatric patients with CML or Ph+ ALL have been published with median doses administered of 21.4 mg/m^2 and 20 mg/m^2 (or 16 mg/m^2 for ALL); treatment efficacy is reported as major molecular response in 50% of cases in one study and as disease burden decrease in 70% of cases in the other.(66, 67)

Asciminib is a BCR-ABL1 inhibitor specifically targeting the ABL Myristoyl Pocket (STAMP) recently approved for the treatment of adult patients with CML, previously treated with two or more TKIs or carrying the T315I mutation. No trial in pediatric are currently available or planned.

2.4 MEK inhibition

MEK kinase is a component of the RAS-RAF-MEK-ERK-pathway. Approximately 40% of R/R ALL carry different *RAS*-pathway mutations (NRAS, KRAS, PTNP11, and *FLT3*). In a mouse model, selumetinib selectively repressed growth of *RAS*-mutated ALL blasts, also reducing CNS disease burden.(68) Selumetinib acts synergistically with dexamethasone. Dexamethasone-induced blast apoptosis is mediated by increasing the pro-apoptotic protein Bim, which is inactivated when phosphorylated by ERK. MEK inhibition prevents this ERK phosporylation, hence inducing synergy. The ITCC-063 trial is evaluating the combination of dexamethasone and selumetinib in pediatric and adult R/R ALL patients (NCT03705507).

The presence of NRAS or KRAS mutations in T-ALL have significant impact on overall survival and mutations occur in 9% of T-ALL relapsed patients.(69) MEK-inhibitors might be able to re-sensitize patients to steroids, which could be of value in this population with a poor prognosis.(70) Moreover, MEK inhibitors effectively killed *RAS*-mutant infant ALL cells *in vitro*, with trametinib being the most potent compound

tested.(71) Trametinib is not yet tested in pediatric patients with acute leukemia; one trial in R/R Juvenile Myelomonocytic Leukemia is currently ongoing (NCT03190915).

2.5 BTK inhibition

Signaling from the B-cell receptor (BCR) is essential for normal B-cell development and controls several cellular functions including proliferation, apoptosis and differentiation. Constitutive activation of the BCR is common in B-cell malignancies, including chronic lymphocytic leukemia (CLL), and mature B-NHL. Ibrutinib is an oral, potent inhibitor of Bruton's Tyrosine Kinase (BTK), the kinase responsible for BCR signal transduction.(72) The SPARKLE study (NCT02703272) investigated ibrutinib in pediatric patients with R/R mature B-cell NHL, in combination with either RICE (rituximab, ifosfamide, carboplatin, etoposide and dexamethasone) or RVICI (rituximab, vincristine, idarubicin, carboplatin, ifosfamide and dexamethasone). The phase 1 part of the study demonstrated that the combination was safe, and preliminary efficacy was promising with 57% response. However, the randomized phase 2 part stopped enrollment for futility as failed demonstrating the superior EFS in the ibrutinib+RICE/RVICI arm compared to RICE/RVICI alone.(73) In addition, *in vitro* data suggest that deletion of BTK may enhance asparaginase-induced apoptosis, suggesting that therefore ibrutinib combined with asparaginase could enhance efficacy.

2.6 JAK/STAT inihibition

Rearrangements of *CRLF2-R* with frequent concomitant *JAK2* point mutations occur in ~50% of Ph-like ALL cases and induce constitutive JAK/STAT and other kinase signaling. An additional 15-20% of Ph-like ALL carries other JAK pathway alterations that similarly activate JAK/STAT signaling. Ruxolitinib is a potent, selective JAK1/JAK2 inhibitor with demonstrated activity in preclinical Ph-like ALL models. In the ongoing phase 2 COG AALL 1521 study (NCT02723994), ruxolitinib is administered in combination with multi-agent chemotherapy in Ph-like ALL patients. The continuous administration of 40 mg/m2/dose × 28 days per cycle was found to be safe and to induce sustained inhibition of phosphorylated STAT5. (74)

2.7 CDK4/CDK6 inhibition

The CDK 4/6-p16-retinoblastoma (Rb) pathway is commonly disrupted in cancer, leading to abnormal cell proliferation. *NOTCH* mutations, a common feature of T-ALL, lead to up-regulation of cyclin D3, resulting in CDK4/6 hyper-activation, phosphorylation of Rb and

cell cycle progression. Ribociclib and palbociclib are selective inhibitors of CDK4 and CDK6.(75) Both ribociclib and palbociclib are FDA approved for hormone receptor-positive/HER2/neu-negative advanced breast cancer when combined with an aromatase inhibitor. A pediatric phase 1 study with ribociclib in rhabdoid tumors and neuroblastoma has been completed; most common grade 3-4 adverse event was hematologic toxicity.(76) The phase 1 COG AINV18P1 trial will test palbociclib in combination with reinduction chemotherapy in children and young adults with relapsed B- or T- lineage ALL(NCT03792256). Another phase 1 trial (DFCI 18-328) investigates ribociclib in combination with the mTOR inhibitor everolimus and dexamethasone in R/R pediatric ALL. An important secondary objective is to determine the pharmacokinetic characteristics of ribociclib and everolimus when given in combination with dexamethasone, due to potential drug-drug interactions related to CYP3A metabolism (NCT03740334).

2.8 IDH inhibition

Mutations in *IDH1/2* generate high levels of 2-hydroxyglutarate (2-HG) that inhibit various components of the epigenetic machinery including histone and DNA demethylases – impairing cellular differentiation. In adult AML, *IDH1/2* mutations occur in approximately 10% of cases, but in pediatric AML only in 2-4% of cases, mostly in adolescents.(77) Enasidenib is a targeted inhibitor of mutated *IDH2*. In adult AML with *IDH2* mutations, continuous daily enasidenib treatment was generally well tolerated, with 19.3% of R/R patients attaining complete remission.(78) The multicentre, single-arm open-label ESMART trial (NCT02813135) aims to evaluate safety and activity of enasidenib as a single agent in up to ten R/R AML *IDH2*-mutated pediatric patients. Ivosidenib is an inhibitor of IDH1, currently FDA approved for adult R/R AML *IDH1* mutated (or first line in elderly patients with AML). Clinical trials in pediatric AML are not open, but the MATCH trial (NCT04195555) is investigating ivosidenib in pediatric solid tumors and lymphomas with *IDH1* mutations.

2.9 Proteasome inhibition

Bortezomib in combination with chemotherapy has shown improved complete remission rate in R/R T/BCP-ALL over historical controls in a COG study.(79) A larger randomized phase 3 trial in pediatric AML failed to show benefit.(80)

Carfilzomib is a second-generation highly selective proteasome inhibitor that showed a better safety profile in terms of peripheral neurotoxicity compared to bortezomib. In 2012, carfilzomib was approved by FDA and EMA for patients with

MM who had received at least two prior therapies. Preclinical models demonstrated that carfilzomib achieved higher levels of proteasome inhibition than bortezomib, suggesting that it may have enhanced clinical activity.(81) The international CFZ2008 study is a phase 1b/2 study of carfilzomib in combination with chemotherapy for R/R pediatric ALL (NCT02303821).(82) During the dose escalation phase, the UKALLR3 regimen (vincristine, dexamethasone, PEG asparaginase, mitoxantrone) was considered too toxic, and was replaced with VXLD (vincristine, dexamethasone, PEG asparaginase, daunorubicin). The overall remission rate with VXLD-carfilzomib was 67% at the end of consolidation. A Phase 2 study is ongoing (NCT02303821).

2.10 Apoptosis pathway

Venetoclax prevents interaction of the anti-apoptotic protein BCL2 with the pro-apoptotic proteins BIM and BAX, allowing the latter proteins to activate the mitochondrial apoptotic pathway. Venetoclax is FDA and EMA-approved for adult patients with CLL or small lymphocytic lymphoma, and in combination with hypomethylating agents or low-dose cytarabine for the treatment of newly diagnosed AML in adults not eligible for intensive induction chemotherapy. A phase 1/2 study of venetoclax in combination with chemotherapy in pediatric patients with different diseases (ALL, AML, NHL, neuroblastoma, and other tumors) showed preliminary efficacy in leukemia patients at an age or weight adjusted adult-equivalent target dose of 800 mg.(83) A phase 1 study of venetoclax in combination with cytarabine, with or without idarubicin, in children and young adults with R/R AML, established the RP2D at 360 mg/m^2 once daily, and overall responses were observed in 69% of the 35 patients.(84) One resistance mechanism to venetoclax may occur through up-regulation of the anti-apoptotic protein MCL1. *TP53* activation by the MDM2 inhibitor idasanutlin inhibits intracellular signaling pathways that lead to MCL1 up-regulation. Addition of venetoclax to idasanutlin seems to switch the cellular context from one that favors cell cycle arrest to one that favors cell death.(85) This combination tested in a phase 1b/2 study (NCT02670044) enrolling adults with R/R AML led to a CR/CRi rate of 33%.(86) The idasanutlin phase 1b/2 pediatric study (NCT04029688) is evaluating idasanutlin in combination with chemotherapy or venetoclax in pediatric AML, ALL and solid tumors including neuroblastoma. Of note, a phase 3 trial in adults testing idasanutlin in combination with cytarabine in patients with R/R AML was stopped early for futility (NCT02545283). Results of another phase 1 study in pediatric and adult ALL/LL showed a good safety profile and a promising CR rate of 60%, when venetoclax was combined with low-dose navitoclax, a BCL-XL/

BCL-2 inhibitor, and chemotherapy.(87) In the PedAL/EuPAL program for R/R AML a randomized trial between FLA-GO +/- venetoclax will be investigated.

2.11 KMT2A targeting

KMT2A-rearrangement occurs both in AML and ALL, involving approximately 15–20% of childhood AML and being particularly common in infant ALL with a prevalence of approximately 75%. Outcomes remain poor for *KMT2A*-r ALL with conventional therapies, and two newer classes of inhibitors have emerged as possible targeted therapies: DOT1L and menin inhibitors. DOT1L is a histone methyltransferase that modifies chromatin of target genes to help maintain the integrity of these genes**.** Preclinical work on cell-lines and mice transformed with *KMT2A*-rearranged leukemia demonstrated activity. However, clinically, the DOT1L inhibitor pinometostat showed only very modest anti-leukemic activity as single agent in R/R patients with *KMT2A-r* acute leukemia (NCT01684150 and NCT02141828).(88) Pinometostat is now under investigation in combination with standard chemotherapy (NCT03724084) or azacitidine (NCT03701295) in *KMT2A*-r AML patients.

Menin inhibitors prevent the binding between MLL and menin, an essential co-factor of the oncogenic component of the *KMT2A*-complex that acts as a histone methyltransferase for transcription regulating genes critical to tumor proliferation. Menin inhibitors have demonstrated downregulation of the *Meis-2* and several *HOX*-genes in *KMT2A*-r cell lines, and a survival benefit in *KMT2A*-r leukemia xenograft mice models across various fusion types.(89) The first in-human trial, NCT04065399, of single agent SNDX-5613 in adults and children with R/R acute leukemia showed an ORR of 44% (49% among patients with *KMT2A*-r leukemia and 30% in patients with *NPM1*-mutated leukemia).(90) Another menin-*KMT2A* inhibitor from Kura Oncology (KO)-539 has been shown to prolong survival in *KMT2A*-rearranged cell lines and *in vivo* models, and has recently entered a Phase I/II trial in adults with r/r AML (NCT04067336). *In-vitro* and *ex-vivo* data suggest that combined treatment with a menin inhibitor combined with a DOT1L inhibitor may induce synergy.(91)

3. OTHERS

3.1 Recombinant arginase

Arginine is a semi-essential amino acid, with a key role in cell survival and proliferation in both normal and malignant cells. Treatment with BCT-100, a pegylated (PEG) recombinant human arginase, results in depletion of arginine and starvation of

malignant cells in different settings, including leukemias, hepatocellular carcinoma, melanoma and prostate cancer.(92) The compound was well tolerated in phase 1 trials in adults, with no neutralizing antibodies or ammonia formation observed.(93) A Phase 2 trial of BCT-100 in combination with cytarabine for older AML patients has recently completed (EuDRACT - 2011-000749-19). The phase 1/2 NCT03455140 trial is currently evaluating the safety and activity of BCT-100 in R/R cancers of children and young adults.

3.2 Vyxeos liposomal

Vyxeos liposomal (CPX-351) is a fixed combination of daunorubicin and cytarabine, encapsulated in liposomes, with a prolonged half-life and a prolonged synergistic effect of the two components, as liposomes accumulate and persist in high concentration in the bone marrow.(94) Given that liposomal daunorubicin is no longer available, which was considered the 'standard of care anthracycline' in Europe for relapsed AML, with the aim to prevent anthracycline-induced cardiac toxicity, this drug is of particular interest for this indication. Vyxeos liposomal is currently approved by FDA and EMA for treatment of adults, and by FDA also for children, with newly diagnosed therapy-related AML, and AML with myelodysplasia-related changes. The COG phase 1/2 AAML 1421 study evaluated CPX-351 followed by fludarabine, cytarabine, and G-CSF (FLAG) in children with relapsed AML (NCT02642965), showing an overall response rate over 80%, and no detectable residual disease by flow cytometry in 84% of patients with CR/CRp. (95) The ongoing phase 1B ITCC-092 (2020-000142-34) study is evaluating Vyxeos in combination with clofarabine in R/R AML. The COG phase 3 AAML1831 study is evaluating Vyxeos liposomal in pediatric patients with *de novo* AML. The front-line AIEOP-BFM AML 2020 study will also randomize Vyxeos liposomal against a standard induction treatment. The possible lower cardiotoxicity could be also relevant for patients with Down syndrome and AML, and a trial for newly diagnosed AML in children with Down syndrome (ML-DS 2018) will test Vyxeos liposomal in induction (EudraCT n. 2018-002988-25).

FUTURE DIRECTIONS

Immunotherapy in the form of targeted antibodies (as well as CAR T-cell therapy) represents at present the most promising future direction for hematologic malignancies. InO and blinatumomab are already introduced in front-line treatment for newly diagnosed ALL, as single-agent cycles during consolidation therapy.

Table 2. Drugs in development for pediatric hematological malignancies

Drug class	Compound	Disease	Trial Number and Reference, if applicable
IMMUNOTHERAPY			
Bispecific antibodies	Blinatumomab	ALL	COG AALL1331 (NCT02101853) (18) COG ALL1731 (NCT03914625) NCT02187354 (75) NCT02393859 (76) Interfant-06 NL7937 AIEOP-BFM ALL 2017 (NCT03643276) ALL together (NCT03911128)
	Blinatumomab + nivolumab	ALL	COG AALL1821 (NCT04546399)
	Flotetuzumab	AML	COG ADVL1812 (NCT0215956)
Monoclonal antibodies	Daratumumab	ALL	NCT03384654
	Isatuximab	AML/ALL	NCT03860844
	Rituximab	NHL	Inter B-NHL Ritux 2010 (NCT01516580) (53)
Antibody-drug conjugates	Inotuzumab ozogamicin	ALL	ITCC-059 (EudraCT 2016-000227-71) (86, 87) COG AALL1621 (NCT02981628)(88) COG AALL1732 (NCT03959085) ALL Together (NCT03911128)
	Anetumab ravtansine	AML	PedAL
	IGMN632	AML	PedAL/EupAL
	Brentuximab	HL/ALCL	NCT01492088 (59)
Immune checkpoint inhibitors	Nivolumab	HL/ALCL	NCT03703050
	Pembrolizumab	HL	NCT03407144
TARGETED THERAPY			
FLT3 inhibition	Midostaurin	AML	NCT03591510 (98)
	Sorafenib	AML	COG AAML1031 (NCT 01371981)
	Quizartinib	AML	COG ADVL1822 (NCT03793478)
	Crenolanib	AML	NCT02270788
	Gilteritinib	AML	COG AAML1831 (NCT04293562)
ALK inhibition	Crizotinib	NHL	ITCC-053 (EudraCT 2015-005437-53)
	Ceritinib	NHL	NCT01742286 (57)
	Brigatinib	NHL	ITCC-098
BCR-ABL inhibition	Ponatinib	ALL/AML/CML	NCT03934372
	Dasatinib	ALL	NCT01460160
	Bosutinib	CML	ITCC-054/COG AAML1921 (EudraCT 2015-002916-34)

Table 2. Continued

Drug class	Compound	Disease	Trial Number and Reference, if applicable
MEK inhibition	Selumetinib	ALL	ITCC-063 (NCT03705507)
BTK inhibition	Ibrutinib	NHL	(NCT02703272) (142)
JAK-STAT inhibition	Ruxolitinib	ALL	COG AALL1521 (NCT02723994)
CDK4/CDK6 inhibition	Ribociclib	ALL	DFCI 18-328 (NCT03740334)
	Palbociclib	ALL	COG AINV18P1 (NCT03792256)
IDH inhibition	Enasidenib	AML	ITCC-057 (NCT02813135)
Proteasome inhibition	Carfilzomib	ALL	ITCC/TACL CFZ2008 (NCT02303821) (128)
BCL2 inhibition	Venetoclax	AML/ALL	NCT03236857 (130)
	Venetoclax + idasanutlin	AML	NCT04029688)
KMT2A inhibition	SNDX-5613 (Menin inhibition)	AML/ALL	PedAL/EupAL
	Pinometostat (DOT1L inhibition)	AML/ALL	NCT03724084
OTHER			
Arginine depletion/ enzyme therapy	BCT-100 (recombinant arginase)	AML/ALL	ITCC-062 (NCT03455140)
Chemotherapy	Vyxeos liposomal	AML	COG AAML1421 (NCT02642965)(141) COG AAML1831 (NCT04293562) ITCC-092 (EudraCT 2020-000142-34) AIEOP-BFM AML 2020

InO is further tested against regular reinduction therapy in relapsed BCP-ALL, and may be more efficacious and less toxic in that setting that regular 4 drug reinduction chemotherapy. Whether similar results may be obtained with anti-CD38 or anti-CD123 (conjugated) antibodies for other subsets of leukemia needs to be awaited. With the focus on immunotherapy the urge to develop targeted agents is somewhat diminished, however, for example BCR-ABL inhibitors have an established role in Ph+-ALL. The development and approval (at least in Europe) of ALK inhibitors is lagging behind despite clear activity in ALCL. The role of MEK-inhibitors, FLT3 inhibitors, menin inhibitors and venetoclax needs to be studied further in order to assess their added value.

Meetings of the ACCELERATE platform have accelerated pediatric drug development, and resulted in the set-up of umbrella trials for AML and NHL lymphoma, respectively referred to as the PedAL project and the GloBNHL project.(16, 43) The PedAL project

is further initiated and supported by the Leukemia Lymphoma Society, to mirror the Beat-AML project for adults.(97) Both projects are collaborative initiatives between Europe and US facilitating recruitment in these rare patient populations. Another umbrella protocol is in the final stages of design (Hem-iSMART), and focusses primarily on T-cell ALL with persisting MRD post re-induction therapy, and multiple relapses of BCP-ALL, often post-CART and post SCT. Molecular profiling and international tumor boards to facilitate allocation of patients to the best suitable target-based trial or therapy are implemented, and in leukemia need to take surface marker expression and potentially also drug response profiling into account.

Considering the growing partnership between collaborative groups in Europe and North-America, harmonization of regulatory incentives on both sites of the Atlantic Ocean is a pre-requisite for timely initiation of these early-phase studies.(98) Further development of the academic networks, including sponsorship capabilities, for early phase studies in children with cancer is needed to accommodate the regulatory requirements associated with 'intent to file' studies sponsored by academia.

Acknowledgments

Most of the agents presented in the manuscript were discussed during two editions of the New Agents in Leukemia/Lymphoma meeting, 16-17 October 2018 and 27-28 October 2020, Utrecht, The Netherlands. The authors would like to thank the ITCC consortium and the COG group for the close collaboration during these meetings and for upcoming early clinical trials in pediatric oncology.

They would also like to thank Leonie Kastaneer for all the logistic support necessary to organize the New Agents in Leukemia/Lymphoma meetings and Astrid Danen-van Oorschot for collecting all the information discussed during the meetings and helping in the writing phase.

References

1. Minard-Colin V, Brugieres L, Reiter A, Cairo MS, Gross TG, Woessmann W, et al. Non-Hodgkin Lymphoma in Children and Adolescents: Progress Through Effective Collaboration, Current Knowledge, and Challenges Ahead. J Clin Oncol. 2015;33(27):2963-74.
2. Zwaan CM, Kolb EA, Reinhardt D, Abrahamsson J, Adachi S, Aplenc R, et al. Collaborative Efforts Driving Progress in Pediatric Acute Myeloid Leukemia. J Clin Oncol. 2015;33(27):2949-62.
3. von Stackelberg A, Locatelli F, Zugmaier G, Handgretinger R, Trippett TM, Rizzari C, et al. Phase I/Phase II Study of Blinatumomab in Pediatric Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia. J Clin Oncol. 2016;34(36):4381-9.
4. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018;378(5):439-48.
5. Reismüller B, Attarbaschi A, Peters C, Dworzak MN, Pötschger U, Urban C, et al. Long-term outcome of initially homogenously treated and relapsed childhood acute lymphoblastic leukaemia in Austria – A population-based report of the Austrian Berlin-Frankfurt-Münster (BFM) Study Group. British Journal of Haematology. 2009;144(4):559-70.
6. Moore AS, Kearns PR, Knapper S, Pearson AD, Zwaan CM. Novel therapies for children with acute myeloid leukaemia. Leukemia. 2013;27(7):1451-60.
7. Mosse YP, Voss SD, Lim MS, Rolland D, Minard CG, Fox E, et al. Targeting ALK With Crizotinib in Pediatric Anaplastic Large Cell Lymphoma and Inflammatory Myofibroblastic Tumor: A Children's Oncology Group Study. J Clin Oncol. 2017;35(28):3215-21.
8. Locatelli F, Mauz-Koerholz C, Neville K, Llort A, Beishuizen A, Daw S, et al. Brentuximab vedotin for paediatric relapsed or refractory Hodgkin's lymphoma and anaplastic large-cell lymphoma: a multicentre, open-label, phase 1/2 study. Lancet Haematol. 2018;5(10):e450-e61.
9. Brugieres L, Pacquement H, Le Deley MC, Leverger G, Lutz P, Paillard C, et al. Single-drug vinblastine as salvage treatment for refractory or relapsed anaplastic large-cell lymphoma: a report from the French Society of Pediatric Oncology. J Clin Oncol. 2009;27(30):5056-61.
10. Parker C, Waters R, Leighton C, Hancock J, Sutton R, Moorman AV, et al. Effect of mitoxantrone on outcome of children with first relapse of acute lymphoblastic leukaemia (ALL R3): an open-label randomised trial. Lancet. 2010;376(9757):2009-17.
11. Bhakta N, Liu Q, Ness KK, Baassiri M, Eissa H, Yeo F, et al. The cumulative burden of surviving childhood cancer: an initial report from the St Jude Lifetime Cohort Study (SJLIFE). Lancet. 2017;390(10112):2569-82.
12. Bhatia S. Long-term health impacts of hematopoietic stem cell transplantation inform recommendations for follow-up. Expert Rev Hematol. 2011;4(4):437-52; quiz 53-4.
13. Hwang TJ, Orenstein L, DuBois SG, Janeway KA, Bourgeois FT. Pediatric Trials for Cancer Therapies With Targets Potentially Relevant to Pediatric Cancers. J Natl Cancer Inst. 2020;112(3):224-8.
14. Pearson AD, Heenen D, Kearns PR, Goeres A, Marshall LV, Blanc P, et al. 10-year report on the European Paediatric Regulation and its impact on new drugs for children's cancers. Lancet Oncol. 2018;19(3):285-7.

15. Pearson ADJ, Rossig C, Lesa G, Diede SJ, Weiner S, Anderson J, et al. ACCELERATE and European Medicines Agency Paediatric Strategy Forum for medicinal product development of checkpoint inhibitors for use in combination therapy in paediatric patients. Eur J Cancer. 2020;127:52-66.

16. Pearson ADJ, Scobie N, Norga K, Ligas F, Chiodin D, Burke A, et al. ACCELERATE and European Medicine Agency Paediatric Strategy Forum for medicinal product development for mature B-cell malignancies in children. Eur J Cancer. 2019;110:74-85.

17. Bras AE, Beishuizen A, Langerak AW, Jongen-Lavrencic M, Te Marvelde JG, van den Heuvel-Eibrink MM, et al. CD38 expression in paediatric leukaemia and lymphoma: implications for antibody targeted therapy. Br J Haematol. 2018;180(2):292-6.

18. Bride KL, Vincent TL, Im S-Y, Aplenc R, Barrett DM, Carroll WL, et al. Preclinical efficacy of daratumumab in T-cell acute lymphoblastic leukemia. Blood. 2018;131(9):995-9.

19. Deckert J, Wetzel MC, Bartle LM, Skaletskaya A, Goldmacher VS, Vallée F, et al. SAR650984, a novel humanized CD38-targeting antibody, demonstrates potent antitumor activity in models of multiple myeloma and other CD38+ hematologic malignancies. Clin Cancer Res. 2014;20(17):4574-83.

20. Baruchel A, Abrahamsson J, Bertrand Y, Gonzalez O, Nysom K, Quinones W, et al. Isatuximab in Combination with Chemotherapy in Pediatric Patients with Relapsed/ Refractory Acute Lymphoblastic Leukemia or Acute Myeloid Leukemia (ISAKIDS): Interim Analysis. Blood. 2021;138(Supplement 1):516-.

21. Minard-Colin V, Aupérin A, Pillon M, Burke GAA, Barkauskas DA, Wheatley K, et al. Rituximab for High-Risk, Mature B-Cell Non-Hodgkin's Lymphoma in Children. New England Journal of Medicine. 2020;382(23):2207-19.

22. Maury S, Chevret S, Thomas X, Heim D, Leguay T, Huguet F, et al. Rituximab in B-Lineage Adult Acute Lymphoblastic Leukemia. New England Journal of Medicine. 2016;375(11):1044-53.

23. Jeha S, Behm F, Pei D, Sandlund JT, Ribeiro RC, Razzouk BI, et al. Prognostic significance of CD20 expression in childhood B-cell precursor acute lymphoblastic leukemia. Blood. 2006;108(10):3302-4.

24. Lussana F, Gritti G, Rambaldi A. Immunotherapy of Acute Lymphoblastic Leukemia and Lymphoma With T Cell–Redirected Bispecific Antibodies. Journal of Clinical Oncology. 2021;39(5):444-55.

25. Locatelli F, Zugmaier G, Mergen N, Bader P, Jeha S, Schlegel P-G, et al. Blinatumomab in pediatric patients with relapsed/refractory acute lymphoblastic leukemia: results of the RIALTO trial, an expanded access study. Blood Cancer Journal. 2020;10(7):77.

26. Brown PA, Ji L, Xu X, Devidas M, Hogan LE, Borowitz MJ, et al. Effect of Postreinduction Therapy Consolidation With Blinatumomab vs Chemotherapy on Disease-Free Survival in Children, Adolescents, and Young Adults With First Relapse of B-Cell Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. JAMA. 2021;325(9):833-42.

27. Locatelli F, Zugmaier G, Rizzari C, Morris JD, Gruhn B, Klingebiel T, et al. Effect of Blinatumomab vs Chemotherapy on Event-Free Survival Among Children With High-risk First-Relapse B-Cell Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. JAMA. 2021;325(9):843-54.

28. Webster J, Luskin MR, Prince GT, DeZern AE, DeAngelo DJ, Levis MJ, et al. Blinatumomab in Combination with Immune Checkpoint Inhibitors of PD-1 and CTLA-4 in Adult Patients with Relapsed/Refractory (R/R) CD19 Positive B-Cell Acute Lymphoblastic Leukemia (ALL): Preliminary Results of a Phase I Study. Blood. 2018;132(Supplement 1):557-.

29. Pieters R, De Lorenzo P, Ancliffe P, Aversa LA, Brethon B, Biondi A, et al. Outcome of Infants Younger Than 1 Year With Acute Lymphoblastic Leukemia Treated With the Interfant-06 Protocol: Results From an International Phase III Randomized Study. J Clin Oncol. 2019;37(25):2246-56.

30. Li AM, Rabin KR, Kairalla J, Wang C, Devidas M, Militano O, et al. Blinatumomab Associated Seizure Risk in Patients with Down Syndrome and B-Lymphoblastic Leukemia: An Interim Report from Children's Oncology Group (COG) Study AALL1731. Blood. 2021;138(Supplement 1):2304-.

31. Vergez F, Green AS, Tamburini J, Sarry JE, Gaillard B, Cornillet-Lefebvre P, et al. High levels of CD34+CD38low/-CD123+ blasts are predictive of an adverse outcome in acute myeloid leukemia: a Groupe Ouest-Est des Leucemies Aigues et Maladies du Sang (GOELAMS) study. Haematologica. 2011;96(12):1792-8.

32. Bras AE, de Haas V, van Stigt A, Jongen-Lavrencic M, Beverloo HB, Te Marvelde JG, et al. CD123 expression levels in 846 acute leukemia patients based on standardized immunophenotyping. Cytometry B Clin Cytom. 2019;96(2):134-42.

33. Uy GL, Aldoss I, Foster MC, Sayre PH, Wieduwilt MJ, Advani AS, et al. Flotetuzumab as salvage immunotherapy for refractory acute myeloid leukemia. Blood. 2021;137(6):751-62.

34. Busfield SJ, Biondo M, Wong M, Ramshaw HS, Lee EM, Ghosh S, et al. Targeting of acute myeloid leukemia in vitro and in vivo with an anti-CD123 mAb engineered for optimal ADCC. Leukemia. 2014;28(11):2213-21.

35. Kovtun Y, Jones GE, Adams S, Harvey L, Audette CA, Wilhelm A, et al. A CD123-targeting antibody-drug conjugate, IMGN632, designed to eradicate AML while sparing normal bone marrow cells. Blood Adv. 2018;2(8):848-58.

36. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab Ozogamicin versus Standard Therapy for Acute Lymphoblastic Leukemia. N Engl J Med. 2016;375(8):740-53.

37. Bhojwani D, Sposto R, Shah NN, Rodriguez V, Yuan C, Stetler-Stevenson M, et al. Inotuzumab ozogamicin in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. Leukemia. 2018.

38. Brivio E, Locatelli F, Lopez-Yurda M, Malone A, Diaz de Heredia C, Bielorai B, et al. A Phase I study of inotuzumab ozogamicin in pediatric relapsed/refractory acute lymphoblastic leukemia (ITCC-059 study). Blood. 2020.

39. Brivio E, Locatelli F, Thano A, Petit A, Vormoor BJ, Rives S, et al. A Phase II Study of Single-Agent Inotuzumab Ozogamicin in Pediatric CD22-Positive Relapsed/Refractory Acute Lymphoblastic Leukemia: Results of the ITCC-059 Study. Blood. 2020;136(Supplement 1):8-9.

40. al. OBMe. A Phase 2 Trial of Inotuzumab Ozogamicin (InO) in Children and Young Adults with Relapsed or Refractory (R/R) CD22+ B-Acute Lymphoblastic Leukemia (B-ALL): Results from Children's Oncology Group Protocol AALL1621 Blood. 2019;134:741.

41. Brivio E, Chantrain CF, Gruber TA, Thano A, Rialland F, Contet A, et al. Inotuzumab ozogamicin in infants and young children with relapsed or refractory acute lymphoblastic leukaemia: a case series*. British Journal of Haematology.n/a(n/a).

42. Daver NG, Montesinos P, DeAngelo DJ, Wang ES, Papadantonakis N, Deconinck E, et al. Clinical Profile of IMGN632, a Novel CD123-Targeting Antibody-Drug Conjugate (ADC), in Patients with Relapsed/Refractory (R/R) Acute Myeloid Leukemia (AML) or Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN). Blood. 2019;134(Supplement_1):734-.

43. Pearson ADJ, Zwaan CM, Kolb EA, Karres D, Guillot J, Kim SY, et al. Paediatric Strategy Forum for medicinal product development for acute myeloid leukaemia in children and adolescents: ACCELERATE in collaboration with the European Medicines Agency with participation of the Food and Drug Administration. Eur J Cancer. 2020;136:116-29.

44. G.R. B. Phase I study of anti-mesothelin antibody drug conjugate anetumab ravtansine (AR). Journal of Clinical Oncology. 2016;34(15_suppl):2509-.

45. Gopalakrishnapillai A, Kaeding A, Schatz C, Sommer A, Meshinchi S, Kolb EA, et al. In Vivo Evaluation of Mesothelin As a Therapeutic Target in Pediatric Acute Myeloid Leukemia. Blood. 2019;134(Supplement_1):1370-.

46. Lowe EJ, Reilly AF, Lim MS, Gross TG, Saguilig L, Barkauskas DA, et al. Brentuximab Vedotin in Combination with Chemotherapy for Pediatric Patients with ALK+ALCL: Results of COG Trial ANHL12P1. Blood. 2021.

47. Gamis AS, Alonzo TA, Meshinchi S, Sung L, Gerbing RB, Raimondi SC, et al. Gemtuzumab ozogamicin in children and adolescents with de novo acute myeloid leukemia improves event-free survival by reducing relapse risk: results from the randomized phase III Children's Oncology Group trial AAML0531. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32(27):3021-32.

48. Meshinchi S, Appelbaum FR. Structural and functional alterations of FLT3 in acute myeloid leukemia. Clin Cancer Res. 2009;15(13):4263-9.

49. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. N Engl J Med. 2017;377(5):454-64.

50. Zwaan CM, Söderhäll S, Brethon B, Luciani M, Rizzari C, Stam RW, et al. A phase 1/2, open-label, dose-escalation study of midostaurin in children with relapsed or refractory acute leukaemia. Br J Haematol. 2019;185(3):623-7.

51. Pollard JA, Alonzo TA, Brown PA, Gerbing RB, Fox E, Choi JK, et al. Sorafenib in Combination with Standard Chemotherapy for Children with High Allelic Ratio FLT3/ITD+ AML Improves Event-Free Survival and Reduces Relapse Risk: A Report from the Children's Oncology Group Protocol AAML1031. Blood. 2019;134(Supplement_1):292-.

52. Burchert A, Bug G, Fritz LV, Finke J, Stelljes M, Röllig C, et al. Sorafenib Maintenance After Allogeneic Hematopoietic Stem Cell Transplantation for Acute Myeloid Leukemia With FLT3-Internal Tandem Duplication Mutation (SORMAIN). J Clin Oncol. 2020;38(26):2993-3002.

53. Zarrinkar PP, Gunawardane RN, Cramer MD, Gardner MF, Brigham D, Belli B, et al. AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute myeloid leukemia (AML). Blood. 2009;114(14):2984-92.

54. Cooper TM, Cassar J, Eckroth E, Malvar J, Sposto R, Gaynon P, et al. A Phase I Study of Quizartinib Combined with Chemotherapy in Relapsed Childhood Leukemia: A Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) Study. Clin Cancer Res. 2016;22(16):4014-22.

55. Scholl S, Fleischmann M, Schnetzke U, Heidel FH. Molecular Mechanisms of Resistance to FLT3 Inhibitors in Acute Myeloid Leukemia: Ongoing Challenges and Future Treatments. Cells. 2020;9(11).

56. Perl AE, Martinelli G, Cortes JE, Neubauer A, Berman E, Paolini S, et al. Gilteritinib or Chemotherapy for Relapsed or Refractory FLT3-Mutated AML. New England Journal of Medicine. 2019;381(18):1728-40.

57. Pearson ADJ, Barry E, Mossé YP, Ligas F, Bird N, de Rojas T, et al. Second Paediatric Strategy Forum for anaplastic lymphoma kinase (ALK) inhibition in paediatric malignancies: ACCELERATE in collaboration with the European Medicines Agency with the participation of the Food and Drug Administration. European Journal of Cancer. 2021;157:198-213.

58. Shen J, Li S, Medeiros LJ, Lin P, Wang SA, Tang G, et al. PD-L1 expression is associated with ALK positivity and STAT3 activation, but not outcome in patients with systemic anaplastic large cell lymphoma. Mod Pathol. 2020;33(3):324-33.

59. Schulte JH, Moreno L, Ziegler DS, Marshall LV, Zwaan CM, Irwin M, et al. Final analysis of phase I study of ceritinib in pediatric patients with malignancies harboring activated anaplastic lymphoma kinase (ALK). Journal of Clinical Oncology. 2020;38(15_suppl):10505-.

60. Fischer M, Moreno L, Ziegler DS, Marshall LV, Zwaan CM, Irwin MS, et al. Ceritinib in paediatric patients with anaplastic lymphoma kinase-positive malignancies: an open-label, multicentre, phase 1, dose-escalation and dose-expansion study. Lancet Oncol. 2021.

61. Camidge DR, Kim HR, Ahn MJ, Yang JCH, Han JY, Hochmair MJ, et al. Brigatinib Versus Crizotinib in Advanced ALK Inhibitor-Naive ALK-Positive Non-Small Cell Lung Cancer: Second Interim Analysis of the Phase III ALTA-1L Trial. J Clin Oncol. 2020;38(31):3592-603.

62. Tasian SK, Loh ML, Hunger SP. Philadelphia chromosome-like acute lymphoblastic leukemia. Blood. 2017;130(19):2064-72.

63. Cortes JE, Kim D-W, Pinilla-Ibarz J, le Coutre P, Paquette R, Chuah C, et al. A Phase 2 Trial of Ponatinib in Philadelphia Chromosome–Positive Leukemias. New England Journal of Medicine. 2013;369(19):1783-96.

64. O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F, et al. AP24534, a Pan-BCR-ABL Inhibitor for Chronic Myeloid Leukemia, Potently Inhibits the T315I Mutant and Overcomes Mutation-Based Resistance. Cancer Cell. 2009;16(5):401-12.

65. Cortes J, Apperley J, Lomaia E, Moiraghi B, Undurraga Sutton M, Pavlovsky C, et al. Ponatinib dose-ranging study in chronic-phase chronic myeloid leukemia: a randomized, open-label phase 2 clinical trial. Blood. 2021;138(21):2042-50.

66. Millot F, Suttorp M, Versluys AB, Kalwak K, Nelken B, Ducassou S, et al. Ponatinib in childhood Philadelphia chromosome-positive leukaemias: an international registry of childhood chronic myeloid leukaemia study. Eur J Cancer. 2020;136:107-12.

67. Rossoff J, Huynh V, Rau RE, Macy ME, Sulis ML, Schultz KR, et al. Experience with ponatinib in paediatric patients with leukaemia. British Journal of Haematology. 2020;189(2):363-8.

68. Irving J, Matheson E, Minto L, Blair H, Case M, Halsey C, et al. Ras pathway mutations are prevalent in relapsed childhood acute lymphoblastic leukemia and confer sensitivity to MEK inhibition. Blood. 2014;124(23):3420-30.

69. van der Zwet JCG, Buijs-Gladdines JGCAM, Cordo V, Debets DO, Smits WK, Chen Z, et al. MAPK-ERK is a central pathway in T-cell acute lymphoblastic leukemia that drives steroid resistance. Leukemia. 2021.

70. Li Y, Buijs-Gladdines JG, Cante-Barrett K, Stubbs AP, Vroegindeweij EM, Smits WK, et al. IL-7 Receptor Mutations and Steroid Resistance in Pediatric T cell Acute Lymphoblastic Leukemia: A Genome Sequencing Study. PLoS Med. 2016;13(12):e1002200.

71. Kerstjens M, Pinhancos SS, Castro PG, Schneider P, Wander P, Pieters R, et al. Trametinib inhibits RAS-mutant MLL-rearranged acute lymphoblastic leukemia at specific niche sites and reduces ERK phosphorylation in vivo. Haematologica. 2018;103(4):e147-e50.

72. Chu Y, Lee S, Shah T, Yin C, Barth M, Miles RR, et al. Ibrutinib significantly inhibited Bruton's tyrosine kinase (BTK) phosphorylation,in-vitro proliferation and enhanced overall survival in a preclinical Burkitt lymphoma (BL) model. Oncoimmunology. 2019;8(1):e1512455.

73. Burke GAA LF, Kabickova E, Beishuizen A, Tacylidiz N, Uyttebroeck A, Kang H, Luisi F, Minard-Colin V, Burkhardt B, Tamegnon M, Sun S, Poggesi I, Curtis M, Deshpande S, Nottage K, Howes A, Cairo M. Ibrutinib+rice/rvici for r/r mature b-nhl in children/young adults: sparkle trial interim analysis. Pediatric Blood & Cancer. 2021;68(S3):e29060.

74. Tasian SK, Assad A, Hunter DS, Du Y, Loh ML. A Phase 2 Study of Ruxolitinib with Chemotherapy in Children with Philadelphia Chromosome-like Acute Lymphoblastic Leukemia (INCB18424-269/AALL1521): Dose-Finding Results from the Part 1 Safety Phase. Blood. 2018;132(Supplement 1):555-.

75. Pikman Y, Alexe G, Roti G, Conway AS, Furman A, Lee ES, et al. Synergistic Drug Combinations with a CDK4/6 Inhibitor in T-cell Acute Lymphoblastic Leukemia. Clin Cancer Res. 2017;23(4):1012-24.

76. Geoerger B, Bourdeaut F, DuBois SG, Fischer M, Geller JI, Gottardo NG, et al. A Phase I Study of the CDK4/6 Inhibitor Ribociclib (LEE011) in Pediatric Patients with Malignant Rhabdoid Tumors, Neuroblastoma, and Other Solid Tumors. Clin Cancer Res. 2017;23(10):2433-41.

77. Mondesir J, Willekens C, Touat M, de Botton S. IDH1 and IDH2 mutations as novel therapeutic targets: current perspectives. J Blood Med. 2016;7:171-80.

78. Stein EM, DiNardo CD, Pollyea DA, Fathi AT, Roboz GJ, Altman JK, et al. Enasidenib in mutant IDH2 relapsed or refractory acute myeloid leukemia. Blood. 2017;130(6):722-31.

79. Horton TM, Whitlock JA, Lu X, O'Brien MM, Borowitz MJ, Devidas M, et al. Bortezomib reinduction chemotherapy in high-risk ALL in first relapse: a report from the Children's Oncology Group. Br J Haematol. 2019;186(2):274-85.

80. Richard A, Soheil M, Lillian S, Todd A, John C, Brian F, et al. Bortezomib with standard chemotherapy for children with acute myeloid leukemia does not improve treatment outcomes: a report from the Children's Oncology Group. Haematologica. 2020;105(7):1879-86.

81. Messinger YH, Gaynon PS, Sposto R, van der Giessen J, Eckroth E, Malvar J, et al. Bortezomib with chemotherapy is highly active in advanced B-precursor acute lymphoblastic leukemia: Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) Study. Blood. 2012;120(2):285-90.

82. Burke MJ, Ziegler DS, Bautista Sirvent FJ, Attarbaschi A, Gore L, Locatelli F, et al. Phase 1b Study of Carfilzomib in Combination with Induction Chemotherapy in Children with Relapsed or Refractory Acute Lymphoblastic Leukemia (ALL). Blood. 2019;134(Supplement_1):3873-.

83. Karol SE, Cooper TM, Bittencourt H, Gore L, O'Brien MM, Fraser C, et al. Safety, Efficacy, and PK of the BCL2 Inhibitor Venetoclax in Combination with Chemotherapy in Pediatric and Young Adult Patients with Relapsed/Refractory Acute Myeloid Leukemia and Acute Lymphoblastic Leukemia: Phase 1 Study. Blood. 2019;134(Supplement_1):2649-.

84. Karol SE, Alexander TB, Budhraja A, Pounds SB, Canavera K, Wang L, et al. Venetoclax in combination with cytarabine with or without idarubicin in children with relapsed or refractory acute myeloid leukaemia: a phase 1, dose-escalation study. The Lancet Oncology. 2020;21(4):551-60.

85. Pan R, Ruvolo V, Mu H, Leverson JD, Nichols G, Reed JC, et al. Synthetic Lethality of Combined Bcl-2 Inhibition and p53 Activation in AML: Mechanisms and Superior Antileukemic Efficacy. Cancer Cell. 2017;32(6):748-60 e6.

86. al. De. Preliminary Results from a Phase Ib Study Evaluating BCL-2 Inhibitor Venetoclax in Combination with MEK Inhibitor Cobimetinib or MDM2 Inhibitor Idasanutlin in Patients with Relapsed or Refractory (R/R) AML Blood. 2017(130).

87. Pullarkat VA, Lacayo NJ, Jabbour E, Rubnitz JE, Bajel A, Laetsch TW, et al. Venetoclax and Navitoclax in Combination with Chemotherapy in Patients with Relapsed or Refractory Acute Lymphoblastic Leukemia and Lymphoblastic Lymphoma. Cancer Discovery. 2021:candisc.1465.2020.

88. Shukla N, Wetmore C, O'Brien MM, Silverman LB, Brown P, Cooper TM, et al. Final Report of Phase 1 Study of the DOT1L Inhibitor, Pinometostat (EPZ-5676), in Children with Relapsed or Refractory MLL-r Acute Leukemia. Blood. 2016;128(22):2780-.

89. Krivtsov AV, Evans K, Gadrey JY, Eschle BK, Hatton C, Uckelmann HJ, et al. A Menin-MLL Inhibitor Induces Specific Chromatin Changes and Eradicates Disease in Models of MLL-Rearranged Leukemia. Cancer Cell. 2019;36(6):660-73.e11.

90. Stein EM, Aldoss I, DiPersio JF, Stone RM, Arellano ML, Rosen G, et al. Safety and Efficacy of Menin Inhibition in Patients (Pts) with MLL-Rearranged and NPM1 Mutant Acute Leukemia: A Phase (Ph) 1, First-in-Human Study of SNDX-5613 (AUGMENT 101). Blood. 2021;138(Supplement 1):699-.

91. Dafflon C, Craig VJ, Méreau H, Gräsel J, Schacher Engstler B, Hoffman G, et al. Complementary activities of DOT1L and Menin inhibitors in MLL-rearranged leukemia. Leukemia. 2017;31(6):1269-77.

92. Vardon A, Dandapani M, Cheng D, Cheng P, De Santo C, Mussai F. Arginine auxotrophic gene signature in paediatric sarcomas and brain tumours provides a viable target for arginine depletion therapies. Oncotarget. 2017;8(38):63506-17.

93. Cheng PNM, Liu AM, Bessudo A, Mussai F. Safety, PK/PD and preliminary anti-tumor activities of pegylated recombinant human arginase 1 (BCT-100) in patients with advanced arginine auxotrophic tumors. Invest New Drugs. 2021.

94. Wang Q, Banerjee K, Vasilinin G, Marier JF, Gibbons JA. Population Pharmacokinetics and Exposure-Response Analyses for CPX-351 in Patients With Hematologic Malignancies. J Clin Pharmacol. 2019;59(5):748-62.

95. Cooper TM, Absalon M, Alonzo TA, Gerbing RB, Leger KJ, Hirsch BA, et al. AAML 1421, a phase I/II study of CPX-351 followed by fludarabine, cytarabine, and G-CSF (FLAG) for children with relapsed acute myeloid leukemia (AML): A Report from the Children's Oncology Group. Journal of Clinical Oncology. 2019;37(15_suppl):10003-.

96. Cooper T, Ayres M, Nowak B, Gandhi V. Biochemical modulation of cytarabine triphosphate by clofarabine. Cancer Chemother Pharmacol. 2005;55(4):361-8.

97. Burd A, Levine RL, Ruppert AS, Mims AS, Borate U, Stein EM, et al. Precision medicine treatment in acute myeloid leukemia using prospective genomic profiling: feasibility and preliminary efficacy of the Beat AML Master Trial. Nature Medicine. 2020;26(12):1852-8.

98. Reaman G, Karres D, Ligas F, Lesa G, Casey D, Ehrlich L, et al. Accelerating the Global Development of Pediatric Cancer Drugs: A Call to Coordinate the Submissions of Pediatric Investigation Plans and Pediatric Study Plans to the European Medicines Agency and US Food and Drug Administration. Journal of Clinical Oncology. 2020;38(36):4227-30.

Chapter 3

A Phase I Study of Inotuzumab Ozogamicin in Pediatric Relapsed7Refractory Acute Lymphoblastic Leukemia (ITCC-059 study)

Brivio E, Locatelli F, Lopez-Yurda M, Malone A, Díaz-de-Heredia C, Bielorai B, Rossig C, van der Velden VHJ, Ammerlaan ACJ, Thano A, van der Sluis IM, den Boer ML, Chen Y, Sleight B, Brethon B, Nysom K, Šrámková L, Øra, Vinti L, Chen-Santel C, Zwaan CM

Blood (2021) 137 (12): 1582–1590.

Key points:

- The recommended phase 2 dose of InO for pediatric ALL patients was established at 1.8 mg/m2/course
- 85% of the patients with multiple R/R ALL reached CR after one course of single-agent InO at the RP2D, 100% of whom with MRD-negativity

Abstract

This Phase I study investigated the recommended Phase II dose (RP2D) of inotuzumab ozogamicin (InO), a CD22-directed antibody-drug conjugate, in pediatric patients with multiple-relapsed/refractory (R/R) CD22-positive acute lymphoblastic leukemia (ALL). Patients (≥1–<18 years) received three InO doses (Days 1, 8, 15) per course. Dose-escalation was based on dose-limiting toxicities (DLT) during Course 1 . Dose level 1 (DL1)=1.4 mg/m^2 (0.6–0.4–0.4 mg/m^2); DL2=1.8 mg/m^2 (0.8–0.5–0.5 mg/m^2). Secondary endpoints included safety, anti-leukemic activity, and pharmacokinetics. Twenty-five patients (23 evaluable for DLT) were enrolled. In Course 1, first cohort, 1/6 (DL1) and 2/5 (DL2) patients experienced DLTs; subsequent review considered DL2 DLTs to be non-dose-limiting. Dose was de-escalated to DL1 while awaiting protocol amendment to re-evaluate DL2 in a second cohort, where 0/6 (DL1) and 1/6 (DL2) patients had a DLT. Twenty-three patients experienced Grade 3–4 adverse events; hepatic sinusoidal obstruction syndrome was reported in two patients after subsequent chemotherapy. Overall response rate after Course 1 was 80% [95% CI: 59–93%] (20/25 patients; DL1=75% [43–95%], DL2=85% [55–98%]); 84% [60–97%] of responders obtained minimal residual disease-negative CR; 12-month overall survival was 40% [95% CI: 25–66%]. Nine patients received hematopoietic stem cell transplant or chimeric-antigen receptor T-cells after InO. InO median maximum concentrations were comparable to simulated adult concentrations. InO was well tolerated, demonstrating anti-leukemic activity in heavily pre-treated children with CD22-positive R/R ALL. RP2D was established as 1.8 mg/m^2/course, as in adults.

Introduction

Acute lymphoblastic leukemia (ALL) is the most frequent malignant disease of childhood with an estimated western European incidence of 40 cases/million/year, in children aged <15 years.(1) In the past two decades, significant improvement has been made in treating childhood ALL, which is now curable in >85% of children with multi-drug front-line protocols.(2-4) However, the prognosis for those children who relapse or who are refractory to conventional therapy (approximately 15%) remains poor.(4-6) Only about 50% of children who have relapsed can be rescued with intensive chemotherapy, followed by allogeneic-hematopoietic stem cell transplant (HSCT) in high-risk cases.(4) Therefore, new therapeutic approaches are urgently needed to overcome chemotherapy resistance and improve outcome.

Inotuzumab ozogamicin (InO) is an antibody–drug conjugate composed of a monoclonal CD22-directed antibody linked to calicheamicin,(7) a potent cytotoxic antitumor antibiotic that causes cell death by inducing double-strand DNA breaks.(8, 9) CD22 is a B-cell adhesion molecule that is expressed on both normal and malignant B-cells. It is expressed in approximately 90% of childhood B-cell precursor (BCP) ALL cases.(10) InO is approved for the treatment of adults with CD22-positive relapsed or refractory (R/R) BCP-ALL, starting at 1.8 mg/m^2/course (fractionated schedule).(7, 11, 12)·(13) Results from an adult Phase III study revealed superiority of weekly InO over standard intensive chemotherapy, with significantly greater rates of complete response (CR), minimal residual disease (MRD)-negativity, and number of patients proceeding to transplant.(9) Liver-related, treatment-emergent events, including hepatic sinusoidal obstruction syndrome (SOS), were the most frequent non-hematologic toxicities.(9)

Given the results of InO in adult ALL and the medical need in pediatric R/R ALL, investigation of InO in pediatric BCP-ALL is highly warranted. *In vitro* data suggested high sensitivity of childhood ALL cells to calicheamicin.(14) Existing data on InO from a Phase II study (five children), and compassionate use programs in Europe/US and France (51 and 12 children, respectively), indicated that InO was well tolerated and effective in children with R/R BCP-ALL.(15-17) The current Phase I investigation (part of an approved Pediatric Investigational Plan(18)) prospectively evaluated the safety/tolerability, efficacy, and pharmacokinetics (PK)/pharmacodynamics (PD) of InO monotherapy, in order to identify a recommended Phase II dose (RP2D) for pediatric patients with CD22-positive R/R ALL.

Methods

ITCC-059 (Dutch Trial Registry: NTR5736) is a Phase I/II multicenter, single-arm, open-label study of InO in childhood CD22-positive R/R BCP-ALL. Here we present results from the Phase I single-agent dose-finding investigation.

The study was conducted in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, ICH Guidelines for Good Clinical Practice, the Declaration of Helsinki, and applicable local regulatory requirements and laws. The study protocol was approved by the Sponsor (Erasmus MC Sophia Children's Hospital, Rotterdam, The Netherlands), and by the Institutional Review Board/Ethics Committee (IRB/EC) in all participating centers. Parents and/or patients provided written informed consent, and were enrolled between January 2017 and April 2019 at 11 sites of the ITCC consortium.(19)

Patients

Eligible patients were aged ≥1 to <18 years at enrolment, had a diagnosis of CD22-positive R/R BCP-ALL, a M2 or M3 bone marrow status, and refractory disease or ≥2nd relapse, or any relapse post-HSCT. Exclusion criteria included any history of prior or ongoing veno-occlusive disease (VOD)/SOS (modified Seattle criteria).(20) For further entry criteria see Supplementary Table 1.

Study design

The primary objective was to determine the maximum tolerated dose (MTD) or RP2D of IV InO monotherapy; secondary objectives included safety, response, and PK/PD.

A modified Rolling-6 escalation design(21) was used, with dose (de-)escalation based on dose-limiting toxicities (DLTs) during Course 1. In the first cohort, 2 patients at DL2 experienced a DLT per protocol definition. Upon steering committee review one of these toxicities (i.e. transaminase elevation) was not considered to have impaired patient safety significantly (see Supplementary Table 2 for details), and an IRB/EC-approved amendment allowed repeating the DL1 and DL2 for DLT assessment in a second cohort of patients with adapted guidelines for transaminase monitoring.

A maximum of six courses were permitted (two or three courses only prior to HSCT). A course of therapy was defined as three doses of InO (IV infusion/60 minutes) weekly on Days 1, 8, and 15. Course 1 was planned to last 22 days, and all subsequent courses 28 days, with delays allowed up to 42 days. To avoid infusion-related side-effects, pre-treatment with methylprednisolone 1 mg/kg IV (maximum 50 mg) prior to each infusion of InO was recommended.

InO dose levels were assigned at study entry (Table 1). In Course 1, dose-level 1 (DL1) was 1.4 mg/m^2 – 80% of the adult dose – administered in a fractionated fashion, with the Day 1 dose (0.6 mg/m^2) higher than the following two doses (0.4 mg/m^2) given the high tumor burden at relapse. In addition, preclinical studies suggest that fractionated schedules of lower InO doses, compared with single-course high dose, may improve anti-ALL activity and reduce toxicities.(16, 22)

Table 1. InO dose escalation/de-escalation schedule

	InO dose, IV (mg/m2)							
	Course 1				Course 2–6a			
Dose level	**Day 1**	**Day 8**	**Day 15**	**Total dose per course**	**Day 1**	**Day 8b**	**Day 15 b**	**Total dose per course**
Level -2	0.4	0.2	0.2	**0.8**	0.2	0.2	0.2	**0.6**
Level -1	0.5	0.3	0.3	**1.1**	0.3	0.3	0.3	**0.9**
Level 1 (Start)	0.6	0.4	0.4	**1.4**	0.4	0.4	0.4	**1.2**
Level 2	0.8	0.5	0.5	**1.8**	0.5	0.5	0.5	**1.5**
Level 3	1.0	0.6	0.6	**2.2**	0.6	0.6	0.6	**1.8**

[a]Following Course 1, in patients who have achieved a complete response, the Day 1 dose is decreased slightly due to no loading dose requirement. In patients who have not yet achieved a complete response after Course 1, a loading dose similar to Course 1 was given in Course 2, but not in subsequent courses.

[b]Visit window of +/- 1 day

Endpoints

The primary endpoint was the incidence of DLTs during the first course of therapy, defined as any of the following toxicities related to InO: any Grade 5 toxicity; absolute neutrophil count <500/μL and/or platelet count <50,000/μL lasting beyond Day 42 in the absence of persisting leukemia; or Grade 3/4 non-hematologic toxicities persisting for >48 hours (>7 days for liver test abnormalities).

Secondary endpoints were safety and tolerability, including adverse events (AEs), death attributable to InO, cumulative incidence of non-relapse mortality, and hepatic SOS during or after InO. Secondary efficacy endpoints (detailed in Supplementary text) were measures of anti-leukemic activity including: overall response rate (ORR); MRD status(23, 24); duration of response (DOR); and number of patients undergoing post-InO treatment, including consolidation with HSCT or chimeric-antigen receptor T-cell (CAR-T) therapy. Other endpoints were serum PK of unconjugated calicheamicin and InO.

Statistical analysis

The dose escalation analysis set (evaluable for establishing MTD/RP2D) included all enrolled patients who received ≥1 dose of InO and experienced a DLT during Course 1; or who received ≥2 doses of InO without DLT during Course 1. MTD/RP2D was the highest dose level tested at which ≤1/6 patients experienced DLT during Course 1 with at ≥2 patients experiencing DLT at the next higher dose. If the MTD was not reached at the highest dose level (DL3), no further dose escalation would be performed. Instead, the highest tested dose would be taken forward as the RP2D. EFS and OS were estimated using the Kaplan–Meier method.

The Full Analysis Set (FAS) included all enrolled patients who received ≥1 dose of study drug and was used for the efficacy and safety analyses. The PK Analysis Set included all FAS patients who provide ≥1 blood sample for PK.

Results

Data cut-off date was March 4, 2020, with median follow-up of 19 months (range 2–21).

Patients

Overall, 27 patients consented and were screened for inclusion. Twenty-five patients were enrolled (two screening failures, both with inadequate liver function); twenty-three were included in the dose escalation analysis set (Supplementary Figure 2). Two patients in the first cohort who received only one dose of InO were non-evaluable for DLTs as defined in the protocol. Treatment discontinuation in these two patients was not related to DLTs, but to graft versus host disease reactivation in one patient, and to sepsis shortly after the first dose of InO in another patient.

Patient baseline characteristics are summarized in Table 2. A total of 130 doses (median six doses/patient; range: 1–12) and 42 courses (median two courses/patient; range: 1–4) were given. Four courses were incomplete (1–2 doses only).

Toxicity

In Course 1 (Cohort 1), DLTs were experienced by 1/6 patients at DL1 (Grade 4 ALT elevation), so this dose level was cleared. At DL2, DLTs were experienced by 2/5 patients (Grade 4 ALT elevation for >7 days; no hematological recovery at Day 42); on review, the Steering Committee considered the hepatic toxicity (transaminase

elevation) as not impairing patient safety significantly, as the patient was in CR after Course 1, and received all three doses without being clinically ill or hospitalized for side-effects. After an IRB/EC approved protocol amendment a second cohort was added with more stringent transaminase monitoring and dose delay criteria in case of transaminase elevation at day 8 after the first InO dose. With this protocol modification, there was not a hepatic DLT at DL1 or DL2 in the second cohort: 0/6 patients at DL1 and 1/6 patients at DL2 had a DLT (no hematological recovery at Day 42), confirming the safety of both dose levels (Supplementary Table 2).

Table 2. Patient characteristics

Characteristic	**n=25**
Age, years; median (range)	11 (1.7–16.9)
Age category, n (%)	
>1– ≤2 years	1 (4)
>2– ≤6 years	4 (16)
>6 years	20 (80)
Gender, n (%)	
Male	17 (68)
Female	8 (32)
Bone marrow status at screening, n (%)	
M3	22 (88)
M2	3 (12)
White blood cell count at screening, per L; median (range)	3.5 x 10^9 (0.19–8.59 x 10^9)
Disease status at enrolment	
1st relapse after HSCT	7 (28)
≥2nd relapse	15 (60)
Refractory	3 (12)
Number of prior treatments; median (range)	2 (2–7)
Specific elements of prior treatment, n (%)	
Prior HSCT	14 (56)
Prior blinatumomab	6 (24)
Prior CAR-T	1 (4)
CD22 expression at screening	
CD22-positive ALL cells, MFI; median (range)	2768 (505–8370)
CD22-positive blasts; % (range)	98 (53–100)
Cytogenetic subtype, n (%)[a]	
Hypodiploid	4 (16)
Hyperdiploid	13 (52)
t[1;19](q23;p13)	2 (8)
t[4;11](q21;q23)	1 (4)
Normal cytogenetics	4 (16)
Not done	1 (4)

[a]Note: patients can have both hypodiploidy and a translocation

ALL, acute lymphoblastic leukemia; CAR-T, chimeric antigen receptor T cell; HSCT, hematopoietic stem cell transplant; MFI, mean fluorescence intensity

Given the very high response rate reached at DL2 (85%, see below) with MRD-negativity, the Steering Committee decided that no further improvements were to be expected with a higher dose of InO – a conclusion supported by preliminary PK data. Therefore, considering the risk/benefit balance, as well as the consideration that DL2 was the approved dose in adults, dose escalation to DL3 was not performed, and DL2 was declared as the RP2D.

During the study, all treated patients had ≥1 AE, most frequently fever (n=16, 64%), decreased platelet count (n=15, 60%) and neutrophil count (n=14, 56%), vomiting (n=12, 48%), and anemia (n=11, 44%) (Supplementary Table 3). Twenty-three patients had ≥1 Grade 3–4 AE (all onset during Course 1; Figure 1). Four patients had Grade 5 AEs (disease progression with fatal outcome, n=2; lung infection, 2 months after administration of a single dose of InO, n=1; sepsis after HSCT, n=1), all of which were considered unrelated or unlikely to be related to InO. Laboratory and hematologic abnormalities are summarized in Supplementary Table 4.

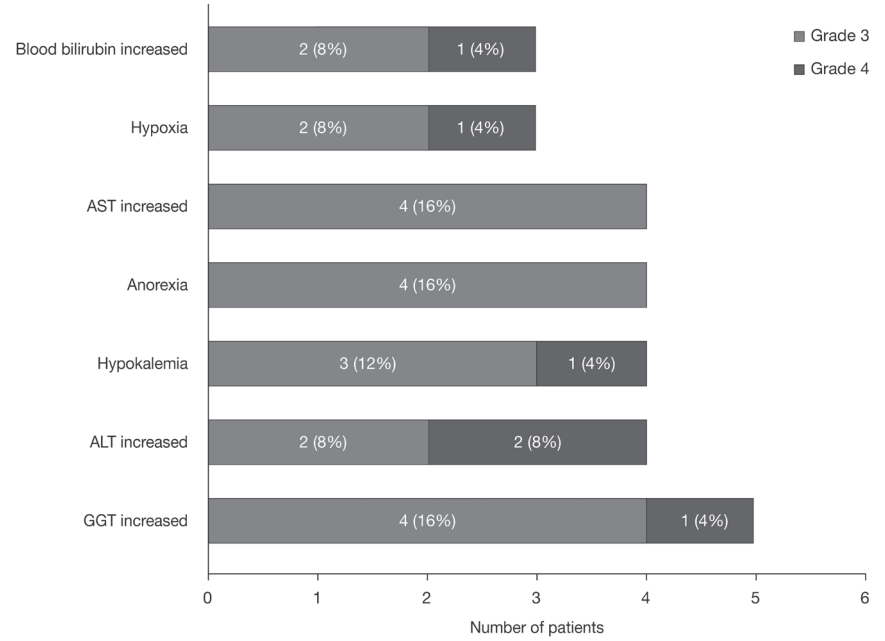


Figure 1. Most common non-hematologic adverse events (Grade 3–5, total >10%) reported as clinically significant by the investigators during the study, N=25[a]

[a]Highest toxicity grade per patient, counted only once per patient

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase

No cases of SOS were reported during InO treatment. However, post-study treatment, two cases of SOS (Grade 3–4, both ongoing at time of death due to infectious events) were recorded, both after subsequent multi-agent chemotherapy (that included high-dose methotrexate and ifosfamide/cyclophosphamide) for R/R disease. One patient received two courses of InO at DL1, and another received one course at DL2, both without relevant signs of liver toxicity. Neither patient received HSCT before or after InO. No SOS occurred in the seven patients who were transplanted after InO: these patients were treated with either one (n=4) or 2 (n=3) courses of InO; only one of these patients received prophylaxis for SOS with defibrotide; conditioning regimens included total body irradiation and etoposide (n=3), and thiotepa-fludarabine combined with either treosulfan (n=2), busulfan (n=1) or melphalan (n=1); 6/7 patients had not been previously transplanted.

Efficacy

ORR after Course 1 was 80% (20/25 patients; 95% confidence interval [CI]: 59–93%); 75% (95%CI: 43–95%) at DL1 and 85% (95%CI: 55–98%) at DL2. Fifteen patients (60%) achieved CR, one patient CR with incomplete platelet recovery (CRp), and four patients CR with incomplete hematologic recovery (CRi). Characteristics of the five non-responders (one refractory, two with 1st relapse after HSCT, and two with $\geq$2nd relapse) are summarized in Supplementary Table 5.

Flow-cytometry MRD data were available for 19/20 responding patients: 16/19 (84%) reached MRD-negative CR (bone marrow; MRD $<$0.01%) as best response (6/9 patients at DL1 and 10/10 patients at DL2), of whom, 14 were MRD-negative after Course 1 (6/9 at DL1 and 8/9 at DL2, one patient had missing data at DL2). Highly comparable MRD data were obtained using molecular analysis (Supplementary Figure 3).

Median DOR after InO treatment was 8 months (range 1–19); 6.9 months for patients treated at DL1, and 12.6 months for patients at DL2 (Figure 2 and Supplementary Table 7). Seven patients received consolidation treatment with HSCT (median 51 days; range 23–125 after the last dose of InO); 4 without any bridging therapy, 3 after receiving additional chemotherapy (oral methotrexate or multiagent reinduction treatment) or Blinatumomab before HSCT. Two patients received CAR-T as consolidation treatment after achieving CR with InO (31 and 97 days after last dose of InO). Cells were harvested prior to InO in one patient and after InO treatment in the other patient. Notably, one patient showed a prolonged CCR (15 months) after 2 courses of InO, without any additional treatment; two other patients were in CCR (both 19 months) without receiving HSCT or CAR-T cell therapy, but one patient did receive additional chemotherapy and the other patient received Blinatumomab (Figure 2) post-InO.

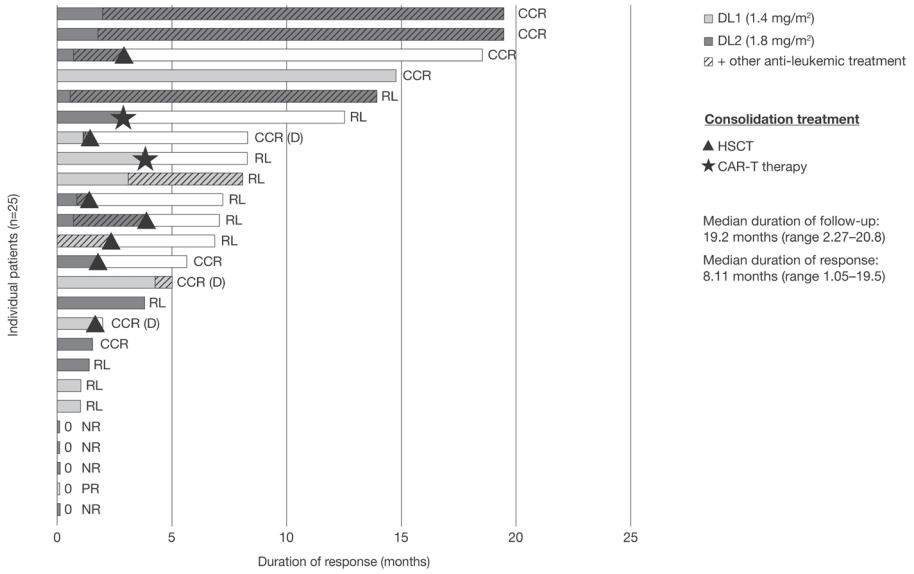


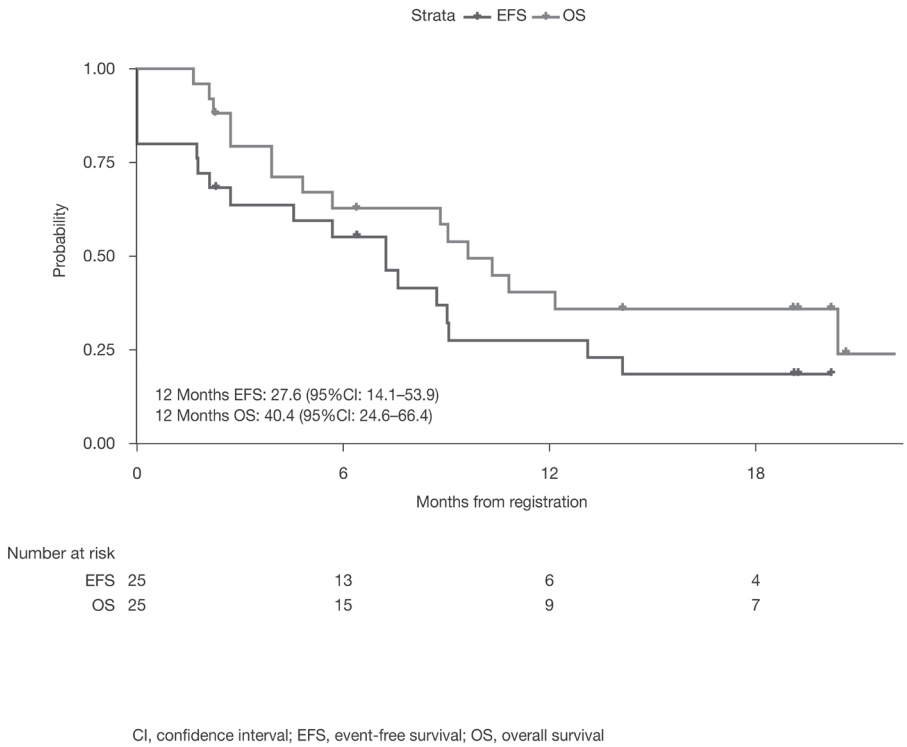
Figure 2. Duration of response to InO treatment

The figure shows the duration of response starting from the first achievement of CR. A different pattern of the box highlights the start of other leukemic anti-treatment, while the white color of the box shows the duration of response without additional treatment after the end of InO treatment. Consolidation treatment with HSCT or CAR-T therapy is also shown with symbols in the figure.

CAR-T, chimeric antigen receptor T cell; CCR, continuous complete remission; CCR (D), death in continuous complete remission; CI, confidence interval; DL1/2, dose level 1/2; HSCT, hematopoietic stem cell transplant; NR, no response; PR, partial response; RL, relapse

Overall survival from the start of InO treatment was 63% (95% CI: 46–85%) at 6 months, and 40% (95% CI: 25–66%) at 12 months (Figure 3). The median overall survival was reached at 7.2 months for patients treated at DL1, while it was not reached for DL2. Event-free survival rate at 6 and 12 months was 55% (95% CI: 39–79%), and 28% (95% CI: 14–54%), respectively (Figure 3). Over the whole study, 15 patients experienced non-response (5 patients) or relapse. The cumulative incidence of non-response or relapse was 36% (95% CI: 18–55%) at 6 months, and 59% (95% CI: 36–77%) at 12 months (Supplementary Figure 1). Fifteen patients died during the study or follow-up, including three non-relapse deaths (two transplant-related, one

multi-organ failure in aplasia after additional chemotherapy for MRD positivity). The cumulative incidence of non-relapse mortality was 8% (95% CI: 1–24%) at 6 months, and 13% (95% CI: 3–31%) at 12 months (Supplementary Figure 1).



CI, confidence interval; EFS, event-free survival; OS, overall survival

Figure 3. Event-free survival and overall survival of the whole population

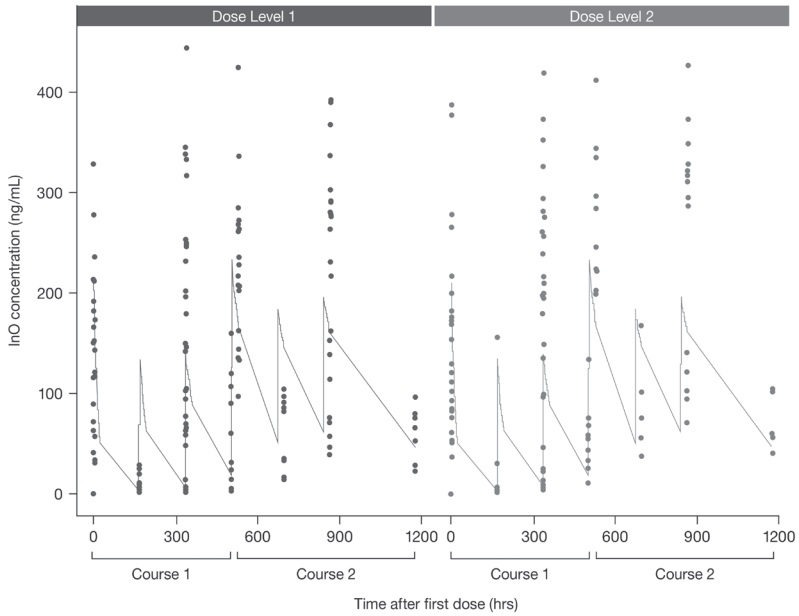
CD22 expression

All patients were CD22-positive at enrolment, as confirmed at the central laboratory (Table 2). The difference in CD22 expression, determined both as mean fluorescence intensity and as percentage of blasts expressing CD22 antigen, was not statistically significant between responder and non- responder patients (both $p>0.05$, Wilcoxon test). During follow-up, 13 patients had material available for reliable CD22 analysis at relapse: 2/13 showed CD22-negativity; in one additional patient 80% of the residual blasts were CD22-negative. In one patient with *KMT2A*-rearranged, CD22 expression was still present at relapse, although 7% of the ALL blasts were CD22 negative. B-cell count was assessed at the end-of-treatment visit in 15 patients in CR: three patients had peripheral B-cell

recovery at that time(median 21 days after last InO dose); one additional patient reached B-cell recovery 3 months after the last InO dose, before receiving CAR-T therapy. Follow-up data are limited due to subsequent HSCT or relapse before recovery.

Pharmacokinetics

The InO serum concentration vs time profile (compared with simulated adult PK data) is shown in Figure 4. InO serum peak and trough concentrations at various visits and treatment courses are summarized in Supplementary Table 6. After a single dose (Day 1, Course 1), InO median maximum concentrations at DL1 (n=12) and DL2 (n=13) were 159 and 130 ng/mL, respectively, compared with the simulated adult concentration of 204 ng/mL at a dose of 1.8 mg/m^2/course. After multiple doses (Day 1, Course 2), InO median maximum concentrations at DL1 (n=9) and DL2 (n=5) were 217 and 246 ng/mL, respectively, which was comparable to the simulated adult concentration of 234 ng/mL at a dose of 1.8 mg/m^2/course (Supplementary Table 2).



Solid black line represents the simulated median concentration achieved in a 70 kg ALL adult patient receiving 1.8 mg/m2/course
Red dots represent the observed InO concentrations in DL1; Blue dots represent the observed InO concentrations in DL2

ALL, acute lymphoblastic leukemia; DL1/2, dose level 1/2; hrs, hours; InO, inotuzumab ozogamicin

Figure 4. Pharmacokinetics – inotuzumab ozogamicin serum concentration vs time profile in Course 1 and Course 2 by dose level

Discussion

In this dose-finding study, InO was well tolerated with notable efficacy in heavily pretreated children with R/R ALL. RP2D was established at 1.8 mg/m^2/course (0.8–0.5–0.5 mg/m^2), as in adults.(7) The ORR was 80%, with a 1-year overall survival probability of 40%. The response rate was higher for patients treated at DL2 (85%) compared to DL1 (75%), although this difference was not statistically significant due to the small sample size; as well as for the MRD-negativity rate, 66% for DL1, while it was 100% in DL2 (n=10). These better results are also reflected in the longer DOR reached by patients treated at DL2 (6.9 months at DL1, versus 12.6 months at DL2), as well as in the median survival (reached at 7.2 months for patients treated at DL1, while it was not reached for DL2) (Supplementary Table 7).

Nine of 25 patients received consolidation therapy (HSCT or CAR-T); 3 patients showed prolonged CCR without subsequent HSCT or CAR-T (two received additional therapy, while one did not receive any treatment post-InO). (Figure 2). Two cases of SOS were recorded after subsequent multiagent chemotherapy, as previously reported in non-transplant-related SOS;(25) no patient given HSCT developed SOS.

The disposition of InO following IV administration is influenced by interaction with CD22-positive B-cells and, as with other therapeutic proteins, in part to binding with the Fc receptor.(26) The PK of InO in adults has been well characterized by a two-compartmental model with linear and time-dependent clearance, using pooled data from 765 adult patients.(27) Steady-state InO levels were achieved after 3–4 cycles of treatment, with exposure increasing with treatment cycle (i.e. clearance decreases over time). This is consistent with the PK of antibodies that target B-cell receptors showing target-mediated drug disposition, as well as a half-life relative to the given dosage interval.(27, 28) The present study is the first including PK of InO in children with ALL. The peak and trough concentrations of InO following single and multiple doses were variably higher or lower in children when compared with the simulated data in a typical adult ALL patient. Given the inconsistent direction of change (lower or higher) and considerable data variability, it is concluded that InO plasma PK is comparable between pediatric and adult patients. Comparable plasma levels were found also in patients treated at DL1 and DL2. The comparable toxicity (Supplementary Table 3-4) and the results indicative of higher activity at DL2 (Supplementary Table 7) lead to the RP2D of 1.8 mg/m2/course.

These preliminary data are the first prospective analysis of InO in pediatric patients, and are consistent with initial observations recorded in retrospective studies of InO in children, including a US/European compassionate use program that reported a CR

rate of 67%; 71% of whom obtained MRD-negativity.(15, 16) There were furthermore no reports of SOS during treatment with InO in a retrospective, compassionate access study in 51 children; however, in contrast with the present trial, SOS was reported in 11/21 patients (52%) after consolidation HSCT.(15) These patients were heavily pretreated, and received a median of five prior lines of therapy.(15)

In a retrospective post-hoc analysis of the present cohort, seven 'very high risk' patients were identified (presenting ≥1 of the following characteristics: low hypodiploidy, n=2; *MLL/AF4*, n=1; t(1;19) (q23;p13), n=2; relapse <18 months post-diagnosis, n=6); of these, only two were non-responders to InO (one with hypodiploidy, and one with t[1;19] and very early relapse). No specific subgroups of patients with decreased efficacy were identified. Ongoing investigations in other cohorts of the current study in R/R pediatric ALL include a Phase II InO monotherapy cohort and a Phase I dose-finding cohort of InO in combination with chemotherapy.

Preliminary results from the COG AALL1621 Phase II study, investigating InO monotherapy in a similar pediatric population at the adult dose, demonstrated a CR/CRi of 58%, with MRD <0.01% in 65% of responders, and minimal hepatic toxicity during InO therapy; however, four out of 13 patients who received a subsequent HCST (30.7%) developed SOS.(29)

InO data from a Phase III randomized controlled study in 326 adults with R/R ALL showed that InO was superior to standard chemotherapy, with greater rates of CR/CRi (73.8% vs 30.9%; one-sided p<0.0001).(30) Similar response rates were observed in the current pediatric study at the same dose established here as single-agent RP2D (1.8 mg/m^2/course). Subsequently, different dosages of InO have been tested in combination with chemotherapy regimens in adult ALL, showing promising results.(31, 32)

Two other immunotherapies are currently indicated for treatment of R/R BCP-ALL. Blinatumomab, a CD19-directed T-cell engager, and tisagenlecleucel, an anti-CD19 CAR-T therapy. The blinatumomab label for pediatric use(33) was supported by a single-arm Phase I/II study in pediatric patients with R/R ALL: 39% of patients achieved CR within two cycles of blinatumomab, 52% of whom achieved complete MRD response.(34) The recent expanded access study showed even higher response rate (CR rate 59%).(35) Although not a direct comparison, these levels were lower than those observed with InO in the present study. Recently, 2 courses of blinatumomab as post-reinduction therapy prior to HSCT showed superiority compared to 2 blocks of standard chemotherapy, leading to improved disease-free and overall survival.(36) Tisagenlecleucel has been approved for treatment of

patients aged ≤25 years with R/R ALL, based on findings from a Phase II study that demonstrated an overall MRD-negative remission rate of 81% at 3 months.(37, 38) As only two patients in the present study received CAR-T, no specific conclusions can be drawn on its use as consolidation therapy after InO. Although prior administration of InO should not lessen the efficacy of tisagenlecleucel, InO induces a post-treatment period of B-cell aplasia by targeting CD22 on both normal and malignant cells and, when there is MRD-negative CR plus B-cell aplasia, the expansion of CAR-T cells may be impaired, potentially requiring a waiting time for B-cells to re-appear prior to CAR-T cell infusion.(39, 40) Other CD22-directed therapies consist of epratuzumab, an unconjugated CD22-directed antibody that showed limited activity,(41, 42) and CD22-directed CAR-T therapy.(43)

In summary, in this Phase I study, InO was well tolerated and demonstrated anti-leukemic activity with high levels of MRD-negative response in children with R/R ALL, in line with observations in adults . The dose taken forward to the ongoing single-agent Phase II cohort of this study was established as 1.8 mg/m^2 (fractionated schedule) during Course 1, as recommended in adults; the dose for subsequent courses remains 1.5 mg/m^2/course up to a maximum of 6 courses, unless HSCT is planned. Findings were consistent with adult PK analyses which indicated that the PK of InO is unaffected by age, thus dose adjustment is not required in children. Dose finding for InO in combination with three-drug re-induction chemotherapy (dexamethasone, vincristine, PEG-asparaginase) in children with R/R ALL is ongoing.

Acknowledgements

The authors would like to thank the ITCC consortium and the I-BFM study group for providing the infrastructure and the collaborative environment to run early clinical trials in pediatric oncology, and Pfizer Inc. for providing funding and a clinical research collaboration to implement the study. They would also like to thank Berna Beverloo and the Department of Clinical Genetics, Erasmus MC for the central review of cytogenetic data.

Sponsorship for the study, including medical writing support, was provided by Erasmus MC Sophia Children's Hospital, Department of Pediatrics, Rotterdam, The Netherlands. The KiKa Foundation provides infrastructural funding for the trial office at ErasmusMC and later at the Princess Máxima Center.

Medical writing support, under the direction of the authors, was provided by Juliet George, PhD, on behalf of CMC AFFINITY, McCann Health Medical Communications, in accordance with Good Publication Practice (GPP3) guidelines.

References

1. WHO: 4.1 Incidence of Childhood Leukaemia, an ENHIS Fact Sheet. Copenhagen, Denmark, World Health Organization, 2009
2. Hunger SP, Mullighan CG: Acute Lymphoblastic Leukemia in Children. N Engl J Med 373:1541-52, 2015
3. Pieters R, de Groot-Kruseman H, Van der Velden V, et al: Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 From the Dutch Childhood Oncology Group. J Clin Oncol 34:2591-601, 2016
4. Henze G, v Stackelberg A, Eckert C: ALL-REZ BFM--the consecutive trials for children with relapsed acute lymphoblastic leukemia. Klin Padiatr 225 Suppl 1:S73-8, 2013
5. Parker C, Waters R, Leighton C, et al: Effect of mitoxantrone on outcome of children with first relapse of acute lymphoblastic leukaemia (ALL R3): an open-label randomised trial. Lancet 376:2009-17, 2010
6. Moricke A, Zimmermann M, Reiter A, et al: Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia 24:265-84, 2010
7. Besponsa™: Prescribing Information, Pfizer Inc, 2018
8. Ellestad GA: Structural and conformational features relevant to the anti-tumor activity of calicheamicin γ 1I. Chirality 23:660-71, 2011
9. Kantarjian HM, DeAngelo DJ, Stelljes M, et al: Inotuzumab Ozogamicin versus Standard Therapy for Acute Lymphoblastic Leukemia. N Engl J Med 375:740-53, 2016
10. Rosenthal J, Naqvi AS, Luo M, et al: Heterogeneity of surface CD19 and CD22 expression in B lymphoblastic leukemia. Am J Hematol 93:E352-E355, 2018
11. Iwamoto S, Deguchi T, Ohta H, et al: Flow cytometric analysis of de novo acute lymphoblastic leukemia in childhood: report from the Japanese Pediatric Leukemia/ Lymphoma Study Group. Int J Hematol 94:185-192, 2011
12. Shah NN, Stevenson MS, Yuan CM, et al: Characterization of CD22 expression in acute lymphoblastic leukemia. Pediatr Blood Cancer 62:964-9, 2015
13. Besponsa: Summary of Product Characteristics, 2020
14. Zwaan CM, Reinhardt D, Jürgens H, et al: Gemtuzumab ozogamicin in pediatric CD33-positive acute lymphoblastic leukemia: first clinical experiences and relation with cellular sensitivity to single agent calicheamicin. Leukemia 17:468-70, 2003
15. Bhojwani D, Sposto R, Shah NN, et al: Inotuzumab ozogamicin in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. Leukemia 33:884-892, 2019
16. Rytting M, Triche L, Thomas D, et al: Initial experience with CMC-544 (inotuzumab ozogamicin) in pediatric patients with relapsed B-cell acute lymphoblastic leukemia. Pediatr Blood Cancer 61:369-72, 2014
17. Calvo C, Cabannes-Hamy A, Adjaoud D, et al: Inotuzumab ozogamicin compassionate use for French paediatric patients with relapsed or refractory CD22-positive B-cell acute lymphoblastic leukaemia. Br J Haematol, 2020
18. (EMA) EMA: Public summary of the evaluation of a proposed paediatric investigation plan Inotuzumab ozogamicin for treatment of acute lymphoblastic leukaemia, 2014

19. ITCC: Innovative Therapies for Children with Cancer in Europe,
20. McDonald GB, Hinds MS, Fisher LD, et al: Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. Ann Intern Med 118:255-67, 1993
21. Skolnik JM, Barrett JS, Jayaraman B, et al: Shortening the timeline of pediatric phase I trials: the rolling six design. J Clin Oncol 26:190-5, 2008
22. de Vries JF, Zwaan CM, De Bie M, et al: The novel calicheamicin-conjugated CD22 antibody inotuzumab ozogamicin (CMC-544) effectively kills primary pediatric acute lymphoblastic leukemia cells. Leukemia 26:255-64, 2012
23. Theunissen P, Mejstrikova E, Sedek L, et al: Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood 129:347-357, 2017
24. van der Velden VH, Panzer-Grümayer ER, Cazzaniga G, et al: Optimization of PCR-based minimal residual disease diagnostics for childhood acute lymphoblastic leukemia in a multi-center setting. Leukemia 21:706-13, 2007
25. Kernan NA, Richardson PG, Smith AR, et al: Defibrotide for the treatment of hepatic veno-occlusive disease/sinusoidal obstruction syndrome following nontransplant-associated chemotherapy: Final results from a post hoc analysis of data from an expanded-access program. Pediatr Blood Cancer 65:e27269, 2018
26. Lobo ED, Hansen RJ, Balthasar JP: Antibody pharmacokinetics and pharmacodynamics. J Pharm Sci 93:2645-68, 2004
27. Garrett M, Ruiz-Garcia A, Parivar K, et al: Population pharmacokinetics of inotuzumab ozogamicin in relapsed/refractory acute lymphoblastic leukemia and non-Hodgkin lymphoma. J Pharmacokinet Pharmacodyn 46:211-222, 2019
28. Dirks NL, Meibohm B: Population pharmacokinetics of therapeutic monoclonal antibodies. Clin Pharmacokinet 49:633-59, 2010
29. O'Brien MM, Ji L, Shah NN, et al: A Phase 2 Trial of Inotuzumab Ozogamicin (InO) in Children and Young Adults with Relapsed or Refractory (R/R) CD22+ B-Acute Lymphoblastic Leukemia (B-ALL): Results from Children's Oncology Group Protocol AALL1621. Blood 134:741, 2019
30. Kantarjian HM, DeAngelo DJ, Stelljes M, et al: Inotuzumab ozogamicin versus standard of care in relapsed or refractory acute lymphoblastic leukemia: Final report and long-term survival follow-up from the randomized, phase 3 INO-VATE study. Cancer 125:2474-2487, 2019
31. Jabbour E, Sasaki K, Ravandi F, et al: Chemoimmunotherapy with inotuzumab ozogamicin combined with mini-hyper-CVD, with or without blinatumomab, is highly effective in patients with Philadelphia chromosome-negative acute lymphoblastic leukemia in first salvage. Cancer 124:4044-4055, 2018
32. Advani AS, Moseley A, Liedtke M, et al: SWOG 1312 Final Results: A Phase 1 Trial of Inotuzumab in Combination with CVP (Cyclophosphamide, Vincristine, Prednisone) for Relapsed/ Refractory CD22+ Acute Leukemia. Blood 134:227-227, 2019
33. (blinatumomab) B: Prescribing Information. Amgen Inc., 2019
34. von Stackelberg A, Locatelli F, Zugmaier G, et al: Phase I/Phase II Study of Blinatumomab in Pediatric Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia. J Clin Oncol 34:4381-4389, 2016

35. Locatelli F, Zugmaier G, Mergen N, et al: Blinatumomab in pediatric patients with relapsed/refractory acute lymphoblastic leukemia: results of the RIALTO trial, an expanded access study. Blood Cancer Journal 10:77, 2020

36. Brown PA, Ji L, Xu X, et al: A Randomized Phase 3 Trial of Blinatumomab Vs. Chemotherapy As Post-Reinduction Therapy in High and Intermediate Risk (HR/IR) First Relapse of B-Acute Lymphoblastic Leukemia (B-ALL) in Children and Adolescents/Young Adults (AYAs) Demonstrates Superior Efficacy and Tolerability of Blinatumomab: A Report from Children's Oncology Group Study AALL1331. Blood 134:LBA-1-LBA-1, 2019

37. Maude SL, Laetsch TW, Buechner J, et al: Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med 378:439-448, 2018

38. Kymriah: Prescribing Information, Novartis Pharmaceuticals, 2018

39. Kansagra AJ, Frey NV, Bar M, et al: Clinical utilization of Chimeric Antigen Receptor T-cells (CAR-T) in B-cell acute lymphoblastic leukemia (ALL)-an expert opinion from the European Society for Blood and Marrow Transplantation (EBMT) and the American Society for Blood and Marrow Transplantation (ASBMT). Bone Marrow Transplant 54:1868-1880, 2019

40. Gardner RA, Finney O, Annesley C, et al: Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. Blood 129:3322-3331, 2017

41. Raetz EA, Cairo MS, Borowitz MJ, et al: Chemoimmunotherapy reinduction with epratuzumab in children with acute lymphoblastic leukemia in marrow relapse: a Children's Oncology Group Pilot Study. J Clin Oncol 26:3756-62, 2008

42. Raetz EA, Cairo MS, Borowitz MJ, et al: Re-induction chemoimmunotherapy with epratuzumab in relapsed acute lymphoblastic leukemia (ALL): Phase II results from Children's Oncology Group (COG) study ADVL04P2. Pediatr Blood Cancer 62:1171-5, 2015

43. Fry TJ, Shah NN, Orentas RJ, et al: CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. Nat Med 24:20-28, 2018

Appendix 1. Supplementary material

Supplementary Table 1. Inclusion/exclusion criteria

INCLUSION ELIGIBILITY CRITERIA	

Age	• ≥1 and <18 years at time of enrollment
	  • The first three patients on dose level 1 must be ≥6 and <18 years
	  • Then ≥2 additional patients ≥1 year and <6 years at the same dose level

Diagnosis	• First relapse of BCP-ALL post allogeneic HSCT • Second or greater R/R BCP-ALL • Refractory disease (newly diagnosed patients who had induction failures after ≥2 previous regimens without attainment of remission, or patients with refractory first relapse after one previous reinduction regimen without attainment of remission) **AND**: • M2 or M3 marrow status (≥5% blasts by morphology) • Malignant clone CD22 surface antigen positive (in either bone marrow or peripheral blood) by institutional standards • The first six patients must have M3 marrow status (≥25% blasts by morphology)
Performance level and life expectancy	• Karnofsky >60% (>16 years) or Lansky >60% (≤16 years) • Life expectancy of ≥6 weeks
Prior therapy	Patients must have recovered from the acute toxic effects of all prior therapy, defined as resolution of non-hematologic toxicities to ≤Grade 2 per the CTCAE 4.03 prior to entering the study a. Chemotherapy ≥7 days since the completion of cytotoxic therapy (exceptions: hydroxyurea, 6-mercaptopurine and steroids which are permitted up until 48 hours prior to initiating protocol therapy) b. Radiotherapy ≥28 days since any prior radiation therapy c. Hematopoietic stem cell transplant ≥90 days since previous allo-HSCT No evidence of active graft vs host disease No GVHD prophylaxis or treatment d. Hematopoietic growth factors ≥7 days since the completion of therapy with GCSF or other growth factors, or ≥14 days since completion of therapy with pegfilgrastim (Neulasta®) e. Immunotherapy ≥42 days after the completion of any type of immunotherapy, e.g. CART therapy. Patients may not have received prior CD22-targeted therapy (immunotoxin or CART therapy) f. Monoclonal antibodies ≥3 half-lives of the antibody must have elapsed after the last dose of a monoclonal antibody (rituximab = 66 days, epratuzumab = 69 days) Exclusion of blinatumomab: patients must have been off blinatumomab infusion for ≥14 days and all drug-related toxicity must have resolved to ≤Grade 2

Supplementary Table 1. Continued

INCLUSION ELIGIBILITY CRITERIA	
Prior therapy (Continued)	g. Investigational drugs ≥7 days or five drug half-lives (whichever is longer) since prior treatment with any experimental drug (with the exception of monoclonal antibodies) under investigation. No residual toxicities should be observed following previous treatment h. Prior calicheamicin exposure Patient has not received prior treatment with a calicheamicin-conjugated antibody (e.g. gemtuzumab ozogamicin)
Renal and hepatic function	• Serum creatinine ≤1.5 x institutional ULN according to age • AST and ALT ≤2.5 x institutional ULN • Total bilirubin ≤1.5 x institutional ULN unless the patient has documented Gilbert syndrome
Cardiac function	• Shortening fraction ≥30% by echocardiogram or an ejection fraction >50% by MUGA.
Reproductive function	• Female patients of childbearing potential: negative urine or serum pregnancy test confirmed prior to enrollment • Female patients with infants must agree not to breastfeed on study • Male and female patients of child-bearing potential must agree to use a *highly effective* method of contraception (≥8 months for females and for ≥5 months for males after the last dose of InO)

EXCLUSION ELIGIBILITY CRITERIA	
Isolated extramedullary relapse	• Patients with isolated extramedullary disease are excluded
VOD/SOS	• Any history of prior or ongoing VOD/SOS as per modified Seattle criteria, or prior liver-failure [defined as severe acute liver injury with encephalopathy and impaired synthetic function (international normalized ratio of ≥1.5)]

Infection	• Systemic fungal, bacterial, viral or other infection that is exhibiting ongoing signs/symptoms
	• The patient may not have:
	  • A requirement for vasopressors
	  • Positive blood culture within 48 hours of study enrollment
	  • Fever above 38.2 degrees Celsius within 48 hours of study enrollment with clinical signs of infection. Fever that is determined to be due to tumor burden is allowed, with documented negative blood cultures for ≥48 hours prior to enrollment and no concurrent signs or symptoms of active infection or hemodynamic instability
	  • A positive fungal culture within 30 days of study enrollment
	  • Active fungal, viral, bacterial, or protozoal infection requiring intravenous or oral treatment. Chronic prophylaxis therapy to prevent infections is allowed

Other anti-cancer therapy	• Patients will be excluded if there is a plan to administer non-protocol anti-cancer therapy during the study period
Allergic reaction	• Patients with prior Grade 3/4 allergic reaction to a monoclonal antibody are excluded

Supplementary Table 1. Continued

EXCLUSION ELIGIBILITY CRITERIA	
Concurrent disease	• Significant concurrent disease, illness, psychiatric disorder or social issue that would compromise patient safety or compliance with protocol therapy, interfere with consent, study participation, follow-up, or interpretation of study results • Children with Down syndrome are excluded from participation in the dose finding parts of the study

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCP-ALL, B-cell precursor acute lymphoblastic leukemia; CART, chimeric antigen receptor T cell; GCSF, granulocyte-colony stimulating factor; GVHD, graft versus host disease; HSCT, hematopoietic stem cell transplant; InO, inotuzumab ozogamicin; MUGA, multiple gated acquisition scan; R/R, relapsed/refractory; SOS, sinusoidal obstruction syndrome; ULN, upper limit of normal; VOD, veno-occlusive disease

Supplementary Table 2. Dose-limiting toxicity

<table>
<tr><th>Dose level</th><th>No. patients enrolled</th><th>Significant safety events</th></tr>
<tr><td colspan="3">First cohort</td></tr>
<tr><td>DL1</td><td>6</td><td>• One DLT: Grade 4 ALT elevation >7 days
This patient with 2nd relapse of ALL (WBC 5.3 x 109/L, no prior SCT) received only one dose of InO and rapidly developed Grade 4 transaminase elevation, without bilirubin elevation, during Course 1. ALT elevation lasted longer than 7 days. The patient was taken off study, despite the fact that response evaluation showed CR with a low level of MRD following one dose of InO.</td></tr>
<tr><td>DL2</td><td>7</td><td>• Two patients not evaluable for DLTs
• Two possible DLTs discussed by the Steering Committee:
– Case 1: Lack of hematologic recovery at Day 42 after Course 1
MRD negativity achieved
– Case 2: Grade 4 ALT elevation >7 days
Very good clinical condition and CR achieved. Hepatomegaly was noted at baseline. At Day 8 there was a sharp rise in ALT and AST, without any increase in bilirubin. The laboratory result became available after the second dose of InO was infused. The ALT then dropped at Days 15 and 22, but increased again later (see table below). This initial rise may have been related to leukemia, although there was no clear explanation for the late rise in transaminases which prevented continuation of InO treatment. The patient was receiving liposomal amphotericin B prophylaxis. No azoles or other co-medication were identified to explain this rise; no infectious complications were documented and viral tests were negative.</td></tr>
</table>

	Date	ALT (IU/L)	AST (IU/L)	Total bilirubin (µmol/L)
Normal ranges		<47	<47	<25
Screening	20-04-2018	13	24	5
Course 1 Day 1	26-04-2018	81	32	10
Course 1 Day 8	03-05-2018	937	484	6
	07-05-2018	515 (declined despite recent InO dose)	93	8
Course 1 Day 14	09-05-2018	368	78	5
	14-05-2018	454		5
Course 1 Day 22	17-05-2018	384	164	5
	22-05-2018	1140	574	3
EOT	07-06-2018	200	110	4

Supplementary Table 2. Continued

Dose level	No. patients enrolled	Significant safety events

The Steering Committee discussed the observed DLTs at DL2 (above) and considered case 2 (Grade 4 ALT elevation >7 days) not truly dose limiting as patient safety was not compromised. As defined in the protocol, transaminase elevation grade ≥3 for >7 consecutive days fulfilled the strict DLT criteria. However, the patient had an atypical late pattern compared to the adult experience with InO. The typical pattern for transaminases is to rise early in the first course, while in this patient the ALT elevation persisted for over 3 weeks. At that time, the protocol did not include dose delays within course 1 for high ALT/AST and so the patient continued to be dosed. This patient was clinically well during this laboratory abnormality and achieved CR after Course 1, suggesting clinical benefit.

After this occurrence, the dose was temporarily de-escalated to DL1, while awaiting approval of protocol amendment. The protocol was amended to add more stringent ALT/AST monitoring, especially at day 8, and dosing criteria within course 1.

Changes in Institutional Review Board-approved protocol amendment:
- Liver functions (bilirubin and transaminases) to be checked at Day 8 prior to InO infusion. In case of a >2.5x ULN elevation in transaminases, or >1.5x ULN for bilirubin, dose delay.
- Grade 3 or 4 elevation of hepatic transaminases >7 days not considered DLT if judged by the investigator to be related to leukemic infiltration of the liver or to another medical complication of therapy such as infection or hepatotoxic concomitant medication(s).

Second cohort
There was no hepatic DLT at DL1 or DL2 in this second cohort (see below). Despite the more stringent ALT/AST monitoring, incidentally no patient had to delay InO doses due to transaminitis during the DLT evaluation period.

DL1	6	No DLTs
DL2	6	One DLT: Lack of platelet recovery at Day 42 *CR and MRD negativity achieved*

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CR, complete remission; DL1/2, dose level 1/2; DLT, dose-limiting toxicity; EOT, end of treatment; InO, inotuzumab ozogamicin; MRD, minimal residual disease; SCT, stem cell transplant; ULN, upper limit of normal; WBC, white blood cell

Supplementary Table 3. Most common adverse events at DL1 and DL2 (total >10% [≥3 patients]), N=25

	Number of patients										
	Grade 1		Grade2		Grade 3		Grade 4		Grade 5		Total
	DL1	DL2	DL1	DL2	DL1	DL2	DL1	DL2	DL1	DL2	
Fever	6	5	2	2	0	1	0	0	0	0	16
Platelet count decreased	0	1	0	0	1	2	6	5	0	0	15
Neutrophil count decreased	0	0	0	0	0	1	7	6	0	0	14
Vomiting	4	3	1	4	0	0	0	0	0	0	12
Anemia	0	0	0	0	6	5	0	0	0	0	11
Cough	2	3	1	3	0	0	0	0	0	0	9
Nausea	1	3	2	3	0	0	0	0	0	0	9
Febrile neutropenia	0	0	0	0	3	4	0	1	0	0	8
WBC decreased	0	0	0	0	2	0	2	4	0	0	8
Anorexia	1	0	0	2	2	2	0	0	0	0	7
AST increased	0	2	0	1	2	2	0	0	0	0	7
Constipation	2	2	0	3	0	0	0	0	0	0	7
Fatigue	2	1	0	3	0	1	0	0	0	0	7
Headache	4	2	0	1	0	0	0	0	0	0	7
Abdominal pain	2	2	1	0	0	1	0	0	0	0	6
GGT increased	0	0	0	1	1	3	1	0	0	0	6
Hypokalemia	1	0	0	1	1	2	0	1	0	0	6
Pain in extremity	3	1	1	1	1	0	0	0	0	0	6
Diarrhea	1	3	0	1	0	0	0	0	0	0	5
Hematoma	2	3	0	0	0	0	0	0	0	0	5
Hypoxia	0	0	1	1	1	1	0	1	0	0	5
Oral hemorrhage	3	1	0	1	0	0	0	0	0	0	5
ALT increased	0	0	0	0	1	1	1	1	0	0	4
Edema, face	0	4	0	0	0	0	0	0	0	0	4
Edema, limbs	2	2	0	0	0	0	0	0	0	0	4
Epistaxis	1	3	0	0	0	0	0	0	0	0	4
Lymphocyte count decreased	0	0	0	0	0	1	2	1	0	0	4
Periorbital edema	3	1	0	0	0	0	0	0	0	0	4
Blood bilirubin increased	0	0	0	0	0	2	0	1	0	0	3
Dyspnea	0	0	1	1	1	0	0	0	0	0	3
Generalized muscle weakness	0	0	1	2	0	0	0	0	0	0	3
Mucositis, oral	0	1	1	0	0	0	1	0	0	0	3
Oral pain	2	0	1	0	0	0	0	0	0	0	3
Pain	1	1	0	1	0	0	0	0	0	0	3
Rhinitis, infective	0	2	0	1	0	0	0	0	0	0	3

[a]Highest toxicity grade per patient, counted only once per patient

ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; WBC, white blood cell

Supplementary Table 4. Chemistry and hematologic laboratory test abnormalities at DL1 and DL2[a,b]

	Number of patients										
	Grade 0		Grade 1		Grade 2		Grade 3		Grade 4		Total
Chemistry analytes	**DL1**	**DL2**	**DL1**	**DL2**	**DL1**	**DL2**	**DL1**	**DL2**	**DL1**	**DL2**	
Albumin (low)	5	3	4	5	3	4	0	1	0	0	25
Alkaline phosphatase (high)	10	9	2	3	0	1	0	0	0	0	25
ALT (high)	1	0	7	8	2	2	1	2	1	1	25
AST (high)	0	1	8	7	2	2	1	3	1	0	25
Calcium (low)	7	4	4	7	0	1	1	1	0	0	25
Creatinine	12	13	0	0	0	0	0	0	0	0	25
Phosphate (low)	6	8	2	2	3	1	0	2	1	0	25
Potassium (low)	5	3	2	6	0	1	5	2	0	1	25
Sodium (low)	7	6	5	7	0	0	0	0	0	0	25
Total bilirubin (high)	7	9	2	1	2	1	0	2	1	0	25
Uric acid (high)	12	13	0	0	0	0	0	0	0	0	25
Amylase (high)	10	11	2	0	0	0	0	1	0	0	24
Lipase (high)	9	8	3	3	0	0	0	0	0	1	24
Urea (high)	7	12	0	0	0	0	0	0	0	0	19
GGT (high)	0	1	0	1	0	1	0	3	0	1	7
Hematologic analytes											
ANC	0	0	0	0	0	0	0	1	12	12	25
Hemoglobin	0	0	0	7	3	1	9	5	0	0	25
Platelet count	0	0	0	1	1	0	2	1	9	11	25
WBC	0	0	0	0	2	1	2	0	8	12	25

[a]Highest toxicity grade per patient, counted only once per patient

[b]Not all abnormalities were reported as AEs

ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; WBC, white blood cell

Supplementary Table 5. Characteristics of patients (n=5) who were non-responders to InO therapy

Subject ID	Disease status at enrolment	WBC per L (x109) at C1D1	% circulating blasts at C1D1	Ploidy	Karyotyping / FISH at enrolment	Early relapse (<18 months after initial diagnosis)	CD22 expression at screening (BM blasts)		Notes
							Blasts, %	ALL cells, MFI	
All study patients, median		2.9	7				98	2949	
1	≥2nd relapse	2.5	45	Hyperdiploid	No abnormalities	No	99	4928	
2	1st relapse after HSCT	3.7	49	Hypodiploid	No abnormalities	No	100	4457	
3	1st relapse after HSCT	0.14	0	ND	ND	No	98	2181	Received only one dose of InO due to rapid onset of infection considered not related to InO
4	≥2nd relapse	2.73	4	ND	No abnormalities	No	94	1054	
5	refractory	0.4	25	Hyperdiploid	49,XY,add(1)(q42),t(1;19)(q23;p13), dup(5)(q13q21),inv(6) (p21q26),+8,+8,+20[14]/ 49,XY,t(1;19),+20,add(21) (q21),+22,+mar[6]	Yes	64	625	

ALL, acute lymphoblastic leukemia; C1D1, Course 1, Day 1; MFI, mean fluorescence intensity; ND, not determined; NR, no response; PR, partial response; WBC, white blood cell

Supplementary Table 6. Summary of pharmacokinetic data in pediatric and adult patients with ALL

Patients	Dose (mg/m2)	Day 1 peak	Day 8 trough	Day 15 trough	Day 15 peak	Day 22/28 trough[a]
Course 1						
Pediatric (n=12)[b]	1.4	158 (87.8) [159]	10.6 (9.51) [8]	33.7 (33.3) [14]	208 (87.7) [202]	57.1 (54.1) [31]
Pediatric (n=13)[c]	1.8	163 (98.6) [130]	21.4 (48.1) [4.5]	25.5 (28.0) [13]	257 (74.1) [259]	51.5 (36.7) [49.5]
Adult[d]	1.8	221 (91) [204]	3.3 (4.1) [1.9]	11 (12) [7]	152 (65) [140]	24.6 (21) [19]
Course 2						
Pediatric (n=9)[e]	1.4	227 (98.9) [217]	62.2 (36.7) [82]	87 (43.0) [73.5]	291 (44.0) [279]	55.5 (28.6) [59.5]
Pediatric (n=5)[f]	1.8	275 (84.3) [246]	87.4 (50.4) [76]	106 (26.6) [103]	325 (16.8) [320]	72.4 (29.0) [60]
Adult[d]	1.8	252 (105) [234]	56 (37) [51]	65 (41) [60]	208 (89) [191]	49 (34) [43]

Data are mean (standard deviation) [median]; summary data are not presented for n <3.

[a]Course 1 refers to the Day 22 trough concentration; Course 2 refers to the Day 28 trough concentration.

[b]n=12 for Day 1 peak; n=11 for Day 8 trough, Day 15 trough and peak, and Day 22 trough.

[c]n=13 for Day 1 peak; n=10 for Day 8 trough, Day 15 peak and Day 22 trough; n=11 for Day 15 trough.

[d]Adult PK data are simulated using the final model described by Garret et al.[27]

[e]n=9 for Day 1 peak and Day 8 trough; n=8 for Day 15 trough and peak, and Day 28 trough.

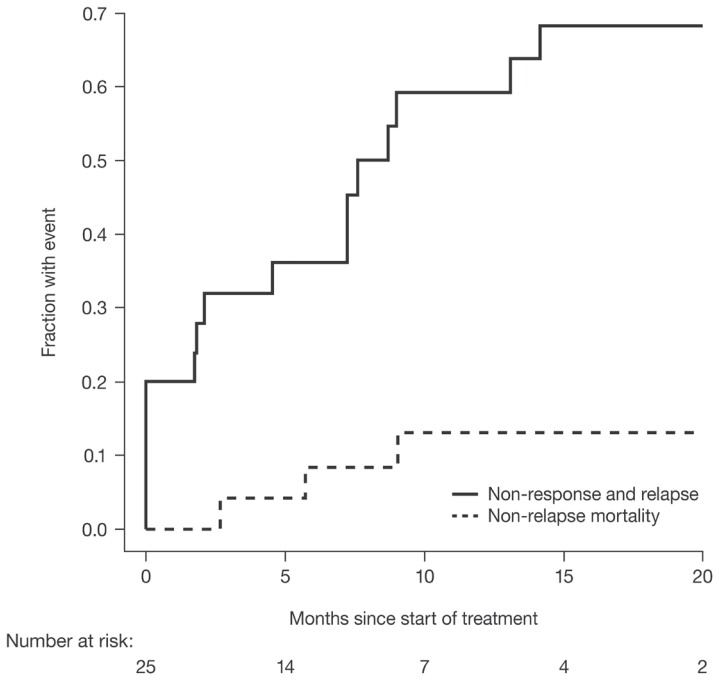
[f]n=5 for Day 1 peak, Day 8 trough, Day 15 trough and Day 28 trough; n=4 for Day 15 peak

ALL, acute lymphoblastic leukemia

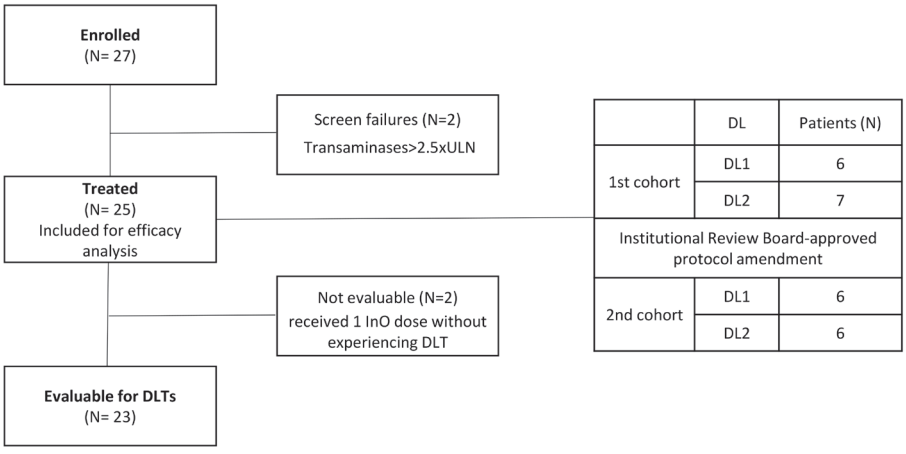
Supplementary Table 7. Response rate, MRD negativity rate, duration of response and survival by DL1 and DL2

	DL1+DL2		DL1		DL2		*p-value*
Response rate	20/25	*80%*	9/12	*75%*	11/13	*85%*	*0.6*
MRD negativity rate	16/19 (1 missing data)	*84%*	6/9	*66%*	10/10 (1 missing data)	*100%*	*0.1*
Median duration of response (months)	8.1		6.9		12.6		*0.1*
Median overall survival (months)	9.6		7.2		Median not reached		*0.06*

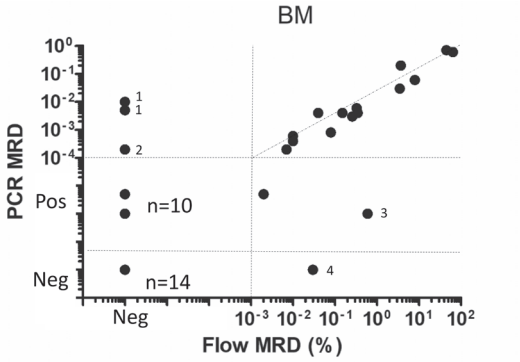
The Fisher's exact test showed no significant difference between DL1 and DL2 in response rate and MRD negativity rate; the Logrank test indicated no significant difference in median duration of response and overall survival between DL1 and DL2.



Supplementary Figure 1. Cumulative incidence of non-response or relapse and non-relapse mortality



Supplementary Figure 2. Consort diagram



Supplementary Figure 3. Comparison between Flow-cytometry and PCR-MRD data on bone marrow samples

Legend: Flow MRD data are expressed as percentage. The sensitivity maximally was 0,001%. PCR MRD data are expressed relative to the diagnostic sample (=1). Data outside the quantitative range but positive are shown as Pos (positive); the top symbols represent data with a quantitative range of 5x10-4, the bottom symbols represent data with a quantitative range of at least 1x10-4. The vertical dotted line represent the sensitivity limit of the flow MRD assay. The horizontal dotted lines represent the borders between quantitative data, positive but non-quantifiable data, and negative data for the PCR MRD assay.

Explanation for the discrepancies:

– Positive RQ-PCR cases with negative flow MRD:

1: This RQ-PCR result was likely falsely positive because it was only observed for the T-cell receptor gamma (TCRG) target, whereas the DH–JH target was negative. This discrepancy is possibly explained by the presence of a reactive T-cell clone, harboring the TCRG gene rearrangement). The prolonged CR in absence of additional therapy for this patient supports this hypothesis

2: Less than 50.000 leukocytes could be measured by flow cytometry and therefore the sensitivity was limited to 0,1%.

– Positive flow with negative RQ-PCR MRD:

3: RQ-PCR around level of 2x10-5. ALL cells are partially CD19-negative, no clear explanation for discordancy but likely false-positive by flow cytometry.

4: By flow cytometry <50 events suspected for BCP-ALL MRD, but hard to distinguish from normal cells. Therefore possibly false positive by flow cytometry.

Supplementary text

Expanded listing/definitions of secondary outcomes

Overall response rate (ORR), defined as complete remission (CR), CR with incomplete hematologic recovery (CRi), or CR with incomplete platelet recovery (CRp; response criteria followed standard procedures in leukemia, as designed by the National Comprehensive Cancer Network, with slight modifications in relation to bone marrow regeneration [CR = platelets >50,000/μL rather than >100,000/μL; and ANC >500/μL rather than >1000/μL]).

MRD status: bone marrow negative if <0.01% with multi-parameter flow cytometry according to EuroFlow protocols[23] or $<1x10^{-4}$ with real-time quantitative-PCR according to EuroMRD.[24]

Event-free survival (EFS), defined as time from start of treatment to first event including failure to achieve CR/CRp/CRi, relapse, death, and second malignancies.

Cumulative incidence of non-response or relapse, defined as the cumulative probability of non-response or relapse, with time calculated between start of study treatment and relapse and with non-responders included as an event on Day 0. Non-relapse death is considered a competing event.

Cumulative incidence *of non-relapse mortality*, defined as the cumulative probability of non-relapse mortality, with time calculated between start of study treatment and death due to other causes than relapsed or refractory leukemia or lymphoma, accounting for competing events.

Overall survival (OS), defined as time to death following start of study treatment.

Pharmacokinetic analysis

Blood samples were collected at multiple, protocol-defined time points. Concentrations of InO and unconjugated calicheamicin in serum were measured using validated high performance liquid chromatography with tandem mass spectrometry. InO peak and trough concentrations were summarized by visit, course, and dose level. PK data for a typical 70 kg adult receiving 1.8 mg/m^2/course administered as three divided doses were simulated using the final population PK model in ALL patients.[27]

Chapter 4

Inotuzumab Ozogamicin as Single Agent in Pediatric Patients with Relapsed and Refractory Acute Lymphoblastic Leukemia: Results from a Phase II Trial

Pennesi E*, Michels N*, Brivio E, van der Velden VHJ, Jiang Y, Thano A, Ammerlaan AJC, Boer JM, Beverloo HB, Sleight B, Chen Y, Vormoor-Bürger B, Rives S, Bielorai B, Rössig C, Petit A, Rizzari C, Engstler G, Starý J, Bautista Sirvent FJ, Chen-Santel C, Bruno B, Bertrand Y, Rialland F, Plat G, Reinhardt D, Vinti L, Von Stackelberg A, Locatelli F, Zwaan CM

* contributed equally

Leukemia. 2022 Jun;36(6):1516-1524.

Abstract

Inotuzumab Ozogamicin is a CD22-directed antibody conjugated to calicheamicin, approved in adults with relapsed or refractory (R/R) B cell acute lymphoblastic leukemia (BCP-ALL). Patients aged 1-18 years, with R/R CD22+ BCP-ALL were treated at the RP2D of 1.8 mg/m^2. Using a single-stage design, with an overall response rate (ORR) $\leq$ 30% defined as not promissing and ORR $>$ 55% as expected, 25 patients needed to be recruited to achieve 80% power at 0.05 significance level. Thirty-two patients were enrolled, 28 were treated, 27 were evaluable for response. The estimated ORR was 81.5% (95%CI: 61.9%-93.7%), and 81.8% (18/22) of the responding subjects were minimal residual disease (MRD) negative. The study met its primary endpoint. Median follow up of survivors was 16 months (IQR: 14.49-20.07). One year Event Free Survival was 36.7% (95% CI: 22.2%−60.4%), and Overall Survival was 55.1% (95% CI: 39.1%−77.7%). Eighteen patients received consolidation (with HSCT and/or CAR T-cells therapy). Sinusoidal obstructive syndrome (SOS) occurred in seven patients. MRD negativity seemed correlated to calicheamicin sensitivity in vitro, but not to CD22 surface expression, saturation, or internalization. InO was effective in this population. The most relevant risk was the occurrence of SOS, particularly when InO treatment was followed by HSCT.

1. Introduction

In pediatric patients with acute lymphoblastic leukemia (ALL), relapse still occurs in 10-15% of the subjects (1,2). Overall survival (OS) after relapse plateaus at 50-60%, while event free survival (EFS) after second and third relapse are approximately 25% and 15%, respectively (3–5). Novel therapies are changing the treatment of children with relapsed/refractory (R/R) B-cell precursor ALL (BCP-ALL), with the approval of blinatumomab, a CD19-antigen directed T-cell engager, and Tisagenlecleucel, a CD19 chimeric antigen receptors (CAR) T-cell therapy. However, cases in which these agents are no longer effective were reported (6,7). Inotuzumab Ozogamicin (InO) consists of an anti-CD22 monoclonal antibody linked to the cytotoxic agent calicheamicin (8,9). InO is approved for adults with CD22-positive R/R BCP-ALL, based on the INO-VATE ALL trial (10). CD22 is an antigen on the cell surface of most normal B-cells (60–90%) (11), and is expressed on the leukemic blasts in more than 90% of childhood BCP-ALL (12,13). Two retrospective studies of InO in pediatric BCP-ALL patients showed a remission rate of approximately 67% (14,15). The results of the phase I pediatric study from our group reported an Overall Response Rate (ORR) of 80% (95% CI: 59-93%) and, among the responders, 84% (95% CI: 60–97%) achieved minimal residual disease (MRD) negativity (16). Recently, the Children's Oncology Group (COG) reported a remission rate of 58.3% (90% CI: 46.5-69.3%), from 48 patients enrolled in a phase II trial using 1.8 mg/m^2 (17). A correlation between clinical response and CD22 alternative splicing and expression has been hypothesized. A study in Acute Myeloid Leukemia (AML) patients treated with gemtuzumab ozogamicin, a CD33 conjugate to calicheamicin, showed a link between alternative splicing of the CD33 antigen and clinical response (18), while an increased expression of a CD19 isoform with intraexonic splicing of exon 2 was found associated with treatment failure on blinatumomab (19). Moreover, a trial in adults treated with the combination of chemotherapy and InO, with or without blinatumomab, identified baseline CD22 expression level <70% as predictor of poor outcome (20). In this paper, we report the clinical results from the phase II InO single-agent ITCC-059 clinical trial and elaborate on the pharmacodynamic (PD) investigations on potential causes of intrinsic resistance to InO.

2. Materials/Subjects And Methods

ITCC-059 (EUDRACT nr 2016-000227-71) is a phase I-II, multicenter, international, single-arm, open-label study conducted in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, International Council

for Harmonization Guidelines for Good Clinical Practice, and the Declaration of Helsinki. Patients were treated under protocol version 2 and 3 following an amendment unrelated to this cohort. Informed consent was obtained from all patients or their parents (as applicable) before enrolment. The study was sponsored by the Erasmus MC and funded by Pfizer inc. in the context of a Pediatric Investigational Plan.

2.1 Patients and treatment

Criteria for enrollment (supplementary table 1) included age ≥1 to <18 years, diagnosis of CD22-positive R/R BCP-ALL with an M2 or M3 bone marrow (BM) status and refractory disease or ≥2nd relapse, or any relapse post-hematopoietic stem cell transplantation (HSCT). Exclusion criteria included isolated extramedullary disease, active infections, and any history of prior or ongoing hepatic sinusoidal obstruction syndrome (SOS). Subjects started InO at the recommended phase II dose (RP2D) of 1.8 mg/m^2/cycle fractionated in three weekly administrations, or 1.5 mg/m^2/cycle once remission was achieved. Intrathecal prophylaxis was administered depending on the central nervous system (CNS) status. A maximum of six cycles were allowed, except for patients proceeding to HSCT for which the recommended number of cycles was two, or three if still MRD positive. Patients attaining an M1 BM with absolute neutrophil count (ANC) ≥ 0.5 x 10^9/L and platelets count ≥ 30 x 10^9/L, and those with M3 BM at study entry attaining an M2 BM irrespective of hematological criteria, could proceed to the subsequent cycles.

2. 3 Endpoints and statistical design

The primary objective was to establish the preliminary activity of InO. Secondary objectives included safety, other measures of antileukemic activity, PD analysis and pharmacokinetic (PK) parameters. PD analysis was performed on patients from phase I and II for whom material was available. The primary endpoint of the study was the overall response rate (ORR), defined as the combined Complete Remission (CR), CR with insufficient platelet recovery (CRp) and without recovery of counts (CRi) rate (supplementary table 2); and measured as best response during the entire treatment. Secondary endpoints included ORR after cycle one, EFS, OS, duration of response (DOR), MRD negativity rate and safety (supplementary table 3). MRD negativity was defined as either a PCR result below 10^{-4}, or a flow cytometry result below 0.01% when the PCR was negative, but the quantitative range (QR) was above 10^{-4} (supplementary text 2) (21,22). PD parameters included the relationship between clinical response (MRD-negativity rate)

and CD22 expression, saturation kinetics, CD22 clonal evolution, alternative splicing of the CD22 transcript, calicheamicin sensitivity, and the percentage of patients who exhibited anti-drug antibodies (ADA). The statistical design consisted of a single-stage design, based on exact binomial distribution. An ORR of $\leq$ 30% was considered not promising (null hypothesis, H_0) and an ORR of > 55% was expected (H_1); 25 patients evaluable for response provided 80% power at a significance level of 0.05 (one-sided).

2.4 Cd22 expression levels

Flow cytometry was used to evaluate CD22 expression levels, by measuring both the mean fluorescent intensity (MFI) of leukemic blasts and the percentage of CD22-positivity at diagnosis on peripheral blood (PB) and BM samples at the Erasmus MC central Immunology laboratory in Rotterdam. Leukemic blast cells were gated based on expression of CD45, CD10, CD20, CD19, CD38, CD81, and CD34. The CD22 antibody RFB4® (Thermo Fisher, Waltham, Massachusetts) was used for flow cytometry. In addition, CD22 saturation (Eq.1 supplementary) and internalization (Eq.2 supplementary) were measured on PB samples taken at day one and day eight of cycle one. The methods for the analysis of CD22 saturation and internalization of InO have been described previously (9).

2.5 In-vitro drug response

In vitro drug response to calicheamicin (MedChemExpress, Monmouth Junction, New Jersey) was assessed with MTT assays in U-bottom 96-well plates. Patient samples from BM or PB were enriched to at least 80% leukemic blasts, based on morphology with a May-Grunwald-Giemsa staining, using a negative magnetic bead enrichment. The concentrations of calicheamicin on the MTT assay plates were tested in duplicates and ranged from 0.4 ng/ml to 400 ng/ml. MTT assays were performed over four days at 1.6 million cells/ml density using medium containing RPMI 1640 Dutch Modified with 20% Fetal calf serum, Penicilline, Streptavidine, and Fungizone. After four days >70% leukemic blast had to be present in the no-drug control wells to construct dose response curves. The absorbance was read on a spectrophotometer at wavelengths of 562 nm and 720 nm and analyzed with Softmax Pro software. Optical density (OD) values after correction for blank wells of >50 were required. Metabolic activity was calculated at each drug concentration relative to control wells after correction for the background OD values of the blank wells. IC50 values represent the concentration of the drug which inhibits 50% of the leukemic cells (23).

2.6 Rna sequencing, cd22 splice variants

The GENCODE reference annotations version 29 for GRCh38 and the GRCh38.p12 compliant Ensembl human genome reference were provided by the CTAT resource bundle (release: 27th of March 2019). Paired-end RNA-sequencing reads were aligned to this human genome reference and, subsequently, read counts per gene were calculated using STAR 2.6.0c. Split-reads were used to evaluate alternative splicing of the CD22 transcript. Only splice variants with at least 10 split reads were considered. Differential gene expression was assessed using TMM normalized counts and the generalized linear model from the EdgeR package in R statistics. Anti- and pro-apoptotic genes were selected based on the hallmark geneset of the GeneSet Enrichement Analysis software.

2.7 Anti-drug antibody (ada) analysis

Blood samples were collected during the screening, prior to each course of treatment, and at the end of treatment study. Samples were tested for ADA using a validated, electro chemiluminescent bridging assay

2.8 Statistical analysis

The response analysis set included all enrolled patients who received at least one dose of InO and completed at least one baseline and one post-baseline disease assessment. The full analysis set consisted of all enrolled patients who received at least one dose of study therapy and was used for the safety analysis. Detailed definitions of outcome measures are provided in supplementary table 4. EFS and OS were estimated using the Kaplan–Meier method. Events defined as non-response (not achieving CR, CRi or CRp, considered as event at day 0), relapse, death or second malignancy. For the analysis of PD parameters, patients were categorized into three groups: CR and MRD negative; CR and MRD positive; and no CR. For RNA sequencing analysis and calicheamicin sensitivity, subject not in CR and subject with CR MRD positive were grouped together, due to limited sample size. The Kruskal-Wallis test was used to test the association between the three response groups and the following PD parameters: CD22 surface expression (as MFI and percentage positive cells), CD22 saturation and InO internalization. The Wilcoxon rank-sum test was used to test calicheamicin sensitivity. As a post-hoc analysis, Fisher's exact test and Mann-Whitney U test were used to test the association between clinical characteristics (eg. sex and age) and MRD response and between potential risk factors (eg. number of InO

cycles received, time to HSCT) and SOS occurrence in post-InO transplanted patients. For all hypothesis tested, p values ≤ 0.05 were considered statistically significant.

3. Results

Results are based on a data cut-off date of 12 October 2021.

3.1 Patients and treatment

Overall, 32 patients consented and were screened for inclusion from 03 June 2019 to 24 April 2020 at 16 sites of the ITCC consortium. In total, 30 patients were enrolled (two screening failures, both with inadequate liver function), 28 started treatment (two patients did not start treatment due to rapidly progressive disease), and 27 were evaluable (disease response not assessed in one patient, who discontinued due to SOS). Patient characteristics are reported in Table 1. A total of 147 doses of InO were given to 28 patients (median six doses/patient, range: 1-12). Thirteen (46.4%) subjects received one cycle, nine (32.1%) received two cycles, five (17.9) received three cycles and one (3.6%) received four cycles.

Table 1. Patient Characteristics

Patients' Characteristics	**Total (n=28)**
Male	19 (67.9%)
Female	9 (32.1%)
Median age in years at enrollment (IQR)	7.5 (4 - 13)
Age at enrollment breakdown	
>1 & ≤ 2 years	2 (7.1%)
>2 & ≤ 6 years	10 (35.7%)
>6 years	16 (57.1%)
Extramedullary Disease (screening)*	
CNS1	21 (75%)
CNS2	4 (14.3%)
CNS3	2 (7.1%)
Testicular involvment	0
Lymph nodes enlarged	1 (3.6%)
Other locations (excluding spleen and liver)	0
Diagnosis	
first relapsed BCP-ALL post allogeneic HSCT	6 (21.4%)
second or greater relapsed BCP-ALL	16 (57.1%)

Table 1. Continued

Patients' Characteristics	Total (n=28)
refractory BCP-ALL	6 (21.4%)
first HSCT prior to study treatment	14 (50.0%)
second HSCT prior to study treatment	1 (3.6%)
WBC (10^9/L) at screening, median (IQR)	3.1 (2.3 - 9)
CD22 PB Blast (%), median (IQR)	96.7 (86.7 - 99.9)
MFI - CD22+ expression, median (IQR)	2296.9 (1025.5-3709.2)
Prior antibody therapy	
Blinatumomab	7 (25%)
Karyotype abnormalities	
Normal	4 (14.3%)
Not Assessed/Available	9 (32.1%)
Hypodiploid (40-45 chromosomes)	2 (7.1%)
Low Hypodiploid (<40 chromosomes)	2 (7.1%)
Hyperdiploid (47-50 chromosomes)	2 (7.1%)
High hyperdiploid (51-65 chromosomes)	2 (7.1%)
Pseudodiploid	7 (25.0%)
t(9;22)(q34;q11.2) and variants	0
t(4;11)(q21;q23)	0
t(12;21)p13;q22)	1 (3.6%)
t(11;v)(q23;v)	1 (3.6%)
t(1;19)(q23;p13)	1 (3.6%)
dic(9,20)(p11;q11)	1 (3.6%)
Down syndrome	0

*in one patient the sample was not evaluable due to red blood cells contamination

MFI: mean fluorescence intensity; PB: peripheral blood; IQR: inter quartile range; BCP-ALL: B cell precursor acute lymphoblastic leukemia; HSTC: hematopoietic stem cell transplantation; SD: standard deviation; InO: Inotuzumab Ozogamicin. Patients can have both hypodiploidy and a translocation.

3.2 Efficacy

Twenty-two patients achieved response (ORR 81.5%; 95%CI: 61.9%-93.7%), in all cases after the first cycle; 14 were in CR, one in CRp and seven in CRi. MRD negativity, as best response, was achieved by 18 out of 22 (81.8%) responding subjects; after the first cycle by 13 (59.1%) patients, and after the second cycle by the other five. All patients were CNS negative at the end of cycle 1 and maintained the response at end of treatment. A total of 18 patients (66.7%) proceeded to consolidation therapy, 14 with HSCT (one after subsequent therapy with blinatumomab due to

loss of response), two with CAR T-cell therapy (supplementary figure 1), and two with CAR T-cell therapy followed by HSCT. Three patients received blinatumomab as bridging therapy before HSCT; one patient received chemotherapy; two received CAR-T as mentioned above; and the others did not receive additional treatment between the last InO administration and transplant. Median time between last InO dose and HSCT was 45 days (IQR:26.5-70.5). Median time between last InO dose and CAR-T therapy was 53.5 days (IQR:46.5-260.75). In two cases lymphocyte apheresis for CAR T-cell therapy was performed before initiating InO and in the other two cases after. Of the other four responding subjects, one proceeded to blinatumomab (in CCR after seven months), one received maintenance therapy with 6-mercaptopurine and intrathecaltriple therapy (relapsed after seven months), one relapsed within one month after end of treatment, and one patient died while in CR due to neurological deterioration attributable to previous therapy and CNS leukemic involvement. The median follow for survival was 16 months (IQR: 14.49-20.07). At six months, EFS probability was 55.6% (95% CI: 39.6-77.8) and OS was 66.7% (95% CI: 51.1-87.0). At 12 months, EFS probability was 36.7% (95% CI: 22.2-60.4) and OS probability was 55.1% (95% CI: 39.1-77.7) (Figure 1). Median DOR was 7.74 months (95% CI: 5.65-not reached). The cumulative incidence of non-response or relapse was 29.63% (95% CI 13.77-47.42) at six months and 40.74% (95% CI 22.03-58.69) at 12 months. Combining phase I (n=25) and II (n=27) results, 52 patients received InO, of which 40 were treated at the RP2D (13 in phase I and 27 in phase II) (16). Considering the combined response data from patients treated at the RP2D, 33 achieved response (ORR 82.5%; 95%CI: 67.2% - 92.7%), and 27 (81.8%) of the responders were MRD negative. EFS at 12 months was 41.3% (95%CI: 28.3%-60.1%); and OS at 12 months was 56.3% (95%CI: 42.6%-74.3%) (supplementary figure 2). In patients treated at RP2D (N= 40), age, gender, previous treatments, WBC, and CD22 MFI were not found to impact the ORR, MRD-negative response (supplementary table 5) or EFS (table 2). In total, 17 patients relapsed (11 in phase I and 6 in phase II) and for 10 of them CD22 expression data were available. CD22 expression turned negative in three cases, and partially negative in two.

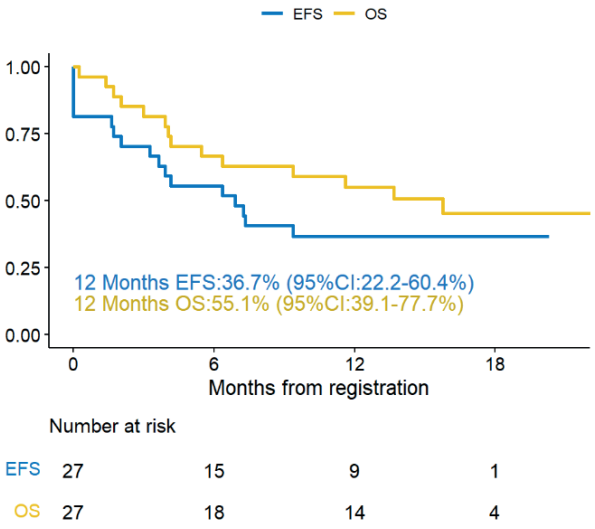


Figure 1. Event-free survival and overall survival of patients treated in phase II
Blue line: Event Free Survival (EFS); Yellow Line: Overall Survival (OS). CI: Confidence Interval

Table 2: Univariable Cox models for EFS of all patients treated at RP2D (N= 40 patients, phase I and II combined, 25 events)

Variable	Events	N	HR	Lower 95%	Upper 95%	Level P	P value
Age at enrolment (per year)	25	40	1.191	0.542	2.617	0.6643	**0.66**
Sex							**0.47**
male	18	29	1				
female	7	11	1.377	0.574	3.305	0.4735	
Diagnosis							**0.17**
first relapse post allogeneic HSCT	6	10	1				
second or greater relapsed	13	23	1.052	0.399	2.775	0.9179	
refractory	6	7	2.452	0.783	7.681	0.1236	
Prior HSCT							**0.19**
no	15	20	1				
yes	10	20	0.589	0.264	1.314	0.1964	
Prior antibody therapy (blinatumomab)							**0.22**
no	18	31	1				
yes	7	9	1.713	0.714	4.108	0.2279	
PB WBC at screening (109/L)							**0.62**
	25	40	0.821	0.374	1.806	0.6246	
MFI - CD22+ expression							**0.35**
	24	38	0.681	0.304	1.526	0.3505	

HR: Hazard Ration; HSCT: Hematopoietic Stem Cell Transplant; PB WBC: Peripheral Blood White Blood Cell Count; MFI: Mean Fluorescence Intensity,

3.3 Safety

All patients (n=28) had at least one adverse event (AE), 20 (71.4%) at least one grade 3-4 AE. The most common AE was fever (n=16, 57.1%). Five (17.6%) patients had infection of grade ≥ 3, and 6 (21.4%) had febrile neutropenia. All patients had at least one grade 3-4 hematologic laboratory test abnormality (neutropenia being the most common, n=26, 92.9%). Only four patients stll had thrombocytopenia grade 3/4 at C1D22, of which one after day 42. Details are provided in supplementary Table 7-9. A total of 26 serious adverse events were observed in 17 (60.71%), patients (supplementary table 10). Seven (25%, n=28) cases of SOS were reported; one grade 2, four grade 3 and two grade 4 (the latter six classified as "severe" according to the EBMT criteria)(24). Six cases occurred after HSCT post-treatment with InO, four resolved after treatment with defibrotide, and two might have contributed to death due to multi organ failure and infection. Another case of SOS occurred after one dose of InO in a patient treated due to relapse three months after HSCT. SOS resolved completely following administration of defibrotide. Including the phase I part of the study, nine SOS cases occurred in 52 treated patients (17.3%), of which six in the 23 transplanted patients (26.1%) (supplementary table 6). In a post-hoc analysis, when considering patients who developed SOS subsequently to transplant post-InO and those who did not, the median time interval between last InO dose and HSCT was shorter in patients developing SOS (24.5 (n=6) vs 54.5 days (n=17), p=0.01). The number of InO cycles received, previous HSCT, defibrotide prophylaxis, conditioning regimen with total body irradiation and dose level, were not statistically significant (supplementary table 6). No cases of toxic death considered related to InO were observed during study treatment. One patient died of encephalopathy considered attributable to prolonged intrathecal chemotherapy due to CNS leukemic involvement. Five additional non-relapse deaths occurred after HSCT due to multiple complications. The cumulative incidence of relapse was 29.63% (95% CI 13.77-47.42) at six months, and 40.74% (95% CI 22.03-58.69) at 12 months. The cumulative incidence of non-relapse death was 14.81% (95% CI 4.47-30.94) at six months, and 22.59% (95% CI 8.8-40.23) at 12 months, including post-HSCT follow-up (supplementary figure 3).

3.4 Pharmacodynamics

In vitro sensitivity to calicheamicin was available for 11 patients, of which 10 had MRD data available. In the latter group, MTT assays were performed, nine from BM

and one from PB. IC_{50} values for calicheamicin ranged from 0.035 to 27.27 ng/ml. The median was twelve times higher in the five MRD-positive patients compared to MRD-negative patients (3.12 ng/ml vs 0.26 ng/ml, p=0.032, n=10; Figure 2). Furthermore, patients with IC_{50} values above the median seemed to have a poorer EFS (supplementary figure 4), despite not statistically significant (n=5, p=0.19). Nevertheless, four of the five poor responders were treated at 1.4 mg/m^2/cycle in phase I (p=0.048).

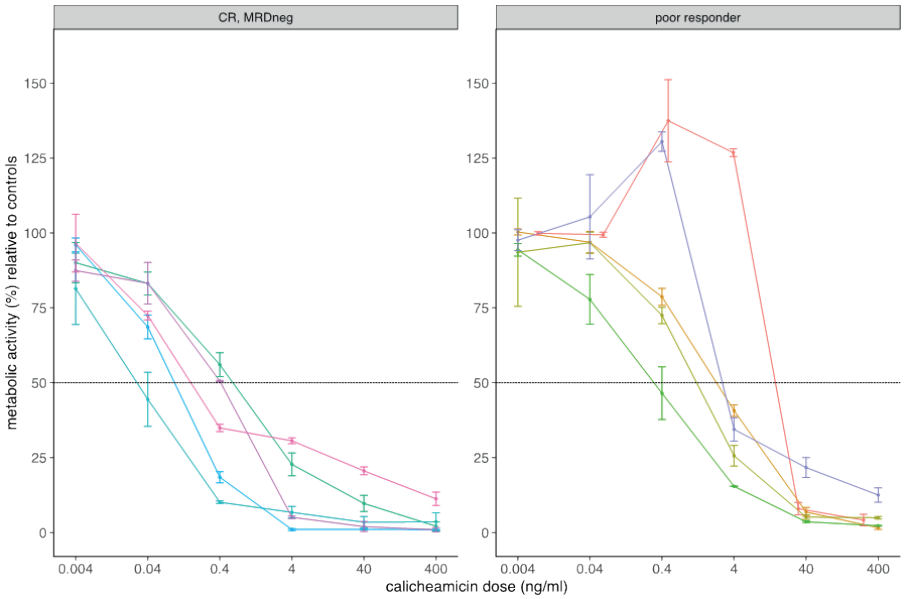


Figure 2. Dose-response curves for calicheamicin based on MTT assays.

Each color represents a different patient. The intersection with the black line at 50% represents the IC50 values. IC50: concentration of drug required for 50% inhibition; MRD neg: minimal residual disease $<10^{-4}$; CR: complete response; poor responders: no CR and/or MRD $\geq 10^{-4}$. The median IC50 value for all patients was 0.75 ng/ml (range 0.035 – 27.27; n=10), median 0.26 ng/ml (range 0.035 – 1.05; n=5) in the good responders (left panel) and median 3.12 ng/ml (range 0.34 – 27.27; n=5) in the poor responders (right panel) (p=0.032). From the literature, the median calicheamicin sensitivity in AML cells was 4.8 ng/ml, ranging between 0.1-1000 ng/ml (de Vries JF, et al.; 2012).

The association of CD22 expression on leukemic blasts in BM samples obtained at baseline with response to InO was not statistically significant, neither the

mean fluorescence index (MFI, range= 479-9619, p=0.37, n=49, Figure 3A), nor the percentage of CD22-positive cells (range=53-100%, p=0.47, n=49, Figure 3B). Additionally, neither the level of saturation of CD22 antigens on PB leukemic blasts after the InO dose (samples taken prior and after infusion at day one) (range=23-100, p=0.52, n=32, Figure 3C), nor the level of internalization of InO after the first InO dose (range=0-90, p=0.55, n=32, Figure 3D) were associated to response.

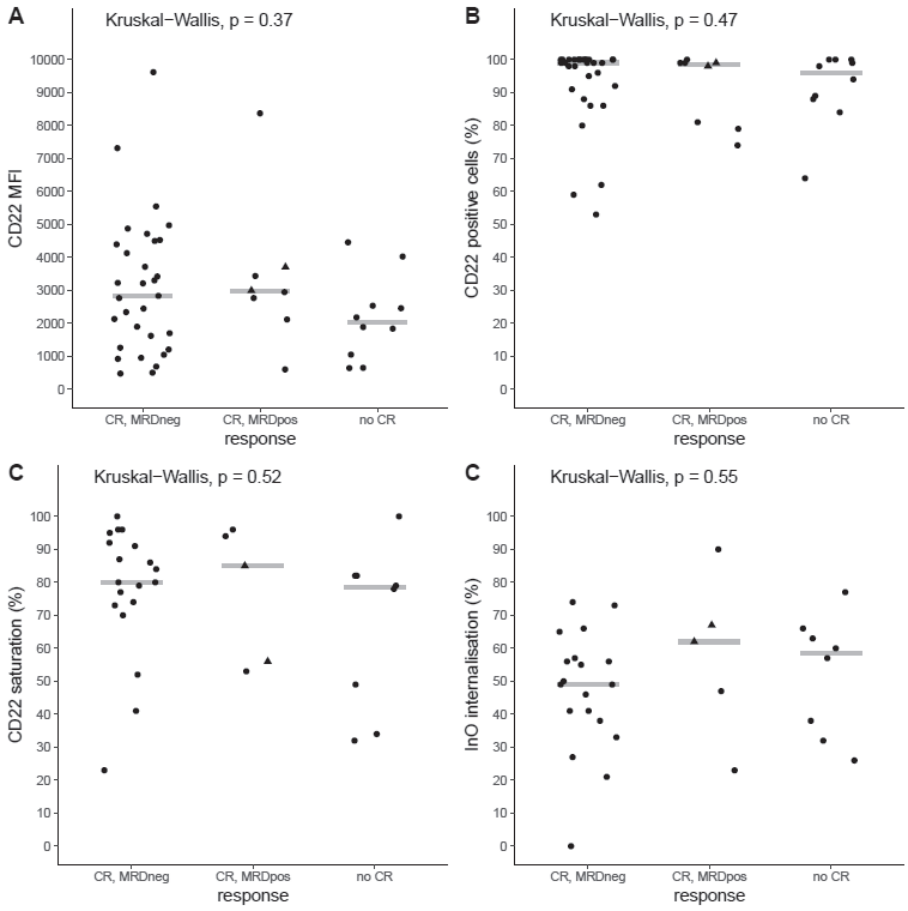


Figure 3. CD22 expression on BM blasts at baseline, saturation and InO internalization on leukemic PB blasts post infusion on day one.

Presented as Mean Fluorescence Intensity (MFI) (A), percentage CD22-positive cells (B), saturation (C) and internalization (D). Grey horizontal lines represent the

median value per group. In all four parameters, there were no statistically significant differences between the response groups as defined in the statistical methods for the PD analysis. Triangles represent patients with PCR-MRD quantitative range $> 10^{-4}$. Alternative splicing was assessed by the number of split reads that included or excluded particular exons. The most prevalent alternative splicing variants of CD22, observed in our samples, were further analyzed. Multiple splicing isoforms involving the skipping of at least exon 2, which contains the start-codon for CD22 translation, were observed in all patients (supplementary figure 5). The splice variant of the CD22 transcript with exclusion of exons two to six (Δex2to6-CD22), encoding part of the extracellular domain, was seen most frequent with variable expression levels among patients . No correlation between alternative splicing of exon 2 and response was seen. Additionally, we did not find any association between the skipping or inclusion of exon 2 in the CD22 transcript and the expression of CD22 antigen on leukemic blasts, the saturation levels of CD22 on leukemic blasts with InO, or the internalization levels of InO (supplementary figure 6). Skipping of exon 5 and 6 was seen in all patients (supplementary figure 7), but did not influence the expression of CD22 antigen on leukemic blasts, the saturation levels of CD22 on leukemic blasts with InO, or the internalization levels of InO (supplementary figure 8). Skipping of exon 12, suggested to negatively affect internalization of InO (25), was found in all patients (n=9, supplementary figure 9), but did not seem to affect internalization levels (supplementary figure 10). RNA sequencing of leukemic cells was performed on nine patients with available material. Since calicheamicin acts by causing DNA double-strand breaks, leading to apoptosis of the cells, we looked at the expression of various anti- and pro-apoptotic genes, including *BCL2* gene family members (9). No significantly different expression of apoptotic genes was seen in the leukemic cells of patients MRD negativity and those MRD positive (supplementary figure 11). Among the 52 patients treated in phase I and II, one (1.9%) patient had positive ADA (titer $\geq$ 2.30) against InO at baseline. The patient was treated at DL1 in Phase I and did not respond to InO. The presence of positive ADA at baseline was likely due to pre-existing host antibodies that were cross-reactive with InO and seemed not to impact on the PK. No treatment-boosted ADA responses were identified.

4. Discussion

This phase II study provides further evidence for the activity of InO in R/R BCP-ALL pediatric patients. No clinical characteristic were found related to ORR or EFS (supplementary 5). InO was generally well tolerated, with a low incidence of infections during treatment (17.8%). SOS remains the most serious AE (25%, n=7), although mostly occurring after subsequent HSCT and only occasionally while on treatment. Most SOS cases (5/7) resolved completely and no cases of toxic death considered related to InO or deaths in CR due to infections were observed during study treatment. Combining data from phase I (all dose levels) and II cohorts, SOS occurred in 26.1% of the patients transplanted post-InO, which is significantly lower than reported by Bhojwani et al. (52%, 11/21) but in line with data from O'Brien et al. (28.6%, 6/21), all treating patients at 1.8 mg/m^2 (14,17). Median time since the last InO dose appeared to be statistically significantly shorter in patients who developed SOS post HSCT. A possible explanation might be the long half-life of InO (12 days), and the inverse relationship between tumor load and the time dependent component of its clearance, generally longer after few weeks of treatment (26). Therefore, InO might still be circulating during the first month after treatment, particularly in patient achieving CR early on treatment. The use of prophylactic defibrotide was left at investigators' discretion, therefore not uniformly performed. This makes difficult to assess its impact as a protective factor for SOS in this small cohort. Taken together, the relationship between risk factors and SOS occurrence should be investigated in larger series. As suggested in other studies, the sensitivity of ALL cells to calicheamicin might contribute to the achievement of MRD negativity (9). When considering all patients from phase I and II, including those treated at 1.4 mg/m^2, MRD negativity was found to correlate to calicheamicin sensitivity in vitro but not to CD22 surface expression, saturation, or internalization. Although CD22 expression is needed for binding of InO, high levels of CD22 expression on leukemic blasts, saturation of CD22 with InO and internalization of InO do not appear to be crucial for clinical response to InO. Our findings are in line with previous *in vitro* studies from our group on BCP-ALL cells which showed that, although CD22 expression was essential for InO binding, efficacy was not dependent on CD22 expression levels, while a clear correlation with calicheamicin sensitivity was noticed (9). In contrast, COG reported lower baseline CD22 density (measured as antibody bound per cell), and a reduction of CD22 percentage over time in the poor responders (17). Nevertheless, they did not find CD22 percentage at baseline as significant, but only four subjects had a CD22 expression $<$ 90%. Instead, CD33 expression on leukemic

blasts does affect clinical response of AML patients to gemtuzumab ozogamicin, (18,27,28). This may be due to AML cells being less sensitive to calicheamicin, higher levels of CD33 on leukemic blasts, higher levels of CD33 saturation, and a continuous loop of internalization and renewed expression of CD33 antigens might be required for a sufficient accumulation of calicheamicin inside AML cells; whereas in ALL cells lower levels of accumulated calicheamicin might be sufficient to cause apoptosis (9). In adults treated with chemotherapy and InO, baseline CD22 expression <70% was independently associated with worse survival, while ORR and MRD negativity rate could not be identified as significantly different in the two groups (20). Moreover, a significant correlation between higher median baseline CD22 levels and achievement of MRD-negative CR was found in patients treated with anti-CD22 CAR T-cells (29). In our cohort, no correlation between CD22 expression and ORR and MRD was found, but only a limited number of patients (n=4) had a CD22 expression <70%. CD22 exon 2 seems to be crucial for both initiating RNA translation into a protein product and for the binding of CD22-directed antibody (30). One patient with only the Δex2 CD22 splice isoform has been reported resistant to InO (30). In our study, alternative splicing of CD22 was observed in all patients, especially the Δex2-6 variant, but did not correlate with response, probably because all patients had at least some normal CD22 expression. Skipping of exon 5-6 (binding region of RFB4 antibody) was observed only in a limited percentage which might explain why it did not correlated to CD22 MFI. Similarly, skipping of CD22 exon 12, previously reported as potentially reducing InO internalization (25), occurred in all patients samples in our cohort (n=9) at various extent, but did not correlate to lower internalization levels. These findings underscore that high levels of full-length CD22 might not be necessary to respond to InO. Nevertheless it is worth noting that CD22 surface expression was tested before study inclusion by using anti-CD22 RFB4 antibody and only CD22-positive cases were included. Our findings confirmed evidence of CD22 negative/dim relapses after treatment with anti-CD22 CAR T-cells, suggesting CD22 downmodulation as possible mechanism of acquired resistance (29). A RNA sequening analysis pre- and post-relapse, which could better highlight the mechanism of CD22 downmodulation, was not performed. Previous studies suggested gene expression as possible cause of resinstance to calicheamicin, but we did not find significant differences between the response groups in the expression of known genes of the apoptotic pathway such as BCL2, albeit in a small sample size (31,32). InO is currently tested in front-line treatment by the COG in a phase III randomized trial for high-risk CD22-positive BCP-ALL (NCT03959085), and in the ALLTogether1 protocol, for patients stratified to the

intermediate-high risk group (NCT03911128). The ITCC-059 study is ongoing, testing InO in combination with chemotherapy in R/R pediatric ALL and as single agent in very high risk first relapse ALL.

References

1. Henze G, Stackelberg A V., Eckert C. ALL-REZ BFM - The consecutive trials for children with relapsed acute lymphoblastic leukemia. Klin Padiatr. 2013;225(SUPPL1).
2. Möricke A, Zimmermann M, Reiter A, Henze G, Schrauder A, Gadner H, et al. Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia. 2010;24(2):265–84.
3. Oskarsson T, Söderhäll S, Arvidson J, Forestier E, Montgomery S, Bottai M, et al. Relapsed childhood acute lymphoblastic leukemia in the Nordic countries: prognostic factors, treatment and outcome. Haematologica. 2016 Jan 1;101(1):68–76.
4. Parker C, Waters R, Leighton C, Hancock J, Sutton R, Moorman AV, et al. Effect of mitoxantrone on outcome of children with first relapse of acute lymphoblastic leukaemia (ALL R3): an open-label randomised trial. Lancet (London, England). 2010;376(9757):2009–17.
5. Ko RH, Ji L, Barnette P, Bostrom B, Hutchinson R, Raetz E, Seibel NL, et al. Outcome of patients treated for relapsed or refractory acute lymphoblastic leukemia: a Therapeutic Advances in Childhood Leukemia Consortium study. J Clin Oncol. 2010 Feb 1;28(4):648–54.
6. Mejstríková E, Hrusak O, Borowitz MJ, Whitlock JA, Brethon B, Trippett TM, et al. CD19-negative relapse of pediatric B-cell precursor acute lymphoblastic leukemia following blinatumomab treatment. Blood Cancer J. 2017 Dec 1;7(12).
7. Dourthe ME, Rabian F, Yakouben K, Chevillon F, Cabannes-Hamy A, Méchinaud F, et al. Determinants of CD19-positive vs CD19-negative relapse after tisagenlecleucel for B-cell acute lymphoblastic leukemia. Leukemia. 2021 Dec 1;35(12).
8. DiJoseph JF, Armellino DC, Boghaert ER, Khandke K, Dougher MM, Sridharan L, et al. Antibody-targeted chemotherapy with CMC-544: A CD22-targeted immunoconjugate of calicheamicin for the treatment of B-lymphoid malignancies. Blood. 2004 Mar 1;103(5):1807–14.
9. De Vries JF, Zwaan CM, De Bie M, Voerman JSA, Den Boer ML, Van Dongen JJM, et al. The novel calicheamicin-conjugated CD22 antibody inotuzumab ozogamicin (CMC-544) effectively kills primary pediatric acute lymphoblastic leukemia cells. Leukemia. 2012;26(2):255–64.
10. Kantarjian HM, DeAngelo DJ, Stelljes M, Liedtke M, Stock W, Gökbuget N, et al. Inotuzumab ozogamicin versus standard of care in relapsed or refractory acute lymphoblastic leukemia: Final report and long-term survival follow-up from the randomized, phase 3 INO-VATE study. Cancer. 2019 Jul 15;125(14):2474–87.
11. Lanza F, Maffini E, Rondoni M, Massari E, Faini AC, Malavasi F. CD22 expression in b-cell acute lymphoblastic leukemia: Biological significance and implications for inotuzumab therapy in adults. Vol. 12, Cancers. MDPI AG; 2020.
12. Iwamoto S, Deguchi T, Ohta H, Kiyokawa N, Tsurusawa M, Yamada T, et al. Flow cytometric analysis of de novo acute lymphoblastic leukemia in childhood: Report from the Japanese Pediatric Leukemia/Lymphoma Study Group. Int J Hematol. 2011 Aug;94(2):185–92.
13. Shah NN, Stevenson MS, Yuan CM, Richards K, Delbrook C, Kreitman RJ, et al. Characterization of CD22 expression in acute lymphoblastic leukemia. Pediatr Blood Cancer. 2015 Jun 1;62(6):964–9.

14. Bhojwani D, Sposto R, Shah NN, Rodriguez V, Yuan C, Stetler-Stevenson M, et al. Inotuzumab ozogamicin in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. Leukemia. 2019 Apr 1;33(4):884–92.

15. Calvo C, Cabannes-Hamy A, Adjaoud D, Bruno B, Blanc L, Boissel N, et al. Inotuzumab ozogamicin compassionate use for French paediatric patients with relapsed or refractory CD22-positive B-cell acute lymphoblastic leukaemia. Vol. 190, British Journal of Haematology. Blackwell Publishing Ltd; 2020. p. e53–6.

16. Brivio E, Locatelli F, Lopez-Yurda M, Malone A, Diaz de Heredia C, Bielorai B, et al. A Phase I study of inotuzumab ozogamicin in pediatric relapsed/refractory acute lymphoblastic leukemia (ITCC-059 study). Blood. 2020 Oct 16.

17. O'Brien MM, Ji L, Shah NN, Rheingold SR, Bhojwani D, Yuan CM, et al. Phase II Trial of Inotuzumab Ozogamicin in Children and Adolescents With Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia: Children's Oncology Group Protocol AALL1621. J Clin Oncol. 2022 Jan 10.

18. Lamba JK, Chauhan L, Shin M, Loken MR, Pollard JA, Wang YC, et al. CD33 splicing polymorphism determines gemtuzumab ozogamicin response in de novo acute myeloid leukemia: Report from randomized phase III children's oncology group trial AAML0531. J Clin Oncol. 2017 Aug 10;35(23):674–2682.

19. Zhao Y, Aldoss I, Qu C, Crawford JC, Gu Z, Allen EK, et al. Tumor-intrinsic and -extrinsic determinants of response to blinatumomab in adults with B-ALL. Blood. 2021 Jan 28;137(4):471–84.

20. Rafei H. CD22 Expression Level As a Predictor of Survival in Patients (Pts) with Relapsed/Refractory (R-R) Acute Lymphoblastic Leukemia (ALL) Treated with Inotuzumab Ozogamicin (INO) in Combination with Low-Intensity Chemotherapy (mini-hyper-CVD) with or without Blinatumomab: Results from a Phase 2 Study. ASH; 2020.

21. Theunissen P, Mejstrikova E, Sedek L, van der Sluijs-Gelling AJ, Gaipa G, Bartels M, et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood. 2017 Jan 19;129(3):347-357. doi: 10.1182/blood-2016-07-726307.

22. van der Velden VH, Panzer-Grümayer ER, Cazzaniga G, Flohr T, Sutton R, Schrauder A, et al. Optimization of PCR-based minimal residual disease diagnostics for childhood acute lymphoblastic leukemia in a multi-center setting. Leukemia. 2007;21(4):706–13.

23. Zwaan CM, Kaspers GJL, Pieters R, Ramakers-Van Woerden NL, Den Boer ML, Wunsche R, et al. Cellular drug resistance profiles in childhood acute myeloid leukemia: differences between FAB types and comparison with acute lymphoblastic leukemia. Blood. 2000 Oct 15;96(8):2879–86.

24. Uckun FM, Goodman P, Ma H, Dibirdik I, Qazi S. CD22 EXON 12 deletion as a pathogenic mechanism of human B-precursor leukemia. Proc Natl Acad Sci U S A. 2010 Sep 28;107(39):16852–7.

25. Garrett M, Ruiz-Garcia A, Parivar K, Hee B, and Boni J. Population pharmacokinetics of inotuzumab ozogamicin in relapsed/refractory acute lymphoblastic leukemia and non-Hodgkin lymphoma. J Pharmacokinet Pharmacodyn. 2019 Jun;46(3):211-222. doi: 10.1007/s10928-018-9614-9. Epub 2019 Mar 11. PMID: 30859374; PMCID: PMC6529376.

26. Zwaan CM, Reinhardt D, Jürgens H, Huismans DR, Hählen K, Smith OP, et al. Gemtuzumab ozogamicin in pediatric CD33-positive acute lymphoblastic leukemia: First clinical experiences and relation with cellular sensitivity to single agent calicheamicin [5]. Vol. 17, Leukemia. Nature Publishing Group; 2003. p. 468–70.

27. Walter RB, Gooley TA, van der Velden VH, Loken MR, van Dongen JJ, Flowers DA, et al. CD33 expression and P-glycoprotein-mediated drug efflux inversely correlate and predict clinical outcome in patients with acute myeloid leukemia treated with gemtuzumab ozogamicin monotherapy. Blood. 2007 May 15;109(10):4168–70.

28. Shah NN, Highfill SL, Shalabi H, Yates B, Jin J, Wolters PL, Ombrello A, et al. CD4/CD8 T-Cell Selection Affects Chimeric Antigen Receptor (CAR) T-Cell Potency and Toxicity: Updated Results From a Phase I Anti-CD22 CAR T-Cell Trial. J Clin Oncol. 2020 Mar 24;38(17):1938–50.

29. Zheng S, Gillespie E, Naqvi AS, Hayer KE, Ang Z, Torres-Diz M, et al. Modulation of CD22 protein expression in childhood leukemia by pervasive splicing aberrations: implications for CD22-directed immunotherapies. Blood Cancer Discov. 2021 Nov 15;bloodcandisc.0087.2021.

30. Prokop A, Wrasidlo W, Lode H, Herold R, Lang F, Henze G, et al. Induction of apoptosis by enediyne antibiotic calicheamicin ϑII proceeds through a caspase-mediated mitochondrial amplification loop in an entirely Bax-dependent manner. Oncogene 2003 2257. 2003 Dec 11;22(57):9107–20.

31. Godwin CD, Bates OM, Jean SR, Laszlo GS, Garling EE, Beddoe ME, et al. Anti-apoptotic BCL-2 family proteins confer resistance to calicheamicin-based antibody-drug conjugate therapy of acute leukemia. https://doi.org/101080/1042819420201786553. 2020 Oct 14;61(12):2990–4.

Supplementary material

Supplementary Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria	
Age	• ≥1 and <18 years at time of enrollment • The first three patients on dose level 1 must be ≥6 and <18 years • Then ≥2 additional patients ≥1 year and <6 years at the same dose level
Diagnosis	• First relapse of BCP-ALL post allogeneic HSCT • Second or greater R/R BCP-ALL • Refractory disease (newly diagnosed patients who had induction failures after ≥2 previous regimens without attainment of remission, or patients with refractory first relapse after one previous reinduction regimen without attainment of remission) **AND**: • M2 or M3 marrow status (≥5% blasts by morphology) • Malignant clone CD22 surface antigen positive (in either bone marrow or peripheral blood) by institutional standards • The first six patients must have M3 marrow status (≥25% blasts by morphology)
Performance level and life expectancy	• Karnofsky >60% (>16 years) or Lansky >60% (≤16 years) • Life expectancy of ≥6 weeks
Prior therapy	Patients must have recovered from the acute toxic effects of all prior therapy, defined as resolution of non-hematologic toxicities to ≤Grade 2 per the CTCAE 4.03 prior to entering the study a. Chemotherapy ≥7 days since the completion of cytotoxic therapy (exceptions: hydroxyurea, 6-mercaptopurine and steroids which are permitted up until 48 hours prior to initiating protocol therapy) b. Radiotherapy ≥28 days since any prior radiation therapy c. Hematopoietic stem cell transplant ≥90 days since previous allo-HSCT No evidence of active graft vs host disease No GVHD prophylaxis or treatment d. Hematopoietic growth factors ≥7 days since the completion of therapy with GCSF or other growth factors, or ≥14 days since completion of therapy with pegfilgrastim (Neulasta®) e. Immunotherapy ≥42 days after the completion of any type of immunotherapy, e.g. CART therapy. Patients may not have received prior CD22-targeted therapy (immunotoxin or CART therapy) f. Monoclonal antibodies ≥3 half-lives of the antibody must have elapsed after the last dose of a monoclonal antibody (rituximab = 66 days, epratuzumab = 69 days) Exclusion of blinatumomab: patients must have been off blinatumomab infusion for ≥14 days and all drug-related toxicity must have resolved to ≤Grade 2

Supplementary Table 1. Continued

Prior therapy (Continued)	g. Investigational drugs ≥7 days or five drug half-lives (whichever is longer) since prior treatment with any experimental drug (with the exception of monoclonal antibodies) under investigation. No residual toxicities should be observed following previous treatment h. Prior calicheamicin exposure Patient has not received prior treatment with a calicheamicin conjugated antibody (e.g. gemtuzumab ozogamicin)
Renal and hepatic function	• Serum creatinine ≤1.5 x institutional ULN according to age • AST and ALT ≤2.5 x institutional ULN • Total bilirubin ≤1.5 x institutional ULN unless the patient has documented Gilbert syndrome
Cardiac function	• Shortening fraction ≥30% by echocardiogram or an ejection fraction >50% by MUGA.
Reproductive function	• Female patients of childbearing potential: negative urine or serum pregnancy test confirmed prior to enrollment • Female patients with infants must agree not to breastfeed on study • Male and female patients of child-bearing potential must agree to use a *highly effective* method of contraception (≥8 months for females and for ≥5 months for males after the last dose of InO)
Exclusion Eligibility Criteria	
Isolated extramedullary relapse	• Patients with isolated extramedullary disease are excluded
VOD/SOS	• Any history of prior or ongoing VOD/SOS as per modified Seattle criteria, or prior liver-failure [defined as severe acute liver injury with encephalopathy and impaired synthetic function (international normalized ratio of ≥1.5)]
Infection	• Systemic fungal, bacterial, viral or other infection that is exhibiting ongoing signs/symptoms • The patient may not have: • A requirement for vasopressors • Positive blood culture within 48 hours of study enrollment • Fever above 38.2 degrees Celsius within 48 hours of study enrollment with clinical signs of infection. Fever that is determined to be due to tumor burden is allowed, with documented negative blood cultures for ≥48 hours prior to enrollment and no concurrent signs or symptoms of active infection or hemodynamic instability • A positive fungal culture within 30 days of study enrollment • Active fungal, viral, bacterial, or protozoal infection requiring intravenous or oral treatment. Chronic prophylaxis therapy to prevent infections is allowed
Other anti-cancer therapy	• Patients will be excluded if there is a plan to administer non-protocol anti-cancer therapy during the study period
Allergic reaction	• Patients with prior Grade 3/4 allergic reaction to a monoclonal antibody are excluded

Supplementary Table 1. Continued

Concurrent disease	• Significant concurrent disease, illness, psychiatric disorder or social issue that would compromise patient safety or compliance with protocol therapy, interfere with consent, study participation, followup, or interpretation of study results • Children with Down syndrome are excluded from participation in the dose finding parts of the study

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCP-ALL, B-cell precursor acute lymphoblastic leukemia; CART, chimeric antigen receptor T cell; GCSF, granulocyte-colony stimulating factor; GVHD, graft versus host disease; HSCT, hematopoietic stem cell transplant; InO, inotuzumab ozogamicin; MUGA, multiple gated acquisition scan; R/R, relapsed/refractory; SOS, sinusoidal obstruction syndrome; ULN, upper limit of normal; VOD, veno-occlusive disease.

Supplementary Table 2. Definitions of Response

Response Category	Criteria*
Complete Response	No evidence of circulating blasts or extramedullary disease; including CNS-1 status; absence of splenomegaly, lymphadenopathy, skin/gum infiltration, testicular mass A bone marrow with <5% blasts (M1 marrow) Recovery of peripheral counts (platelets >50,000/μL and transfusion independent, and ANC >500/μL)
Complete Response with Insufficient Platelet Recovery	No evidence of circulating blasts or extramedullary disease A bone marrow with <5% blasts (M1 marrow) An ANC > 500/μL but Platelets ≤ 50,000/μL
Complete Response without Recovery of Counts	No evidence of circulating blasts or extramedullary disease A bone marrow with <5% blasts (M1 marrow) An ANC ≤ 500/μL and / or Platelets ≤50,000/μL
Partial Response	Greater than 50% relative reduction (with a minimum of 10% absolute reduction) in the bone marrow aspirate leukemic cell count, irrespective of recovery of the peripheral blood counts
Stable Disease/ No Response	Stable disease is present when the patient fails to qualify for CR, CRi, PR, or PD
Progressive Disease	Progressive disease is defined as an increase of at least 25% of the absolute number of bone marrow or circulating leukemic blasts, development of extramedullary disease, or other laboratory or clinical evidence of PD, with or without recovery of ANC or platelets
Relapse	After documentation of remission, a bone marrow aspirate and/or biopsy showing ≥5% leukemic blasts using morphology with flow cytometric confirmation, and/or pathological/radiological evidence of extramedullary disease, including development of CNS3 status or clinical CNS-involvement with radiological confirmation (MRI).
Refractory	Any patient not achieving CR, CPp or CRi after induction therapy (cycle 1 and/or cycle 2).

ANC: Absolute Neutrophile Count; CNS 1/2/3: Central Nervous System Disease Status; CR: Complete Response; CRi: Complete Response without Recovery of Counts; CRp: Complete Response with Insufficient Platelet Recovery; PD: Progressive Disease; SD: Stable Disease.

* All criteria must be present to define each response category

Supplementary Table 3. Study Endpoints

Primary endpoints
ORR, defined as the percentage of patients with CR, CRi, CRp, measured as best response during InO treatment
Secondary endpoints
1. Safety: • AEs, as characterized by type, frequency, severity (as graded using CTCAE v4.03), timing, seriousness, and relation to study therapy, during the first and subsequent cycles of therapy. • Occurrence of toxic death; i.e., death attributable to InO therapy. • Occurrence of VOD/SOS during or after therapy with InO. • Laboratory abnormalities as characterized by type, frequency, severity and timing. • The cumulative incidence of non-relapse mortality, defined as the cumulative probability of non-relapse mortality, with time calculated between start of study treatment and death due to other causes than relapsed or refractory leukemia or lymphoma, accounting for competing events. **2. Other measures of anti-leukemic activity:** • ORR after cycle 1. • Minimal residual disease levels, including the percentage of patients who become MRD-negative (complete MRD response defined as an MRD-level < $1x10^{-4}$), after cycle 1, as well as the best response (MRD-negativity) over multiple cycles. • Duration of response, defined as the time between achieving response (CR, CRi or CRp) after starting study treatment and documented relapse or death. • Number and percentage of patients being transplanted and those receiving CAR T-cell therapy after treatment with InO. • EFS, defined as the time between start of study treatment and first event including failure to achieve CR/CRp/CRi (calculated as an event on day 0), relapse, death of any cause and second malignancies. • Survival, defined as time to death following start of study treatment. • The cumulative incidence of non-response or relapse, defined as the cumulative probability of non-response or relapse, with time calculated between start of study treatment and relapse and with non-responders included as an event on day 0. Non-relapse death is considered a competing event. **3. Serum pharmacokinetic parameters of InO and unconjugated calicheamicin.** **4. Pharmacodynamics parameters*** • Relationship between response (ORR) and CD22 expression levels and WBC. • Relationship between response (ORR) and CD22 saturation kinetics. • Relationship between response (ORR) and calicheamicin sensitivity. • Clonal evolution (CD22-negativity) and relation to loss of response.
Other endpoints
The percentage of patients responding to InO (ORR) without adequate recovery of CD19-positive B-cells (below LLN for age) or immunoglobulins (below LLN for age) following 4 weeks, 10 weeks, 3, 6 and 12 months after treatment with InO, excluding patients who have been transplanted from the date of HSCT or have received CAR-T cells therapy.
Percentage of patients who exhibit ADA

LLN: lower limit for normality; ADA: anti-drug antibodies; WBC: with blood cells; ORR: overall response rate; MRD: Minimal residual disease; SOS/VOD: Sinusoidal occlusive syndrome/veno-occlusive disease; EFS: event free survival; HSCT: Hematopoietic stem cell transplantation. * in post hoc analysis we also investigated the relationship between PD parameters and achievement of MRD negativity as defined in the main paper.

Supplementary Table 4: Expanded definitions of secondary outcomes

Term	Definition
Overall response rate (ORR)	defined as complete remission (CR), CR with incomplete hematologic recovery (CRi), or CR with incomplete platelet recovery (CRp; response criteria followed standard procedures in leukemia, as designed by the National Comprehensive Cancer Network, with slight modifications in relation to bone marrow regeneration [CR = platelets >30,000/μL rather than >100,000/μL; and ANC >500/μL rather than >1000/μL]).
Minimal Residual Disease (MRD) status:	bone marrow negative if MRD $<1x10^{-4}$ with real-time quantitative-PCR or <0.01% with multi-parameter flow cytometry according to EuroFlow protocols when PCR was negative but the QR was > 10^{-4}.[1,2]
Event-free survival (EFS)	defined as time from start of treatment to first event including failure to achieve CR/CRp/CRi, relapse, death, and second malignancies.
Cumulative incidence of non-response or relapse	defined as the cumulative probability of non-response or relapse, with time calculated between start of study treatment and relapse and with non-responders included as an event on Day 0. Non-relapse death is considered a competing event.
Cumulative incidence of non-relapse mortality	defined as the cumulative probability of non-relapse mortality, with time calculated between start of study treatment and death due to other causes than relapsed or refractory leukemia or lymphoma, accounting for competing events.
Duration of response	defined as the time between achieving response (CR, CRi or CRp) after starting study treatment and documented relapse or death
Overall survival (OS)	defined as time to death following start of study treatment.

Additional specifications on the methodology

Supplementary text 1. CD22 saturation and internalization

Saturation was defined as (Eq. 1)

$$\frac{(\text{specific fluorescence intensity of bound Ino})}{(\text{specific fluorescence intensity maximal Ino binding})} \cdot 100\%$$

Internalisation was defined as (Eq. 2)

$$1 - \left(\frac{\text{specific fluorescence intensity of bound Ino}_{\tau+1}}{specific\ fluorescence\ intensity\ maximal\ Ino\ binding_{\tau}}\right) \cdot 100\%$$

Where τ stands for time. Data were acquired on a FACSCanto flow cytometer (BD Biosciences) using EuroFlow instrument settings and analyzed using DIVA (BD Biosciences) and Infinicyt (Cytognos) software.

Supplementary text 2: Determination of MRD levels by PCR and Flowcytometry

Molecular MRD levels were centrally determined by RQ-PCR of leukemia-specific rearranged immunoglobulin (IG) and T-cell receptor (TR) genes (van der Velden and Van Dongen, 2009). Quality control and standardized interpretation of RQ-PCR data were achieved following the guidelines of the European Study Group on MRD detection in ALL (EuroMRD) (Van der Velden et al, Leukemia 2007). For flowcytometric MRD analysis, also centrally performed, bone marrow samples were bulk-lysed and subsequently stained using 8 color stainings according to EuroFlow protocols (Theunissen et al, Blood 2017; Kalina et al, Leukemia 2012). Four million cells (if available) were acquired and MRD positivity was defined if at least 20 ALL cells could be detected. Flow MRD negativity was defined as MRD < 0,01% using an assay with a sensitivity of at least 0,01%. MRD negativity was defined as PCR below 10^{-4} or flow cytometry below 0.01% when PCR was negative but the Quantitative Range was above 10^{-4}.

Supplementary Table 5: Risk factors analysis for MRD response of all patients treated at RP2D (N= 40 patients, phase I and II combined)

	MRD neg	MRD pos	P value
Age at enrollment			0.73
<10 years	13	7	
>=10 years	14	5	
Sex			0.26
male	21	7	
female	6	5	
Diagnosis			0.27
first relapse post allogeneic HSCT	7	2	
second or greater relapsed	17	6	
refractory	3	4	
Prior HSCT			0.73
no	13	7	
yes	14	5	
Prior antibody therapy (blinatumomab)			0.42
no	22	8	
yes	5	4	
PB WBC at screening			0.73
<median	13	7	
>=median	14	5	
IC50 Calicheamicin (N=10)			0.21
<median	4	1	
>=median	1	4	

HSCT: Hematopoietic Stem cell transplant; PB WBC: Peripheral Blood With Blood Count; IC50: values represent the concentration of the drug which inhibits 50% of the leukemic cell

Supplementary Table 6. SOS cases in patients receiving transplantation after InO for combined cohort of phase I and phase II

Age at enrollment	HSCT Conditioning regimen	Defibrotide prophylaxis	Previous HSCT	Days since last InO dose	Number of InO courses	Dose level* (mg/m2)	SOS	Grade	Outcome SOS
6	Etoposide; TBI	no	no	125	1	1.8	no		
4	Fludarabine; Thiotepa; treosulfan	no	no	83	1	1.4	no		
14	Fludarabine; busulfan; Thiotepa	na	no	99	1	1.8	no		
12	Etoposide; TBI	no	no	26	2	1.4	no		
11	Fludarabine; treosulfan; Thiotepa	no	no	23	2	1.4	no		
15	Etoposide; TBI	na	no	39	2	1.8	no		
16	Fludarabine; melphalan; Thioptepa	na	yes	51	1	1.8	no		
4	Etoposide; TBI	yes	yes	55	2	1.8	no		
13	Etoposide; TBI	yes	no	47	3	1.8	yes	3	Resolved
6	Fludarabine; Thiotepa; TBI	no	no	72	4	1.8	no		
12	Fludarabine; busulfan; Thiotepa	no	yes	54	3	1.8	no		
2	Busulfan; fludarabine; Thiotepa	yes	no	27	2	1.8	yes	3	Resolved
1	Fludarabine;treosulfan; Thiotepa	yes	no	35	2	1.8	no		
8	Treosulfan;fludarabine; Thiotepa	no	yes	20	1	1.8	yes	3	Ongoing at time of death (due to MOF)
14	Fludarabine; TBI	yes	no	22	2	1.8	no	3	Resolved
14	Fludarabine; TBI	yes	no	22	2	1.8	yes	2	Resolved

Supplementary Table 6. Continued

Age at enrollment	HSCT Conditioning regimen	Defibrotide prophylaxis	Previous HSCT	Days since last InO dose	Number of InO courses	Dose level* (mg/m2)	SOS	Grade	Outcome SOS
13	Etoposide; TBI	yes	yes	110	1	1.8	no		
5	Etoposide; TBI	no	no	25	3	1.8	no		
7	Thiotepa; fludarabine	no	no	70	2	1.8	no		
7	Etoposide; TBI	no	no	182	2	1.8	no		
13	Etoposide; TBI	no	yes	78	2	1.8	no		
4	Etoposide; TBI	yes	yes	35	1	1.8	yes	4	Resolved
8	Etoposide; TBI	yes	yes	43	1	1.8	no		
17	Etoposide; TBI	yes	no	22	3	1.8	yes	4	Ongoing at time of death (due to MOF)

To identify the risk factors for SOS, Fisher's exact test (for categorical variables) and Mann-Whitney U test (for continuous variables) were used to compare the patients who received transplantation after Inotuzumab Ozogamicin (InO) (6 who later developed SOS vs 18 who did not). Days from last InO dose was found to be statistically significant with a p value of 0.014. The median value of days from last InO dose in patients with SOS was 24.5 (IQR: 21.5-38) while the median in patients with no SOS was 54.5 (IQR: 30.5-75). Dose level 1.4 mg/m2 refers to phase I only. Conditioning regimen with TBI (p=1.0), age at enrollment (p=0.84), defibrotide prophylaxis (p=0.06), previous HSCT (p=1.0), and number of InO courses received (p=0.69) were not statistically significant. SOS: sinusoidal obstruction syndrome; HSCT hematopoietic stem cell transplantation; TBI: total body irradiation; MOF: multi organ failure. SOS was diagnosed based on Seattle criteria and was graded according to the CTCAE v. 4.03 under hepatobiliary disorders, and AE term: Other, specify.

Supplementary Table 7: AEs listing for the patients include in phase 2 (N=28) as reported by the local investigators

	Grade					
Adverse Event Term	**1**	**2**	**3**	**4**	**5**	**Total**
Abdominal pain	1	0	0	0	0	1
Alanine aminotransferase increased	1	1	4	1	0	7
Alkaline phosphatase increased	0	0	1	0	0	1
Allergic reaction	1	1	0	0	0	2
Alopecia	1	0	0	0	0	1
Anal mucositis	0	0	1	0	0	1
Anal pain	0	0	1	0	0	1
Anemia	0	1	5	0	0	6
Anorexia	1	0	0	0	0	1
Anxiety	0	1	0	0	0	1
Arthralgia	0	0	1	0	0	1
Aspartate aminotransferase increased	0	3	5	0	0	8
Back pain	1	2	0	0	0	3
Bloating	1	0	0	0	0	1
Blood bilirubin increased	1	0	1	1	0	3
Bronchial obstruction	1	0	0	0	0	1
Cardiac disorders - hypotension	0	0	1	0	0	1
Left ventricular systolic dysfunction	0	0	0	1	0	1
Catheter related infection	0	0	1	0	0	1
Chest wall pain	1	0	0	0	0	1
Constipation	1	1	1	0	0	3
Cough	2	0	0	0	0	2
Cytokine release syndrome	0	1	1	0	0	2
Death NOS	0	0	0	0	1	1
Depressed level of consciousness	0	0	0	1	0	1
Diarrhea	2	0	0	0	0	2
Dry skin	1	0	0	0	0	1
Dysgeusia	1	0	0	0	0	1
Encephalopathy	0	0	0	0	1	1
Enterocolitis infectious	0	1	0	0	0	1
Epistaxis	1	1	0	0	0	2
Erythema multiforme	2	0	0	0	0	2
Eye disorders - right eyelid retraction	1	0	0	0	0	1
Fatigue	2	0	0	0	0	2
Febrile neutropenia	0	1	6	0	0	7
Fever	12	2	2	0	0	16

Supplementary Table 7. Continued

	Grade					
Adverse Event Term	**1**	**2**	**3**	**4**	**5**	**Total**
Flank pain	1	0	0	0	0	1
Gait disturbance	1	0	0	0	0	1
Gastrointestinal disorders - anal fissure	1	0	0	0	0	1
Gastrointestinal disorders - bleeding gums	1	0	0	0	0	1
General disorders - bleeding lip	1	0	0	0	0	1
General disorders - dry lips	1	0	0	0	0	1
General disorders - infusion related reactions at Amphotericin B	0	1	0	0	0	1
General disorders - left lower lobe Infiltrate	0	1	0	0	0	1
General disorders - mood swings (anger, sadness)	1	0	0	0	0	1
General disorders - blood blister in oral cavity	1	0	0	0	0	1
Gamma glutamil transferasi increased	1	1	1	0	0	3
Headache	6	0	0	0	0	6
Hematoma	2	0	1	0	0	3
Hematuria	0	1	0	0	0	1
Hemorrhoidal hemorrhage	1	0	0	0	0	1
Hepatobiliary disorders - SOS/VOD	0	1	4	2	0	7
Hiccups	1	0	0	0	0	1
Hypercalcemia	1	0	0	0	0	1
Hyperuricemia	1	0	0	0	0	1
Hypoalbuminemia	1	1	0	0	0	2
Hypocalcemia	0	1	0	0	0	1
Hypokalemia	1	1	2	0	0	4
Hypotension	0	0	2	0	0	2
Hypoxia	0	1	0	0	0	1
Immune system disorders - engraftment syndrome	0	0	1	0	0	1
Infections and infestations - bacteremia	0	1	0	0	0	1
Infections and infestations - infection CMV	0	0	1	0	0	1
Infections and infestations - septicemia Pseudomonas plecoglossicida	0	0	1	0	0	1
Infections and infestations - thrush	1	0	0	0	0	1
INR increased	1	0	0	0	0	1
Investigations - blood lactate dehydrogenase increased	1	0	0	0	0	1
Investigations - candida albicans antibodies increased	0	1	0	0	0	1
Investigations - elevated urea	1	0	0	0	0	1
Kidney infection	0	1	0	0	0	1
Localized edema	1	1	0	0	0	2
Lung infection	0	0	2	0	1	3

Supplementary Table 7. Continued

	Grade					
Adverse Event Term	**1**	**2**	**3**	**4**	**5**	**Total**
Lymphocyte count decreased	0	0	3	0	0	3
Mucositis oral	1	1	2	0	0	4
Musculoskeletal and connective tissue disorder - back pain	1	0	0	0	0	1
Nausea	9	1	0	0	0	10
Neck pain	1	0	0	0	0	1
Neoplasms benign malignant and unspecified - disease progression	0	0	0	0	1	1
Neutrophil count decreased	0	0	1	1	0	2
Otitis media	0	1	0	0	0	1
Pain	4	0	0	0	0	4
Pain in extremity	2	1	0	0	0	3
Paronychia	0	1	0	0	0	1
Platelet count decreased	0	1	0	4	0	5
Pruritus	4	1	0	0	0	5
Purpura	1	0	0	0	0	1
Rash acneiform	0	1	0	0	0	1
Rash maculo-papular	1	1	1	0	0	3
Respiratory thoracic and mediastinal disorders - rhinitis	3	0	0	0	0	3
Respiratory thoracic and mediastinal disorders - tachypnea	1	0	0	0	0	1
Respiratory thoracic and mediastinal disorders - tachypnea	1	0	0	0	0	1
Sepsis	0	0	2	0	0	2
Serum amylase increased	1	0	0	0	0	1
Sinus bradycardia	1	0	0	0	0	1
Sinus tachycardia	2	0	0	1	0	3
Sinusitis	0	1	0	0	0	1
Skin and subcutaneous tissue disorders - papillo macular exanthema	1	0	0	0	0	1
Skin and subcutaneous tissue disorders - rash face	1	0	0	0	0	1
Skin and subcutaneous tissue disorders - macular rash	0	1	0	0	0	1
Skin and subcutaneous tissue disorders - rash (unspecified)	1	0	0	0	0	1
Skin and subcutaneous tissue disorders - erythema	1	0	0	0	0	1
Skin and subcutaneous tissue disorders - xerosis	1	0	0	0	0	1
Skin infection	1	0	0	0	0	1
Somnolence	1	0	0	0	0	1
Sore throat	1	0	0	0	0	1
Stoma site infection	1	0	0	0	0	1

Supplementary Table 7. Continued

	Grade					
Adverse Event Term	1	2	3	4	5	**Total**
Stomach pain	3	0	0	0	0	3
Surgical and medical procedures - removal of wisdom teeth	1	0	0	0	0	1
Tumor lysis syndrome	0	0	3	0	0	3
Upper gastrointestinal hemorrhage	0	0	1	0	0	1
Upper respiratory infection	0	1	0	0	0	1
Urinary tract infection	0	1	0	0	0	1
Vascular disorders - hematoma	1	0	1	0	0	2
Vascular disorders - Petechiae	1	0	0	0	0	1
Vomiting	9	2	1	0	0	12
Weight gain	1	0	1	0	0	2
White blood cell decreased	0	2	0	2	0	4

A total of 28 patients had at least one adverse event (AE) during the study, of which 20 patients had at least one grade 3/4 AE. SOS/VOD: sinusoidal obstruction syndrome/hepatic veno-occlusive disease.

Supplementary Table 8. List of chemistry laboratory abnormalities and grade (highest toxicity grade per patient) as compared to local normal ranges.

Grades	0	1	2	3	4	NA	Total
Albumin (low)	5	13	9	0	0	1	28
Alkaline phosphatase (high)	22	6	0	0	0	0	28
ALT (high)	2	16	5	5	0	0	28
Amylase (high)	23	2	2	0	0	1	28
AST (high)	3	16	5	4	0	0	28
Calcium (low)	12	12	3	0	1	0	28
Creatinine	21	7	0	0	0	0	28
GGT (high)	6	8	8	3	0	1	26
Lipase (high)	20	2	4	2	0	0	28
Phosphate (low)	19	3	3	3	0	0	28
Potassium (low)	16	9	0	3	0	0	28
Sodium (low)	15	12	0	1	0	0	28
Total Bilirubin (high)	22	4	1	1	0	0	28
Uric acid (high)	19	7	0	0	0	2	28

The highest toxicity grade per patient was counted only once per patient. This table includes also abnormalities not reported by the local investigator as AEs.

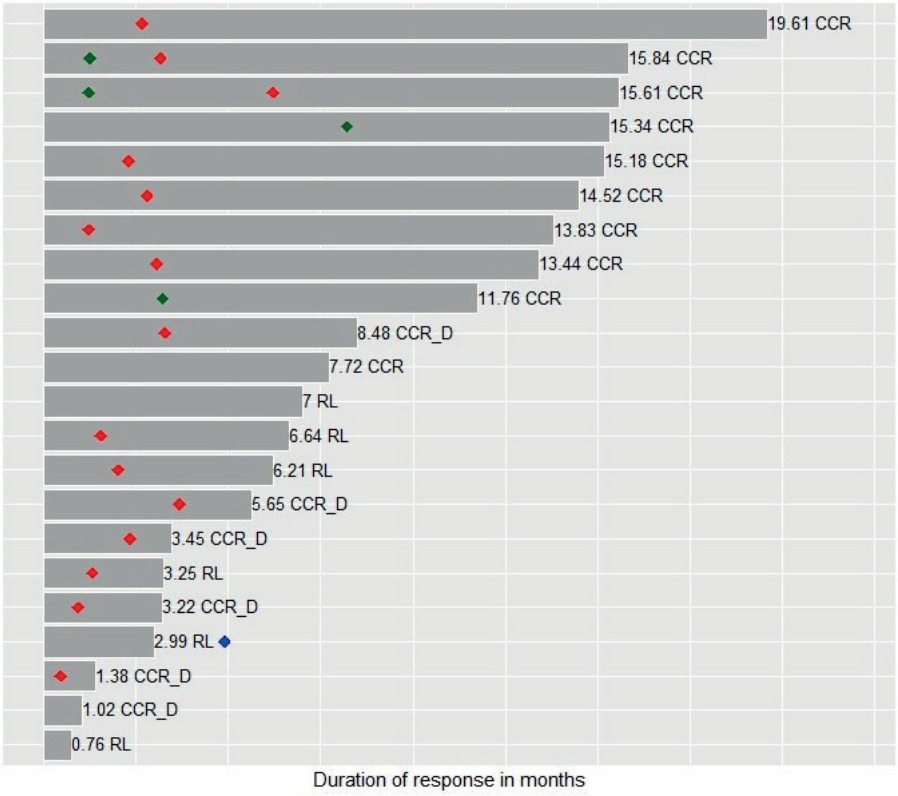
Supplementary Table 9. List of Hematologic laboratory abnormalities and grade (highest toxicity grade per patient) as compared to local normal ranges.

Grade	0	1	2	3	4	Total
Absolute neutrophil count decrease	1	1	0	2	24	28
Hemoglobin decrease	1	3	16	8	0	28
Platelet count decrease	0	3	3	5	17	28
White blood cell count decrease	1	1	1	7	18	28

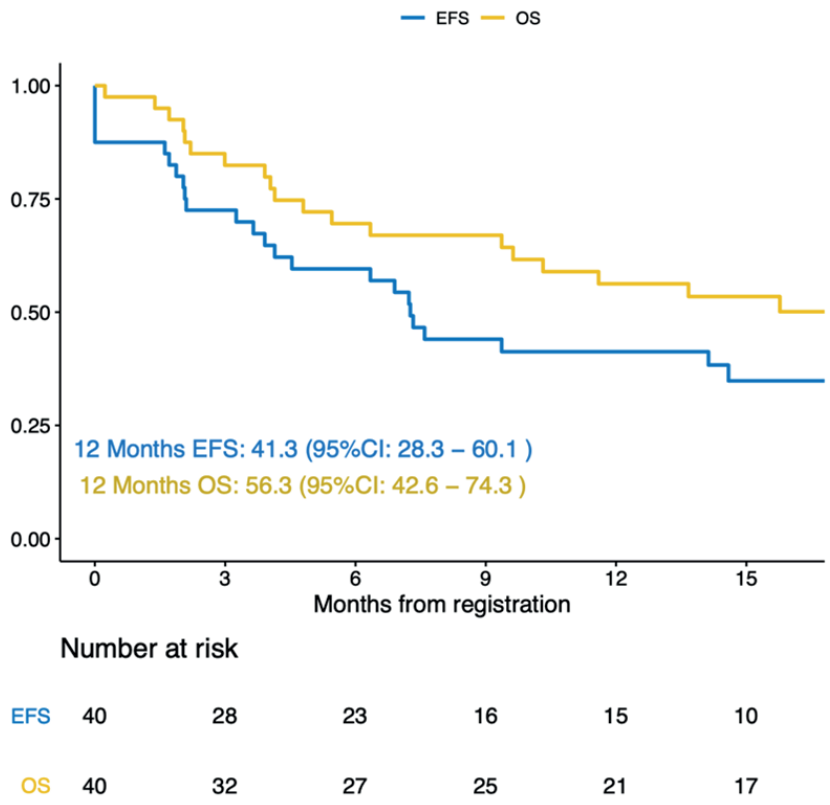
The highest toxicity grade per patient was counted only once per patient. This table includes also abnormalities not reported by the local investigator as AEs.

Supplementary Table 10. Serious Adverse Events (SAE) observed in the phase II

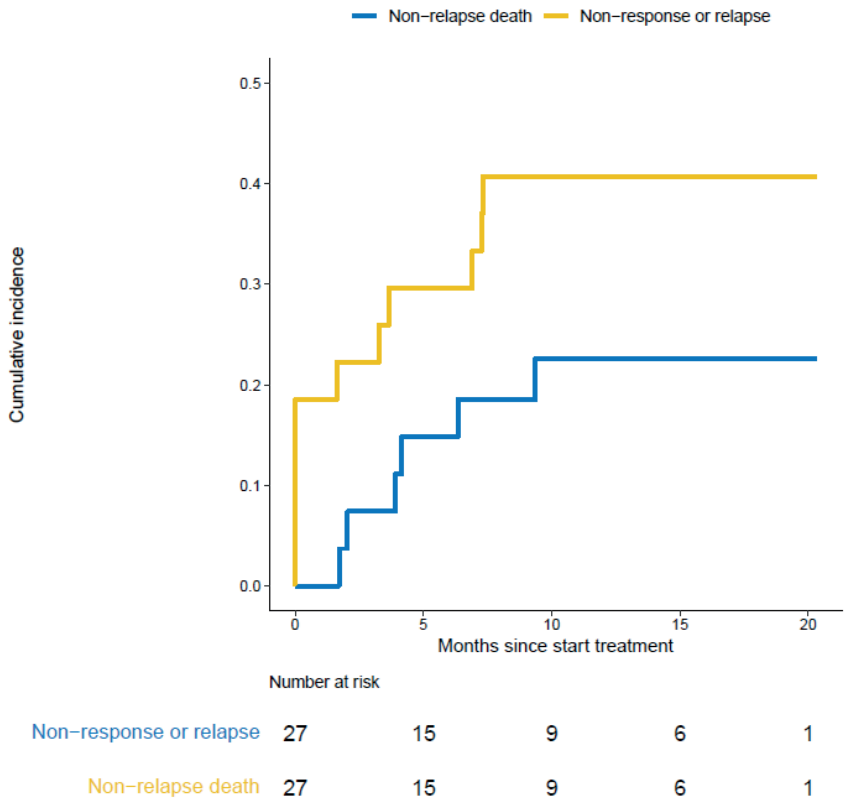
SAE Term	SAE Category	Grade	Outcome
Intestinal massive hemorrhage	Life threatening	4	Ongoing
Febrile neutropenia	Prolongation of hospitalization	3	Resolved
Sepsis	Prolongation of hospitalization	3	Resolved
Febrile neutropenia	Prolongation of hospitalization	3	Resolved
Febrile neutropenia	Prolongation of hospitalization	3	Resolved
Febrile neutropenia	Prolongation of hospitalization	3	Resolved
Catheter related infection	Prolongation of hospitalization	3	Resolved
Hematuria	Prolongation of hospitalization	2	Resolved
Engraftment syndrome	Life threatening	3	Resolved
Mycosis pneumopathy	Life threatening	4	Death
Sinusoidal obstruction syndrome	Prolongation of hospitalization	3	Resolved
Encephalopathy	Death	5	Death
Maculo-papular rash	Prolongation of hospitalization	2	Resolved
Upper respiratory tract infection	Prolongation of hospitalization	2	Resolved
Sepsis	Prolongation of hospitalization	4	Resolved
Sinusoidal obstruction syndrome	Life threatening	4	Resolved
Pain	Prolongation of hospitalization	3	Resolved
Sinusoidal obstruction syndrome	Life threatening	4	Ongoing
Febrile neutropenia	Prolongation of hospitalization	2	Resolved
Bacteremia	Other medically important condition	2	Ongoing
Sinusoidal obstruction syndrome	Other medically important condition	3	Resolved
Multi organ failure	Death	5	Death
Sinusoidal obstruction syndrome	Other medically important condition	3	Ongoing
Sinusoidal obstruction syndrome	Other medically important condition	2	Resolved
Disease progression	Death	5	Death
Sinusoidal obstruction syndrome	Other medically important condition	3	Resolved



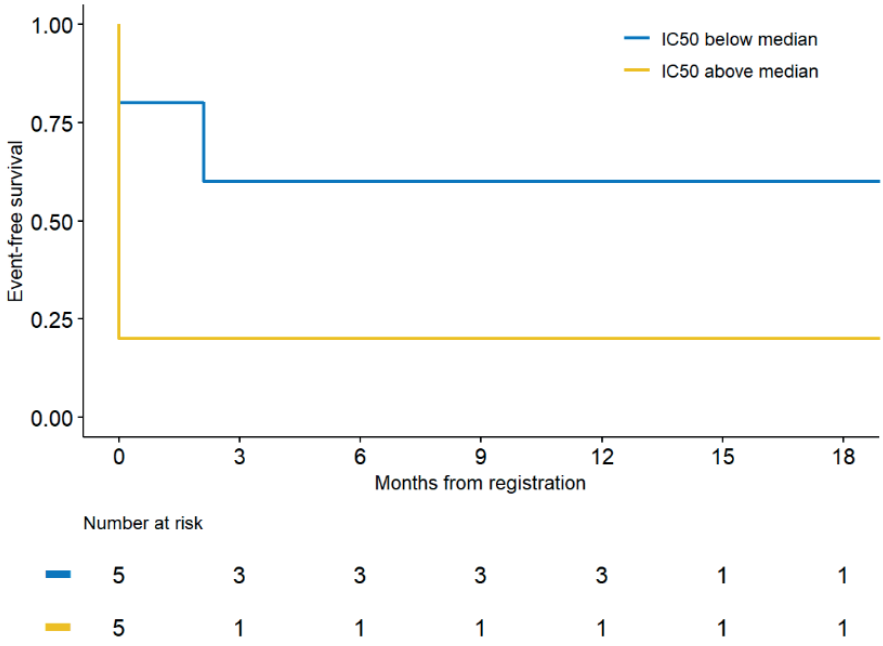
Supplementary Figure 1. Duration of response bar plot (n=22).
CCR: Continuous Complete Remission; RL: Relapse; CCR_Death: Death while in Continuous Complete Remission. Red squares: HSCT treatment; Blue square: in this patient HSCT was performed after additional treatment with blinatumomab due to loss response; Green squares: CAR-T treatment. Numbers at the end of each bar represent the duration of response in months. Four patients were non-responders (not reported here).



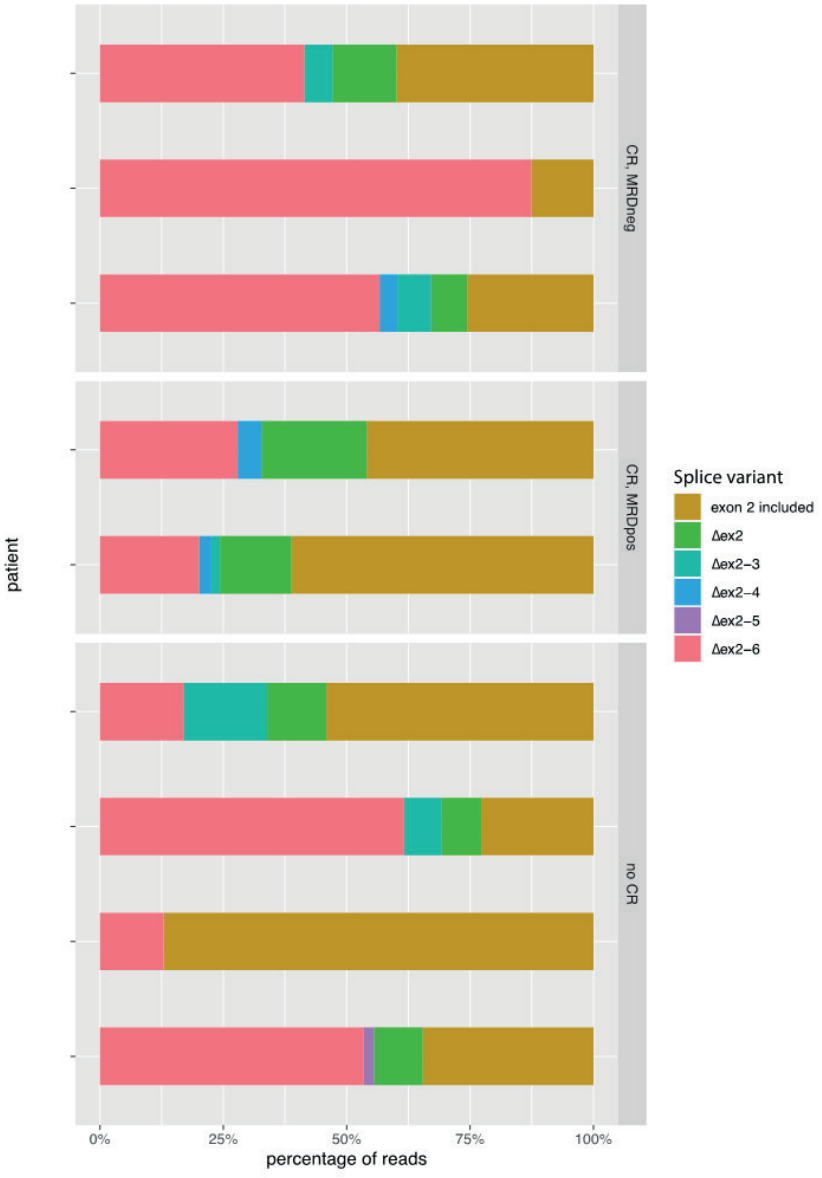
Supplementary Figure 2. EFS and OS for all patients from phase I treated at the RP2D, and phase II patients (n=40). Number at risk is presented below the graph. Blue line: Event Free Survival. Yellow line: Overall Survival. Non responders are added as event on day 0. RP2D: Recommended Phase II dose = 1.8 mg.m^2



Supplementary Figure 3. Cumulative incidence of refractory/relapse and non-relapse death. The number at risk is presented below the graph. Blue line: refractory/relapse cumulative incidence. Yellow line: non-relapse death cumulative incidence. Non responders are added as event on day 0. Five non-relapse death occurred after hematopoietic stem cell transplant procedure.

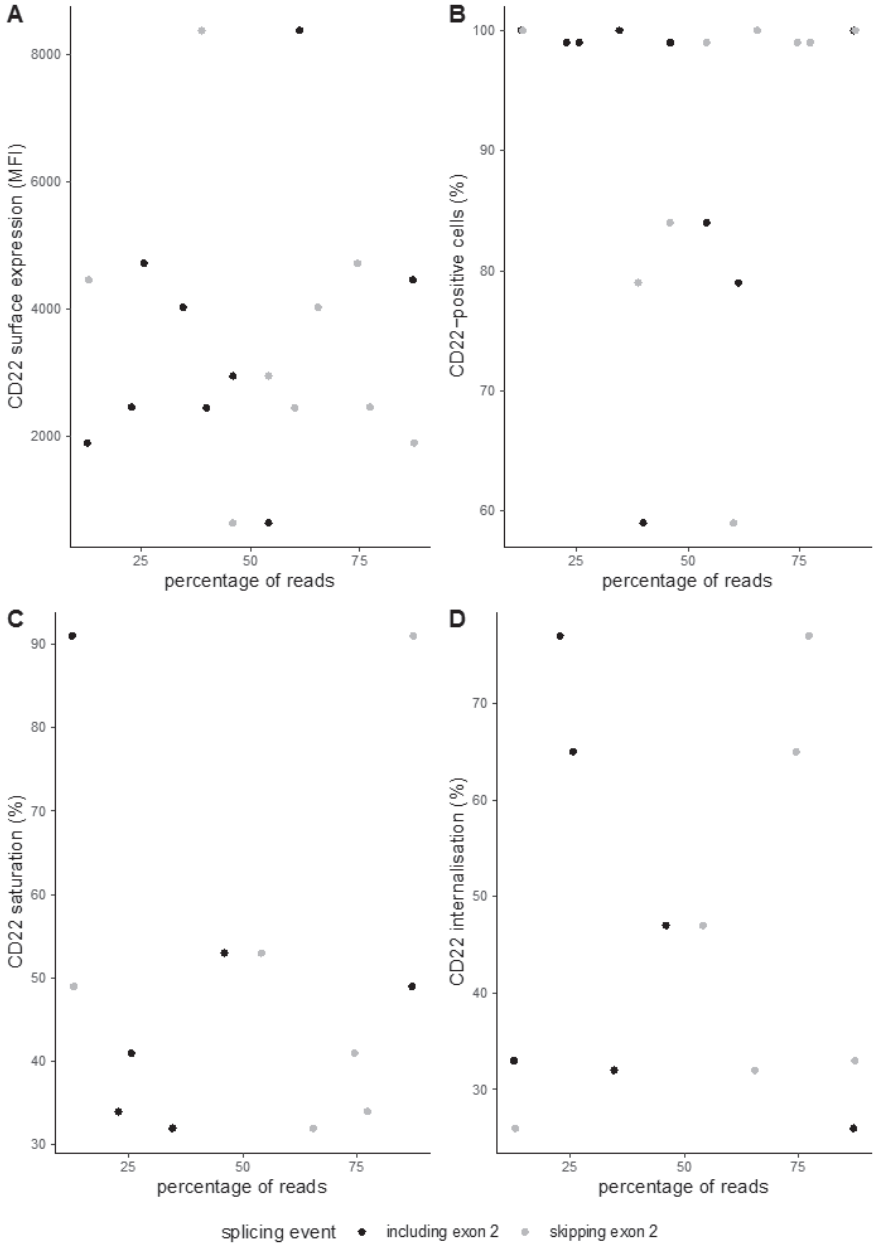


Supplementary Figure 4. EFS in patients with calicheamicin IC50 above the median (yellow line) and in those with calicheamicin IC50 below the median (blue line), n=10. The event definition is this graph differs in that not achieving MRD neg CR is calculated as an event at day 0. Number at risk is presented below the graph. Patients with *in vitro* calicheamicin sensitivity have a better one year EFS than patients with an IC50 above the median (23% (95%CI 29.3-100%) versus 20% (95%CI 3.46-100%)), although the difference is not statistically significant (p=0.19).

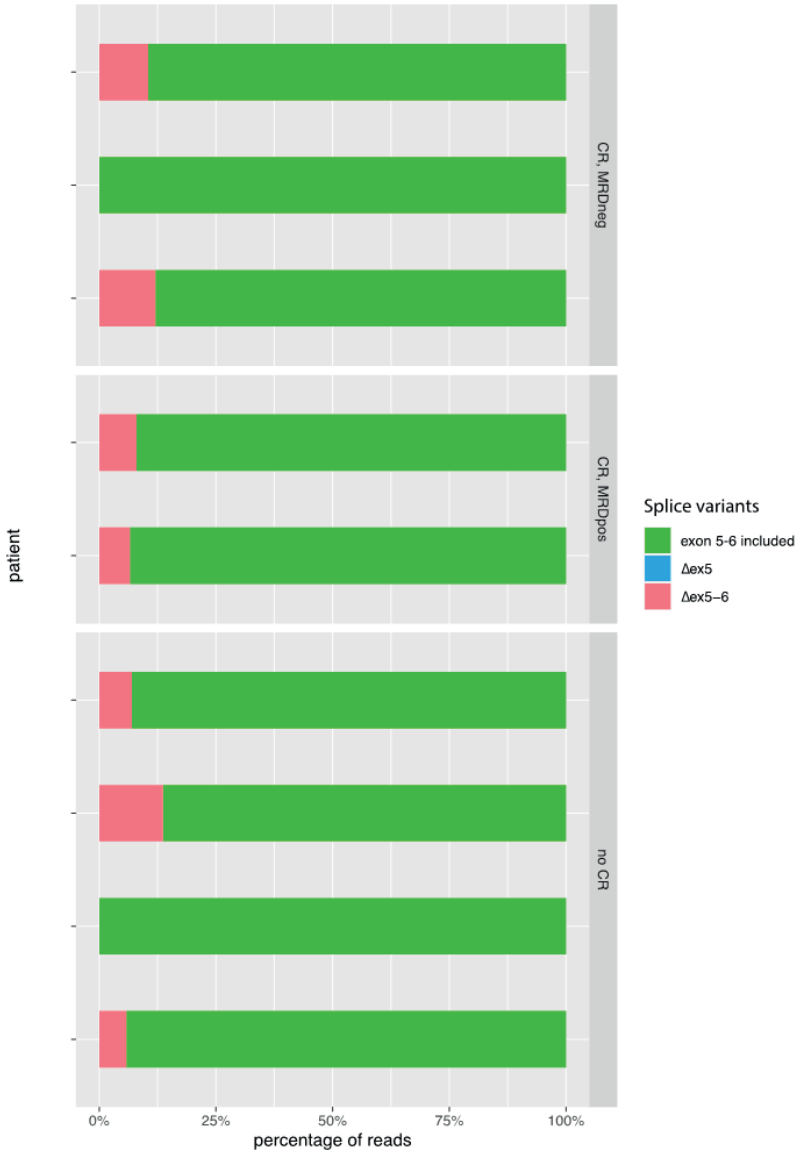


Supplementary Figure 5. Splice variants concerning exon 2 of the CD22 transcript in the 9 patients with RNA sequencing data available.

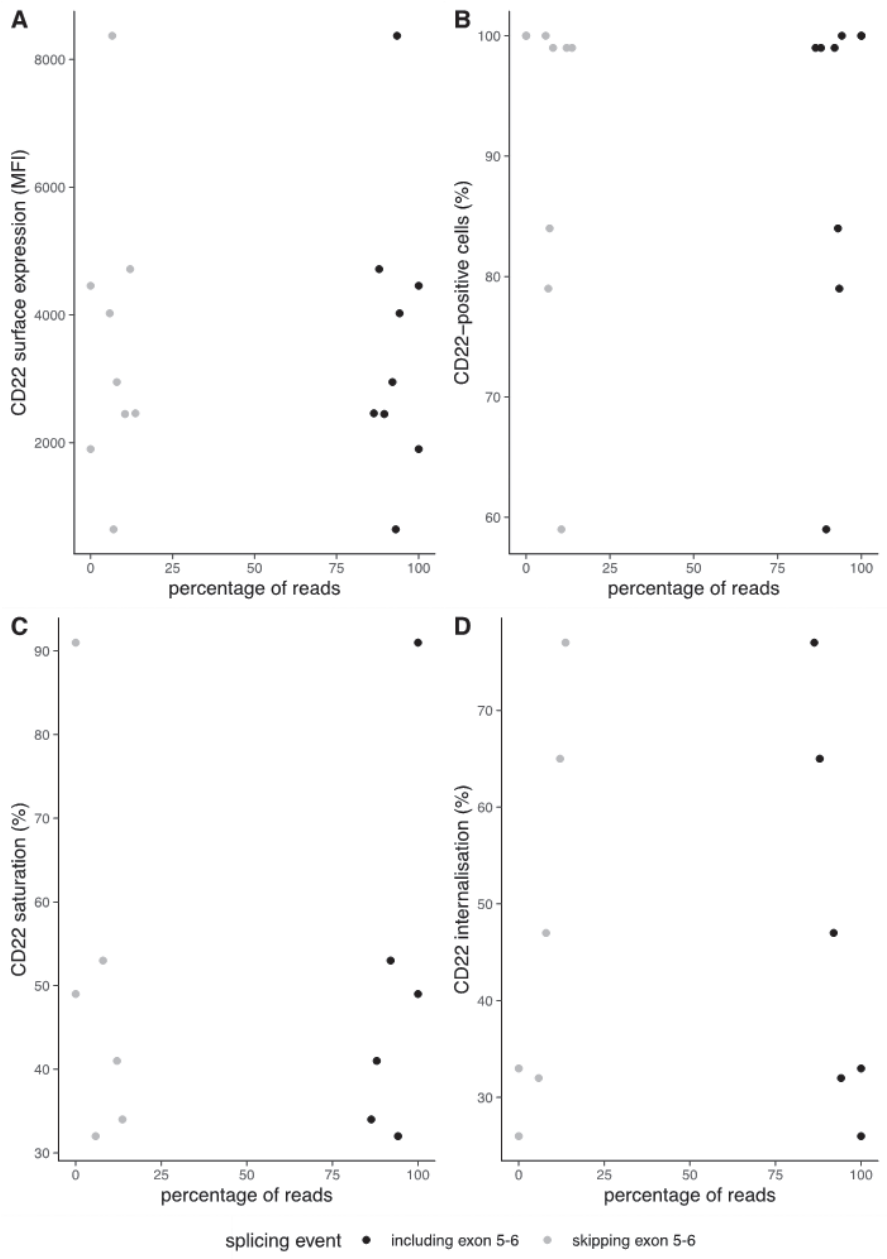
Percentages represent the number of split reads either in- or excluding the exons as percentage of the total number of split reads. All patients had at least some RNA copies including exon 2. The variant delta ex2-6 seems the most prevalent in this sample.



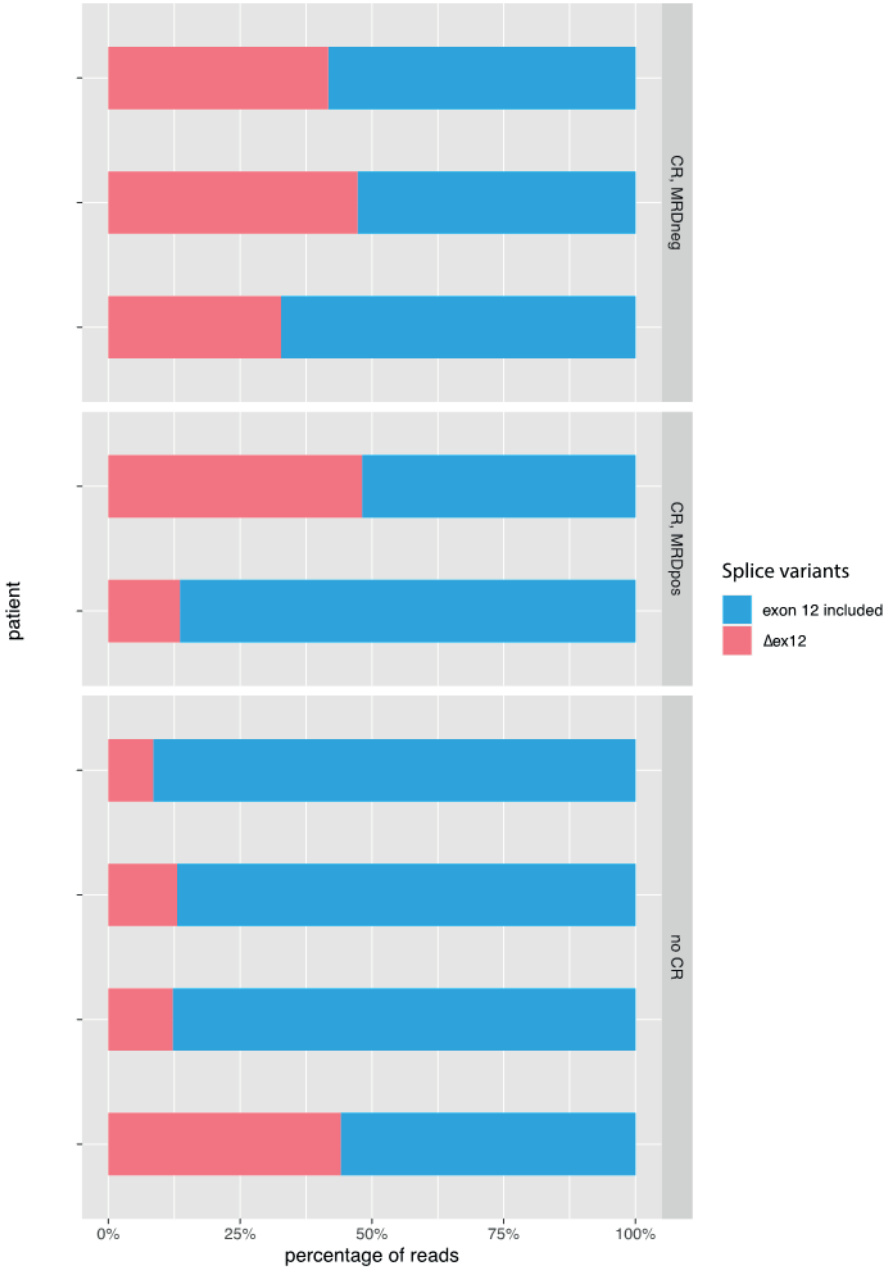
Supplementary Figure 6. Correlation between all splice variants skipping or including exon 2 and CD22 on leukemic blasts as MFI (A), and percentage CD22-positive cells (B), saturation (C) and internalization (D). No trend of association between the in- and exclusion of exon 2 and the expression of CD22 on leukemic blasts, the saturation levels of CD22 on leukemic blasts with InO, or the internalization levels of InO was found.



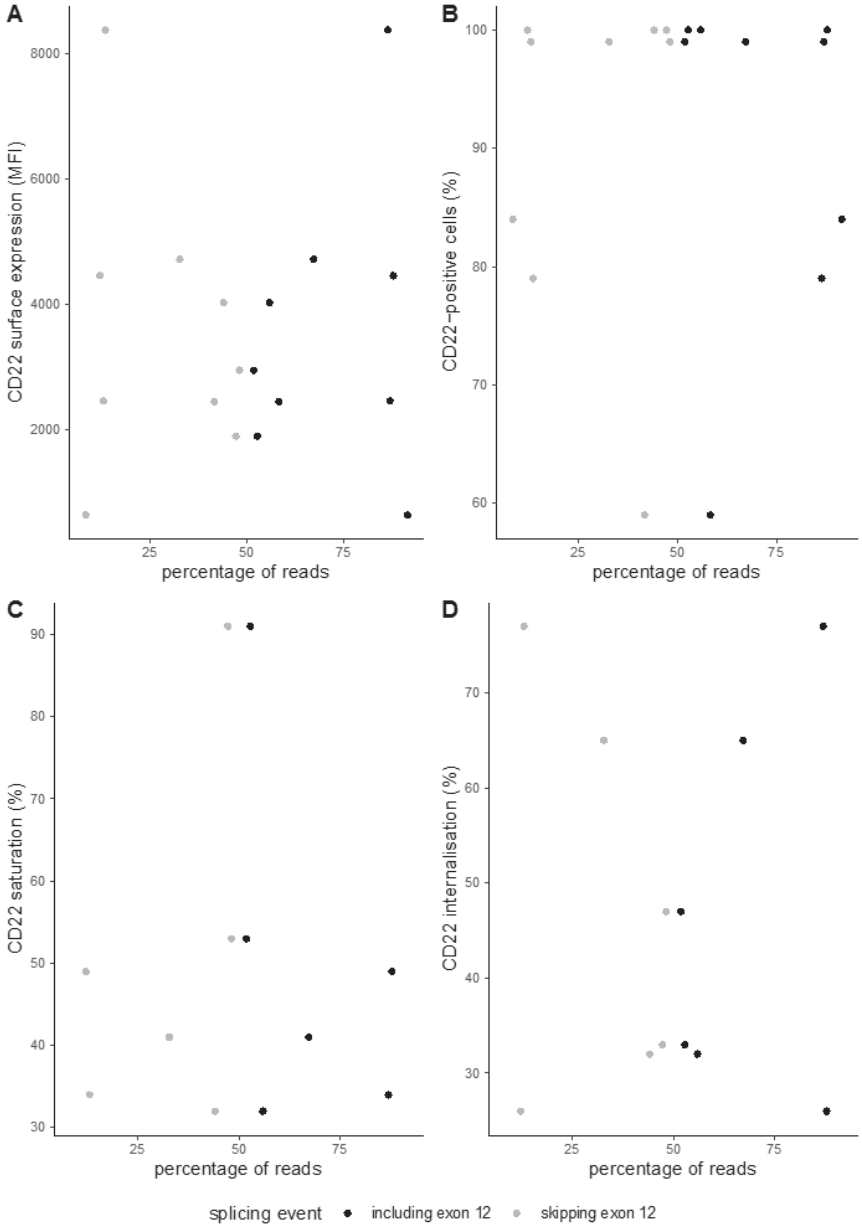
Supplementary Figure 7. Splicing variants concerning exon 5 and 6 of the CD22 transcript in the 9 patients with RNA sequencing data available.
Percentages represent the number of split reads either in- or excluding the exons as percentage of the total number of split reads. All patients had at least some RNA copies including exon 5 and 6.



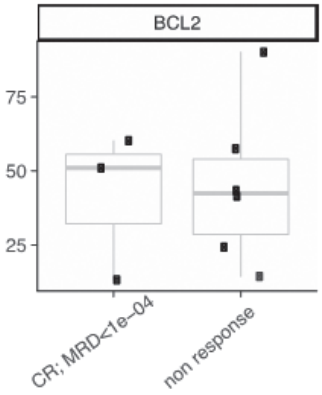
Supplementary Figure 8. Correlation between splicing variants skipping or including exon 5-6 and CD22 on leukemic blasts as MFI (A), and percentage CD22-positive cells (B), saturation (C) and internalization (D). No trend of association between the in- and exclusion of exon 5-6 and the expression of CD22 on leukemic blasts, the saturation levels of CD22 on leukemic blasts with InO, or the internalization levels of InO was found.



Supplementary Figure 9. Splicing variants concerning exon 12 of the CD22 transcript in the 9 patients with RNA sequencing data available. Percentages represent the number of split reads either in- or excluding the exons as percentage of the total number of split reads. All patients had at least some RNA copies including exon 12.



Supplementary Figure 10. Correlation between the splice variant skipping or including exon 12 and CD22 on leukemic blasts as MFI (A), and percentage CD22-positive cells (B), saturation (C) and internalization (D). No trend of association between the in- and exclusion of exon 12 and the expression of CD22 on leukemic blasts, the saturation levels of CD22 on leukemic blasts with InO, or the internalization levels of InO was found.



Supplementary Figure 11. *BCL2* gene expression per response group. Presented as fragments per kilobase per million (FPKM), which correct for library size and gene length. No difference in *BCL2* gene expression between response groups was observed.

References (for supplementary files)

1. Theunissen P, Mejstrikova E, Sedek L, et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. *Blood*. 2017;129(3):347-357. doi:10.1182/blood-2016-07-726307
2. van der Velden VH, Panzer-Grümayer ER, Cazzaniga G, et al. Optimization of PCR-based minimal residual disease diagnostics for childhood acute lymphoblastic leukemia in a multi-center setting. *Leukemia*. 2007;21(4):706-713. doi:10.1038/sj.leu.2404535
3. Kalina T, Flores-Montero J, van der Velden VH, Martin-Ayuso M, Bottcher S, Ritgen M, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia 2012; 26:1986-2010.
4. Theunissen P, Mejstrikova E, Sedek L, et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood. 2017;129(3):347-357.
5. van der Velden VH, van Dongen JJ. MRD detection in acute lymphoblastic leukemia patients using Ig/TCR gene rearrangements as targets for real-time quantitative PCR. Methods Mol Biol. 2009;538:115-150.
6. van der Velden VH, Panzer-Grümayer ER, Cazzaniga G, et al. Optimization of PCR-based minimal residual disease diagnostics for childhood acute lymphoblastic leukemia in a multi-center setting. Leukemia. 2007;21(4):706-713.

Chapter 5

Inotuzumab Ozogamicin in Infants and Young Children with Relapsed or Refractory Acute Lymphoblastic Leukemia: a case series

Brivio E, Chantrain CF, Gruber TA, Thano A, Rialland-Battisti F, Contet A, Elitzur S, Dalla-Pozza L, Kállay KM, Li CK, Kato M, Markova I, Schmiegelow K, Bodmer N, Breese EH, Hoogendijk R, Pieters R, Zwaan CM

Br J Haematol. 2021 Jun;193(6):1172-1177

Abstract

No data on Inotuzumab Ozogamicin (InO) in infant ALL have been published to date. We collected data internationally on infants/young children (<3 years) with ALL treated with InO. Fifteen patients (median 4.4 months at diagnosis) received InO due to R/R disease. Median percentage of $CD22^+$ blasts was 72% (range 40-100%, n=9). The median dose in the first course was 1.74 mg/m^2 (fractionated). Seven patients (47%) achieved complete remission one additional MRD-positive patient became MRD-negative. Six months overall survival was 47% (95%CI 27%-80%). Two patients developed veno-occlusive disease after transplant. Further evaluation of InO in this subgroup of ALL is justified.

Introduction

In the past decades, significant improvement has been made in the treatment of childhood acute lymphoblastic leukemia (ALL), with current event-free survival (EFS) rates close to 90%.(1)

In this context, infants (<1 year of age) with ALL represent a more challenging cohort of patients with an EFS of only 47%.(2) Relapses occur early, and survival after relapse is dismal, with a 3-year overall survival (OS) of 20%.(2, 3) These patients have a special need for innovative strategies.

Inotuzumab Ozogamicin (InO) is a monoclonal CD22-targeted antibody linked to calicheamicin, approved for adults with relapsed/refractory (R/R) CD22+ B-cell precursor ALL (BCP-ALL).

Phase I-II trials with InO in pediatric R/R ALL did not included infants. (4, 5). Pediatric results with InO reflect the positive experience reported in adults, with response rates ranging from 58% to 80%, and the majority of responders becoming MRD negative (65%-84%). (4-7)

We retrospectively collected data of infants and young children diagnosed with R/R ALL and treated with InO.

Subjects and Methods

This is an international retrospective cohort study of infants and young children with R/R BCP-ALL who received InO via a compassionate access program or were treated with InO at physician discretion from commercial source. The study was approved by the Ethics Committee of the Erasmus MC and registered in the Dutch Trial Registry nr NL7937.

Patients were considered eligible for the analysis if treated with at least one dose of InO for R/R BCP-ALL and were less than three years old at diagnosis.

The participating international pediatric oncology centers obtained regulatory approval according to local law and regulations, and collected retrospective demographic, outcome, and toxicity data in case report forms (CRF) developed for this registry. Cytogenetic characteristics and CD22 antigen expression performed locally prior to the start of InO treatment were collected, when available. One course consisted of three weekly doses of InO using the fractionated schedule. Disease response was determined by local institutions at the completion of each course at approximately day 22, right before the start of the next course.

Complete remission (CR) was defined as <5% bone marrow blasts by morphology and absence of extra medullary disease, and partial response (PR) as the reduction

of marrow disease burden from >25% blasts (M3) to 5–25% blasts (M2). Non-responders (NR) included those patients who did not meet the criteria for CR or PR. In case of CR, MRD confirmation either by flow cytometry or by PCR was reported for all cases. MRD was considered negative if <0.01%, for either of the methods.
Toxicities during and up to 30 days after InO treatment were collected. Additional clinical information regarding post-hematopoietic stem cell transplant (HSCT) VOD was captured. Severity of VOD was graded according to the EBMT criteria.(8)
A summary of these data have been presented at the American Society of Hematology 2019 annual meeting.

Statistical analysis

Analytic endpoints included disease response, measured as remission status and MRD, EFS defined as the time from start of treatment to earliest occurrence of treatment failure (non-remission was calculated as an event at day 0), disease relapse, or death from any cause, and OS defined as the time from start of treatment to death from any cause. The latter two endpoints were censored at the time of last reported follow-up. Estimates of EFS or OS probability were based on the Kaplan-Meier method. Fisher's exact test and Wilcoxon rank test were used with a significant level of 0.05.

Results

Between 2015 and 2019, 12 infants (<1 year of age at initial diagnosis) and three young children were initially diagnosed with ALL at a median age of 4.4 months (range 0.1-27 months). After developing relapse or refractory disease, these patients received 1-3 courses of InO.
The median age was 19.6 months (range 7.5-35 months) at the time of InO treatment; median BSA was 0.5 m^2 (range 0.32-0.63) and median weight 11.5 Kg (range 5.8-15.9 Kg).
Patients were heavily pretreated, median three prior treatment regimens (range 2-8). Patients characteristics are listed in Supplementary Table 1.
Twelve out of 15 (80%) patients were *KMT2A*-rearranged *(KMT2A*-r*)*, with a predominance of the *AFF1-KMT2A (MLL)* translocation (seven out of 12).
At the time of InO administration, the leukemia burden was an M3 bone-marrow in 10 patients (66%), M2 in two (13%), and M1 with MRD positivity in three patients (20%), one of whom had extra-medullary disease (subcutaneous nodules); no CNS involvement was reported. An overview of patient data is summarized in Table 1.

Table 1. Overview of patient characteristics

	Age at diagnosis (m)	Age at InO (m)	Disease status	BM at start InO	*KMT2A* status	% CD22+ blasts	Dose/m2 in C1	N InO courses	Best response/ MRD	N courses to response	HSCt after InO	Status at last FUP
1	1,1	7,5	1st rel. (refractory)	M3	*KMT2A-r*	80%	1,8	3	CR/0.1	3	Yes	Alive in remission
2	3,5	18	1st rel. (post-HSCT)	M1	KMT2A-r	N/A	1,8	2	NR	-	No	Died with disease
3	10,5	19,7	1st rel. (refractory)	M3	KMT2A-r	72%	1,6	1	NR	-	Yes	Died with disease
4	4,4	14,6	2nd rel.	M3	wild-type	91,60%	1,5	1	PR	1	Yes*	Died with disease
5	1,9	14,6	1st rel.(post-HSCT)	M3	*KMT2A-r*	52%	1,2	3	CR/0.1	2	No	Died with disease
6	7,3	30,9	1st rel. (refractory)	M3	*KMT2A-r*	100%	1,6	1	NR	-	No	Died with disease
7	26,9	35,3	1st rel. (refractory)	M2	wild-type	N/A	1,8	3	CR/0.01	2	No	Died with disease
8	1,4	14,1	1st rel. (refractory)	M3	*KMT2A-r*	N/A	1,8	2	CR/0.7	1	No	Died with disease
9	21,8	32,5	2nd rel.	M2	*KMT2A-r*	66,80%	1,8	1	NR	-	No	Died with disease
10	1,9	25,9	≥3rd rel.	M3	*KMT2A-r*	N/A	1,5	2	CR/0.003	1	Yes	Died with disease
11	17,9	24,9	2nd rel.	M1	*KMT2A-r*	N/A	1,4	1	CR/neg.	1	Yes	Died with disease
12	11,6	33,3	2nd rel.	M3	wild-type	100%	1,8	1	CR/0.08	1	No	Died in CR
13	0,1	11,8	1st rel.	M3	*KMT2A-r*	65%	1,8	1	PR	1	Yes	Alive with disease
14	3	23,2	≥3rd rel.	M3	*KMT2A-r*	40%	1,8	1	NR	-	No	Died with disease
15	5,3	16,9	1st rel. (refractory)	M1/EMD Subcut. nodules	*KMT2A-r*	N/A	1,5	1	CR/neg.	1	Yes*	Alive in remission

*Patient #4 and #15 developed VOD after HSCT. #4: VOD occurred at day 10 after a haploidentical HSCT performed in PR. #15: VOD occurred at day 7 after HSCT, patient fully recovered after the event.

BM: bone marrow; C1: course 1; CR: complete remission; EMD: extramedullary disease; FU: follow up; HSCT: hematopoietic stem cell transplant; MRD: minimal residual disease; N/A: data not available; NR: no response; PR: partial response; Rel.: relapse;

In total, 24 InO courses were administered. Seven patients (46%) received only one course.

The median administered dose of InO at first course was 1.74 mg/m2 (divided in three weekly doses): eight patients received 1.8 mg/m2 (the adult approved dose), five intermediate dosages as 1.5 or 1.6 mg/m2, and two lower dosages (one at 1.4 mg/m2 and one at 1.2 mg/m2). Two patients were <1 year at the time of InO treatment: both received 1.8 mg/m2; four additional patients were ≤ 10 kg, all but one received 1.8 mg/m2 of Ino in course 1.

Seven patients (46.6%) achieved a CR, four after one course and three after 2 courses. One of these patients was treated for extra-medullary disease (subcutaneous lesions) and MRD positivity without overt bone marrow involvement. One additional MRD-positive patient turned MRD-negative (<0.01%). In total, seven of these eight responsive patients achieved an MRD level ≤0.1%, of whom three had levels <0.01%. Two patients had a partial morphological response (M2 marrow), and five patients had no response (35.7%).

No statistically significant difference was found in median age at diagnosis, WBC, sex, CD22 expression and KMT2A-rearrengment status between responders (*n*=8) and non-responders (*n*=7) (Supplementary Table 2).

Seven (46.6%) patients proceeded to HSCT after InO (4 in CR, one in PR and two after additional treatment following stable disease or partial response after InO), at a median time of 34 days (range 20-139) after the last InO dose. (Supplementary Figure 1)

Three patients were still alive at the time of last follow-up, all of whom underwent HSCT. One patient died in CR due to neurotoxicity described as toxic leukoencephalopathy; one patient due to VOD after HSCT performed in PR; the remaining 10 patients due to progressive disease (in four cases after post-HSCT relapse).

Median follow-up was 342 days (range 19–361 days). EFS at six months was 17.8% (95%CI 5.6%- 56.7%), and OS at six months was 46.7% (95%CI 27.2%-80.2%) (Figure 1).

Limited data about toxicity were reported for 11 out of 15 patients. Hematologic toxicity was the most frequently reported(thrombocytopenia 6/11 patients, grade 2-4). Febrile neutropenia and infectious toxicities were reported in four patients (36%). In the two patients <1 year at the time of InO treatment, thrombocytopenia and fever were reported as adverse events and no specific concern were raised for these two patients, as well as in the other four patients ≤10 kg.

One case of neurotoxicity described as toxic leukoencephalopathy was reported. About 3 months after HSCT, treatment with InO was started after a second relapse (previous treatment Interfant06, UKALLR3+Bortezomib). After one course of InO,

the patient experienced progressive reduced consciousness. Repetitive magnetic resonance imaging scans revealed progressive white matter changes compatible with toxic leukoencephalopathy. This event was considered potentially related to InO treatment by the treating physicians and resulted in death in CR.

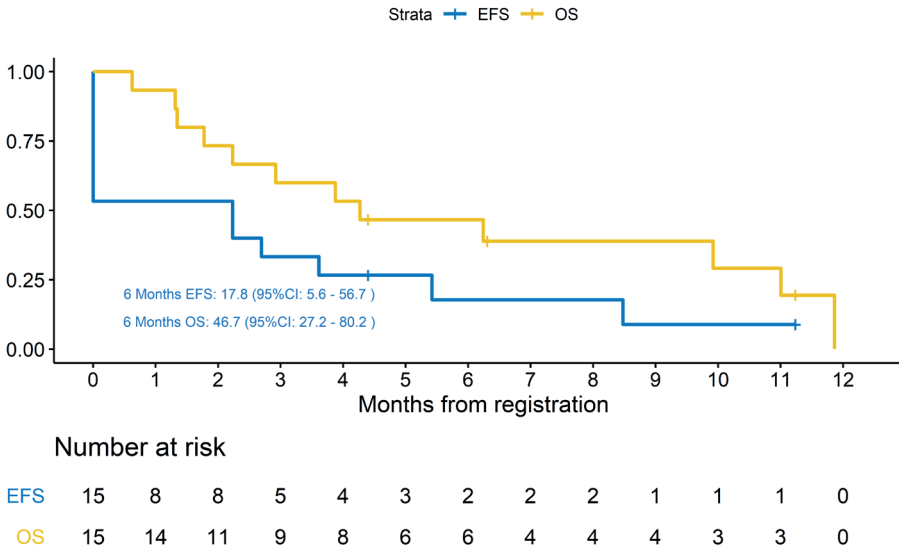


Figure 1. Event-free and overall survival

No patient developed VOD while receiving InO, including a patient treated with two courses of InO despite a previous event of VOD. Two patients developed VOD, after HSCT performed 20 and 21 days after the last Ino dose (1 course administered). Of these, one patient fully recovered and is still alive in CR. In the other case, the event occurred at day 10 after a haploidentical HSCT performed in PR and was considered the main cause of death. Both patients were not previously transplanted.

The percentage of blasts expressing CD22 at InO start was reported in 9/15 pts (median 72%, range 40%-100%); three patients had >90% of blasts expressing CD22. No difference was found in the median percentage of blasts expressing CD22 between patients achieving CR and non-responders (Supplementary Table 2).

Data on post-InO CD22 expression after relapse was available for three patients, and no CD22 negative relapse occurred.

Discussion

Single agent InO in infants and younger children resulted in a CR rate of 47% in this cohort of heavily pretreated patients with acceptable safety, comparable to the 40% ORR induced by a second-line chemotherapy reported by Tomizawa et al. (9) However, in our cohort, InO was used in all but one case as third line of treatment.

Our cohort showed a lower MRD-negativity rate compared both to the adult and the available pediatric data in older children treated with InO.(4-7) No descriptive results of the three patients aged 2-4 years included in the retrospective cohort published by Bohjwani; one KMT2A-r patient in the ITCC-059 cohort achieved CR and at relapse 7% of the blasts CD22 negative. (6, 10)

CD22 is highly expressed in more than 90% cases of childhood B-precursor ALL. Previous studies on *KMT2A*-r leukemia have shown a lower average CD22 antigen density, and a proportion of patients had sub-populations of blasts lacking CD22 expression.(11) However, to date, the level of CD22 expression has been reported not to correlate with response to CD22 targeted therapy (12). This is confirmed in our case series. (Supplementary Table 2)

The management of drug administration in very young patients is always challenging. Dosing is usually calculated according to the body weight, for patients below one year of age and/or below 10 kg for cytotoxic chemotherapeutic agents, to avoid high exposure due to the relatively high BSA in infants compared to body weight and due to impaired drug elimination. BSA and percentage of blasts in the peripheral blood are covariates that may be important predictors of variability in InO distribution and target mediated clearance.(13) InO dosing in our series was based on BSA, , although in some children dose reductions were applied, but , it did not result in increased toxicity. The median WBC in this series of R/R patients was similar to the data reported in other series.(7)

Infants and children below two years of age have a higher risk of VOD after transplant. In our case series, two cases of VOD in 7 patients treated with InO followed by HSCT were reported, but no cases of VOD occurred during InO treatment. No death was related to infections; one fatal event due to toxic leukoencephalopathy was considered possibly related to InO.

In pediatric BCP-ALL, salvage treatment options significantly improved with the use of blinatumomab and chimeric antigen receptor T-cells (CAR T-cells).(14, 15) A trial with blinatumomab after frontline induction chemotherapy, in medium and high risk infant ALL, is currently ongoing (EudraCT number: 2016-004674-17). Retrospective data on 11 infants, in first remission or after first relapse, who received blinatumomab

for persistent MRD prior to HSCT, showed that nine patients became MRD negative. (16) In our cohort, 53% of the patients were already treated with blinatumomab and two with CAR-T cells.

This is the first report focused on InO in a relatively large series of infant R/R ALL. Within the limitation of the retrospective nature of the study, InO appeared to be generally well tolerated and active. The median dose used was close to the adult recommended dose, suggesting that infants/young children might be treated at the same BSA-based recommended phase 2 dose established in the pediatric Phase I study on InO (ITCC-059 study).

Acknowledgements

All authors have contributed to the manuscript in significant ways: Zwaan CM and Brivio E designed the study; Chantrain CF, Gruber TA, Rialland-Battisti F, Contet A, Elitzur S, Dalla-Pozza L, Kállay KM, Li CK, Kato M, Markova I, Schmiegelow K, Bodmer N, Breese EH contributed in patients treatment and data collection and interpretation; Brivio E, Hoogendijk R, Thano A and Zwaan CM contributed to collection and assembly of data and provided data analysis and interpretation; Zwaan CM and Brivio E contributed also in literature search and editing the manuscript. All authors both have reviewed and agreed upon the manuscript content.

The authors would like to thank Andrea Biondi and the iBFM network for sharing the study within the collaborative group centers.

References

1. Pieters R, Groot-Kruseman Hd, Velden VVd, Fiocco M, Berg Hvd, Bont Ed, et al. Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 From the Dutch Childhood Oncology Group. Journal of Clinical Oncology. 2016;34(22):2591-601.
2. Pieters R, De Lorenzo P, Ancliffe P, Aversa LA, Brethon B, Biondi A, et al. Outcome of Infants Younger Than 1 Year With Acute Lymphoblastic Leukemia Treated With the Interfant-06 Protocol: Results From an International Phase III Randomized Study. J Clin Oncol. 2019;37(25):2246-56.
3. Driessen EMC, de Lorenzo P, Campbell M, Felice M, Ferster A, Hann I, et al. Outcome of relapsed infant acute lymphoblastic leukemia treated on the interfant-99 protocol. Leukemia. 2017;31(12):2854.
4. O'Brien MM, Ji L, Shah NN, Rheingold SR, Bhojwani D, Yi JS, et al. A Phase 2 Trial of Inotuzumab Ozogamicin (InO) in Children and Young Adults with Relapsed or Refractory (R/R) CD22+ B-Acute Lymphoblastic Leukemia (B-ALL): Results from Children's Oncology Group Protocol AALL1621. Blood. 2019;134(Supplement_1):741-.
5. Brivio E, Locatelli F, Lopez-Yurda M, Malone A, Diaz de Heredia C, Bielorai B, et al. A Phase I study of inotuzumab ozogamicin in pediatric relapsed/refractory acute lymphoblastic leukemia (ITCC-059 study). Blood. 2020.
6. Bhojwani D, Sposto R, Shah NN, Rodriguez V, Yuan C, Stetler-Stevenson M, et al. Inotuzumab ozogamicin in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. Leukemia. 2019;33(4):884-92.
7. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab Ozogamicin versus Standard Therapy for Acute Lymphoblastic Leukemia. N Engl J Med. 2016;375(8):740-53.
8. Corbacioglu S, Carreras E, Ansari M, Balduzzi A, Cesaro S, Dalle JH, et al. Diagnosis and severity criteria for sinusoidal obstruction syndrome/veno-occlusive disease in pediatric patients: a new classification from the European society for blood and marrow transplantation. Bone Marrow Transplant. 2018;53(2):138-45.
9. Tomizawa D, Koh K, Hirayama M, Miyamura T, Hatanaka M, Saikawa Y, et al. Outcome of recurrent or refractory acute lymphoblastic leukemia in infants with MLL gene rearrangements: A report from the Japan Infant Leukemia Study Group. Pediatr Blood Cancer. 2009;52(7):808-13.
10. Calvo C, Cabannes-Hamy A, Adjaoud D, Bruno B, Blanc L, Boissel N, et al. Inotuzumab ozogamicin compassionate use for French paediatric patients with relapsed or refractory CD22-positive B-cell acute lymphoblastic leukaemia. British Journal of Haematology. 2020;190(1):e53-e6.
11. Shah NN, Stevenson MS, Yuan CM, Richards K, Delbrook C, Kreitman RJ, et al. Characterization of CD22 expression in acute lymphoblastic leukemia. Pediatr Blood Cancer. 2015;62(6):964-9.
12. Kantarjian H, Thomas D, Jorgensen J, Kebriaei P, Jabbour E, Rytting M, et al. Results of inotuzumab ozogamicin, a CD22 monoclonal antibody, in refractory and relapsed acute lymphocytic leukemia. Cancer. 2013;119(15):2728-36.

13. Garrett M, Ruiz-Garcia A, Parivar K, Hee B, Boni J. Population pharmacokinetics of inotuzumab ozogamicin in relapsed/refractory acute lymphoblastic leukemia and non-Hodgkin lymphoma. J Pharmacokinet Pharmacodyn. 2019;46(3):211-22.
14. von Stackelberg A, Locatelli F, Zugmaier G, Handgretinger R, Trippett TM, Rizzari C, et al. Phase I/Phase II Study of Blinatumomab in Pediatric Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia. J Clin Oncol. 2016;34(36):4381-9.
15. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. New England Journal of Medicine. 2018;378(5):439-48.
16. Clesham K, Rao V, Bartram J, Ancliff P, Ghorashian S, O'Connor D, et al. Blinatumomab for infant acute lymphoblastic leukemia. Blood. 2020;135(17):1501-4.

Supplementary Material

Supplementary Tables

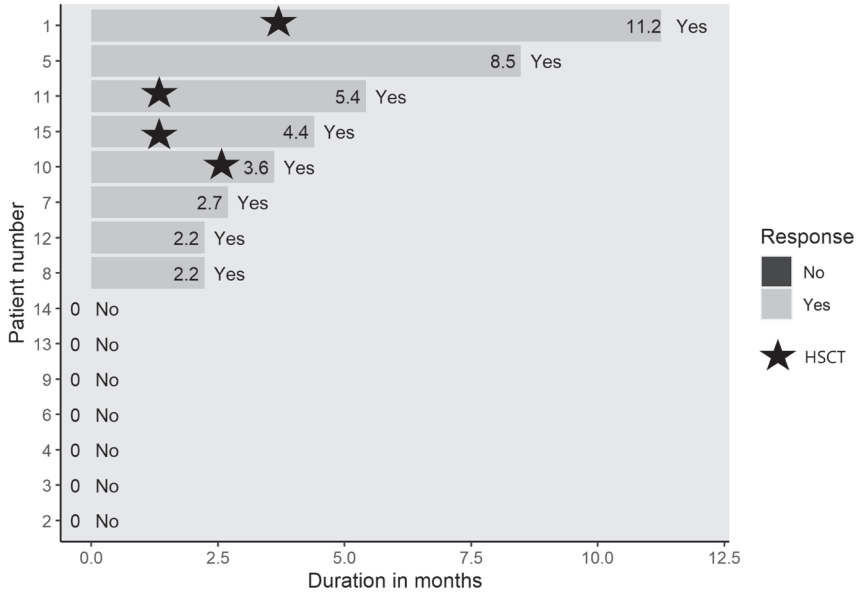
Supplementary table 1. Patients characteristics at start of Inotuzumab Ozogamicin treatment

Patient characteristics	**(n= 15)**	
Age, median (range), months	19.6 (7.5-35)	
Weight, median (range), kg	11.5 (5.8-15.9)	
BSA, median (range), m^2	0.5 (0.32-0.63)	
WBC, median (range), $x10^9/L$	4.3 (0.2-36.9)	
Time from diagnosis to Ino median (range), months	13.6 (6.4- 24)	
BM status		
MRD pos	3	*20.0%*
M2	2	*13.3%*
M3	10	*66.7%*
Disease status		
≥2nd relapse	6	*40.0%*
refractory 1st relapse	7	*46.7%*
1st relapse after HSCT	2	*13.3%*
Prior HSCT	6	*40.0%*
Prior anti-CD19 therapy	9	*60%*
Blinatumomab	7	
CAR-T	1	
Blinatumomab and CAR-T	1	
***KMT2A*-rearranged**	12	*80%*

BM: bone marrow; BSA: body surface area; HSCT: hematopoietic stem cell transplant; MRD: minimal residual disease; WBC: white blood cells.

Supplementary Table 2. Characteristics of responders and non-responders

	Responders	(n)	Non-responders	(n)	Total	(n)	*p-value*
Median % blasts expressing CD22 *(Range)*	80% *(52%-100%)*	(3)	69% *(40%-100%)*	(6)	72% *(40%-100%)*	(9)	*0.52*
Median age at diagnosis, months *(Range)*	3.6 *(0.1 – 27)*	(8)	4.4 *(0.1 – 22)*	(7)	4.4 *(0.1 – 27)*	(15)	*0.95*
Median WBC, x10.9/l *(Range)*	3.4 *(0.2 – 37)*	(8)	7.6 *(0.5 – 12)*	(7)	4.3 *(0.2 – 37)*	(15)	*0.28*
Sex - Female	63%	(5)	71%	(5)	67%	(10)	*>0.99*
KMT2A-*r*	75%	(6)	86%	(6)	80%	(12)	*0.55*



Supplementary Figure1. Duration of response
The figure shows the duration of response form the first achievemnt of CR after the start of InO treatment.

Chapter 6

Bosutinib in Resistant and Intolerant Pediatric Patients with Chronic Phase Chronic Myeloid Leukemia: Results from the Phase I part of Study ITCC054/COG AAML1921

Brivio E*, Pennesi E*, Willemse ME, Huitema ADR, Jiang Y, van Tinteren H, van der Velden VHJ, Beverloo BH, den Boer ML, Rammeloo LAJ, Hudson C, Heerema N, Kowalski K, Zhao H, Kuttschreuter L, Bautista Sirvent FJ, Bukowinski A, Rizzari C, Pollard J, Murillo-Sanjuán L, Kutny M, Zarnegar-Lumley S, Redell M, Cooper S, Bertrand Y, Petit A, Krystal J, Metzler M, Lancaster D, Bourquin JP, Motwani J, van der Sluis IM, Locatelli F, Roth ME, Hijiya N, Zwaan CM

*contributed equally

Submitted

Key points:

- The recommended phase 2 dose of Bosutinib for pediatric resistant or intolerant Ph+ CML patients was established at 400 mg/m^2/day
- The recommended phase 2 dose of Bosutinib for pediatric newly diagnosed Ph+ CML patients was based on bosutinib target exposure in adult newly diagnosed patients and established at 300 mg/m^2/day, extrapolated from safety and exposure data in R/I pediatric patients,
- Bosutinib shows an efficacy comparable to published data for other second generation TKIs approved in children with R/I Ph+ CML
- Gastro-intestinal adverse events are the most commonly reported side effects among these pediatric R/I patients

Abstract

PURPOSE Bosutinib is approved for adults with Chronic Myelogenous Leukemia (CML): 400 mg QD in newly diagnosed (ND); 500 mg in resistant/intolerant (R/I) patients with food. Bosutinib has a different tolerability profile than other TKIs, and potentially less impact on growth. The primary objective of this first-in-child trial was to determine the recommended phase 2 dose (RP2D) for pediatric R/I and ND patients.

METHODS: In the phase I part of this international, open-label trial (NCT04258943), children age 1 to <18 with R/I (per ELN 2013) Ph+ CML were enrolled using a 6+4 design , testing 300, 350 and 400 mg/m^2 QD with food. The RP2D was the dose resulting in 0/6 or 1/10 DLTs during the first cycle, and achieving adult target AUC levels for the respective indication. As ND participants were only enrolled in Phase 2, the ND RP2D was selected based on data in R/I patients.

RESULTS: Thirty patients were enrolled; 27 were evaluable for DLT: 6 at 300 mg/m^2, 11 at 350 mg/m^2 (1 DLT), and 10 at 400 mg/m^2 (1 DLT). The mean AUCs at 300 mg/m^2, 350 mg/m^2 and 400 mg/m^2 were 2203 ng·hr/mL, 2516 ng·hr/mL and 2661 ng·hr/mL. Most common AE was diarrhea (93%; ≥ grade 3: 11%). Seven patients stopped due to intolerance, eight due to insufficient response. All patients achieving or entering the study in Complete Cytogenetic Response and/or Major Molecular Response maintained the response except two.

CONCLUSION: Bosutinib was safe and effective. The pediatric RP2D was 400 mg/m^2 QD (max 600 mg/day) with food in R/I patients and 300 mg/m^2 QD (max 500 mg/day) with food in ND patients, which achieved targeted exposures from adult experience.

Context panel

key Objectives

The primary objective of the Phase I part of this trial was to define the RP2D of bosutinib in R/I and ND pediatric patients with Ph+ CML, based on tolerability and pharmacokinetic exposure. The RP2D for ND patients (enrolled only in the Phase II part of the study) was based on the exposure (adult AUC target for ND patients at 400 mg/day) and safety data in R/I patients. We also aimed at assessing bosutinib preliminary anti-leukemic activity in this R/I cohort.

Knowledge Generated

The RP2D of bosutinib in R/I pediatric patients with Ph+ CML was established at 400 mg/m^2 QD (max 600 mg/day), based on safety data and on target exposure as defined in adults. Only 2 DLTs occurred during Phase 1 dose escalation, the first consisting of both nausea/vomiting and diarrhea (350 mg/m^2 dose level), and the second was transaminases elevation (400 mg/m^2 dose level). The most commonly occurring adverse event was low grade gastrointestinal toxicity, with more than 90% of patients experiencing diarrhea. The cumulative incidence of Major Cytogenetic Response (MCyR) and Complete Cytogenetic Response (CCyR) at six months, excluding patients with CCyR at screening, was 83.3% (95% CI: 40.5−96.4%), and the cumulative incidence of MMR, excluding patients with MMR at screening, was 26.1% (95% CI: 10.3−45.2%). The dose for ND patients was based on the safety and PK data in R/I children and target exposure as derived from adults, and established as 300 mg/m^2 (max 500 mg/day). The effect on bone growth will be better characterized in patients naïve to TKIs and with a longer follow up, which are enrolled in the ongoing phase II part of the ITCC-054/COG AAML1921 study.

Relevance

Approximately one in three children has an unsatisfactory response or intolerance to imatinib, which is often used as first line of treatment in children with Ph+ CML. For these patients dasatinib QD and nilotinib BID are currently approved, but the latter requires administration under fasting conditions. Bosutinib might represent an alternative in pediatric patients not responding or intolerant to the other TKIs, with a different safety profile (more gastrointestinal and less musculoskeletal toxicity). In addition, if the data on preservation of bone growth observed in animal models are confirmed in the clinical setting, bosutinib might have an advantage for children with Ph+ CML, as it might avoid impairment of longitudinal growth, particularly in those diagnosed at prepubertal age. This however requires longer follow-up of patients recruited in this study, especially in the newly diagnosed cohort in the phase 2 part of the study (not reported here)

1. Introduction

Chronic Myeloid Leukemia (CML) is a rare disease in children, accounting for approximately 3% of all pediatric leukemias, and an annual incidence of around 1 per million children under the age of 15 years.(1,2) The leukemia is caused by the characteristic t(9;22)(q34;q11.2) translocation resulting in the *BCR-ABL1* fusion oncogene (Ph+). CML in children is considered a more aggressive disease than in adults.(3)

The introduction of tyrosine kinase inhibitors (TKIs) targeting the BCR-ABL1 protein, such as imatinib, has drastically improved the prognosis of Ph+ CML.(4) In 2003, the FDA (Food and Drug Administration) approved imatinib for children with Ph+ CML, followed by EMA (European Medicines Agency). With imatinib more than 90% of children achieve complete hematologic response (CHR) and around 60% achieve Complete Cytogenetic Response (CCyR) after 1 year of treatment.(5–8) However, in the long term, approximately 30% of children have an unsatisfactory response or intolerance to imatinib.(8) Dasatinib is the best studied second generation TKI, approved for pediatric indication.(9–12) In imatinib resistant/intolerant (R/I) patients with chronic phase Ph+ CML, 82% achieved CCyR with dasatinib.(11) Later, nilotinib was also approved for paediatric patients. In those resistant to previous TKIs, Nilotinib led to around 80% achieving or maintaining CCyR after two years, but it requires twice daily dosing under fasting conditions, as food intake might increase exposure. (13,14) Side effects of imatinib and dasatinib mostly consist of musculoskeletal pain, asthenia and skin rash.(1,8,11,12) Nilotinib may less frequently cause musculoskeletal pain, but more frequently increased bilirubin, nausea, and vomiting.(2,4,13)

Bosutinib is a dual Src and Bcr-Abl inhibitor, approved for adults with ND chronic phase (CP) Ph+ CML at the recommended dose of 400 mg orally once daily (max 600 mg), and 500 mg once daily (max 600 mg) for patients in CP, accelerated phase (AP), and blast phase (BP) previously treated with one or more TKIs.(15) Treatment with bosutinib in adults is mostly associated with transient gastrointestinal toxicities, such as diarrhea, nausea, abdominal pain, rash, and increased transaminases, while musculoskeletal pain is less frequent than observed with imatinib and dasatinib. (2,4,16) Furthermore, when compared with nilotinib, it has the advantage of once daily administration without the need of fasting.

Of particular relevance for children, there is also evidence that long-term exposure to imatinib results in growth impairment and dysregulation of bone metabolism, especially in prepubertal children.(1,17) The mechanism of impaired growth may be related to *"off-target"* TKI inhibition, such as inhibition of c-KIT and PDGF-R, and/

or the development of an acquired growth hormone deficiency.(18–21) On the contrary, preclinical data indicated that this toxicity may not be observed or be less prominent with bosutinib.(22)

We here report the results of the phase I part of the ITCC-054/COG AAML 1541 trial which aims at evaluating the safety, tolerability and pharmacokinetics (PK) and preliminary efficacy of bosutinib, in order to identify a recommended phase II dose (RP2D) for R/I and ND pediatric patients with Ph+ CML. For ND patients (not enrolled here), the RP2D was derived from the exposure and safety data obtained from R/I patients and compared to the recommended exposure in ND adults.

2. Patients and methods

2.1 Study Design and Patients

ITCC-054/COG AAML1921 (NCT04258943) is a phase I/II multicenter, single-arm, open-label study conducted in Europe, Israel and the USA in the context of a Pediatric Investigation Plan and a Pediatric Written Request. The study was conducted in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, ICH Guidelines for Good Clinical Practice, the Declaration of Helsinki, and approved by the Institutional Review Board or Ethics Committee in all participating centers. The study is sponsored by the Erasmus Medical Center in Europe and the Children's Oncology Group (COG) in the United States, it is open in 21 sites in Europe, most of them involved in the Innovative Therapies for Children with Cancer (ITCC) Consortium, and 45 COG sites, and funded by Pfizer.

Eligible patients were aged ≥1 to <18 years at enrolment, had a diagnosis of Ph+ CML (either in chronic, AP, or BP phase), and were resistant or intolerant to at least one prior TKI treatment per 2013 European Leukemia Net criteria, and did not suffer from major organ toxicities.[23] Main exclusion criteria consisted of known T315I or V299L BCR-ABL1 mutations, leptomeningeal leukemia for AP/BP patients, and extramedullary disease only (full list of in/exclusion criteria in Supplementary Table 1). A modified rule based design (6+4) following the principles of the Rolling six design was used. We defined the RP2D as the dose resulting in 0/6 or 1/10 dose limiting toxicities (DLTs, definition in table 1) in patients who received ≥ 75% of the planned dose in cycle 1, and a geometric mean area under the concentration-time curve (AUC) of 3150 ng·hr/mL (±20%) for R/I patients, and 2270 ng·hr/mL (±20%) for ND patients (equivalent to the exposure in adults at approved dosages of 500 mg and 400 mg once daily respectively).

Table 1. Definition of Drug Limiting Toxicity

Non-hematologic AEs*	Hematologic AEs*
Any Grade ≥3 toxicity, despite optimal treatment	Grade 4 neutropenia or thrombocytopenia AND lasting ≥7 days (not explained by persistent leukemia)
Any Grade ≥2 toxicity requiring discontinuation/interruption for ≥7 days	
Clinically significant laboratory abnormality Grade ≥3 AND lasting ≥7 days despite optimal treatment	

*AEs were graded based on the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 and assessed only during the first cycle of treatment (28 days)

Target AUC levels were based on an updated population PK analysis conducted by Pfizer Inc using pooled data (n=1401) from ten clinical studies in adult patients with Ph+ CML or solid tumors and in healthy volunteers treated with bosutinib (in press). The 6+4 design was chosen to allow a better characterization of the PK parameters defining the RP2D, based on a simulation study showing that six to ten patients are needed to demonstrate that target exposure is in the adult range.(24) Pharmacokinetic sampling was mainly performed on day 14 of cycle 1, after the achievement of the steady-state. The RP2D in ND patients was extrapolated from PK and safety data obtained in R/I subjects, as well as target exposure based on adult data. The protocol was amended (Version 4.0 10-Oct-2019), to add a new cohort of ND patients in chronic phase in the phase 2 part of the study, after approval of bosutinib for this indication in adults (Amendment history is Supplementary Table 2).

2.2 Study Treatment

Dose levels were assigned at study entry and a treatment cycle was defined as 28 days, regardless of missed doses or dose delays. Available formulations included tablets (dissolved and used for nasogastric administration if needed) and capsules (which could be opened and sprinkled on food, eg. either apple sauce or yoghurt, and consumed immediately), which could be used in combinations. The bioequivalence of these formulations was established based on data from the trial NCT04549480 and capsule administration instructions with food and vehicle were supported by NCT05032690 and NCT04916769 provided by Pfizer Inc (data in press).

Dose levels were amended in version 4 as the exposure observed at in the first patients treated at 300 mg/m^2 were insufficient to yield the target AUC for R/I patients. Finally, we tested daily doses of 300 mg/m^2, 350 mg/m^2 and 400 mg/m^2 (BSA was calculated per the

Mostellar formula(25)), taken once daily with food, and rounded up to the closest capsule or tablet equivalent (supplementary table 3). Maximum daily dose was capped at 600 mg in R/I patients as per the bosutinib adult label. Moderate or strong CYP3A inducers and inhibitors were prohibited, as well as proton pump inhibitors (short-acting antacids or H2 blockers were allowed). During the first cycle, primary prophylaxis for diarrhea, nausea and vomiting was not permitted. For subsequent cycles the decision was left at investigator discretion. Specific guidance for the management of gastrointestinal side-effects was provided (protocol section 7.10.5, provided as supplement).

2.3 Endpoints and Assessment

Initially, the primary objective was to determine the RP2D of bosutinib for R/I pediatric patients with chronic phase Ph+ CML, based on DLTs in the first cycle and exposure levels recommended in adults. Upon approval of bosutinib for ND adults, the protocol was amended to include determining the RP2D also for ND children (protocol v. 4, 2019) as a primary objective. However, as ND subjects were not enrolled in this phase I, the RP2D for ND patients was based on safety and exposure obtained in R/I subjects, and based on exposure levels recommended in ND adult patients. Secondary objectives included overall safety and preliminary anti-leukemic activity.

The primary endpoints (Supplementary Table 4) were the incidence of DLTs during the first 28 days of treatment and PK parameters such as the maximum observed plasma concentration (Cmax), time to Cmax (Tmax), and AUC. Secondary endpoints for toxicity included the estimation of the frequency and severity of adverse events (AEs), laboratory abnormalities, electrocardiogram (ECG) abnormalities (graded using CTCAE 4.03). Secondary endpoints for efficacy included the proportion and cumulative incidence of CHR, Major CyR (MCyR), CCyR and Major Molecular response (MMR),; and the Event Free Survival and Overall Survival (EFS and OS) probabilities (endpoints definitions are provided in Supplementary Table 5). As a post hoc analysis the cumulative incidence of treatment discontinuation due to unsatisfactory response and intolerance was calculated. Exploratory endpoints included effects of bosutinib on growth and bone metabolism and palatability of the bosutinib formulations (not presented here).

2.4 Sample size and Statistical analysis

The sample size of phase I was dependent on the number of dose levels tested, using a modified rule based design (6+4), as cohorts of 6-10 patients were needed to properly assess PK.(24)

The safety analysis set included all enrolled patients who received ≥1 dose of study drug. The per protocol analysis set (evaluable for DLT/RP2D) included patients who received ≥75% (>21 doses) of the planned 28 doses of bosutinib in cycle 1, unless less dosages were given due to a DLT, and who provided a sufficient number of PK samples to estimate the AUC of bosutinib. The PK concentration set included all enrolled patients who had at least one reportable bosutinib plasma concentration. Cumulative incidence of CHR, MCyR, CCyR and MMR (with/without patients already with response at study entrance as an event at time zero), EFS, and OS were estimated using the Kaplan–Meier method; while the cumulative incidence of treatment discontinuation was estimated using a competing risk setting (insufficient response versus intolerance).

3. Results

3.1. Patients

Between 14 November 2016 and 19 September 2022 (data cut-off for this analysis), 30 patients were screened, 29 were enrolled, 28 were treated (safety and efficacy analysis set), 27 were evaluable for DLT, and 26 patients were evaluable for the RP2D set. One patient was a screen failure; another patient did not start the treatment (due to low absolute neutrophil count (ANC)); one patient was not evaluable for DLT because the treatment lasted <21 days in cycle 1; and another patient was not evaluable for the RP2D due to prohibited use of proton pump inhibitors during cycle 1 (Supplementary Figure 1). Baseline demographics of all treated patients are summarized in Table 2. All patients were in CP at enrolment. Median age was 12 years (range, 1 - 17), 16 (57.1%) were male, 23 (82.1%) were resistant to prior TKIs, while 5 (17.9%) were intolerant, 15 (53.6%) patients were in CCyR at study entry, of which 5 (17.9%) were in MMR; 16 (53.6%) patients received one prior TKI, 9 (32.1%) two lines of treatment and 3 (10.7%) three previous TKIs.

Table 2. Patient characteristics (N=28, all patients receiving at least one dose of Bosutinib)

Characteristic	Total number of patients (%)
Gender	
Male	16 (57.1%)
Female	12 (42.9%)
Age at enrollment (in years)	
Median (range)	12 (1-17)
Age category, n (%)	Age category, n (%)
>1– ≤6 years	6 (21.4%)
>6– ≤12 years	12 (42.9%)
>12 years	10 (35.7%)

Table 2. Continued

Characteristic	Total number of patients (%)
Reason of enrollment	
Resistant	23 (82.1%)
Intolerant	5 (17.9%)
Previous line of treatment (Number of previous TKIs)	
One	16 (57.14%)
Two	9 (32.14%)
Three	3 (10.7%)
Time from diagnosis (in months)	
Median	17
Range	3 - 82
Response status at enrolment	
Complete Cytogenetic Response (CCyR)	15 (53.6%)
Major Molecular response (MMR/≥MR3)	4 (14.3%)
MR2	9 (32.1%)
MR1	7 (25.0%)
No MR	5 (17.9%)
MR n/a	3 (10.7%)

Overall, 490 bosutinib 28-day cycles (median cycles per patient: 15; range: 1–66) were administered. Eleven patients were still on treatment at time of data cut-off. The median follow-up was 23.8 months (range 1.8 - 61.5).

3.2 Safety

Six patients were enrolled at 300 mg/m^2 (median number of cycles 17; range 1-66), without DLTs. The dose was escalated to 350 mg/m^2, and 11 patients instead of 10 were enrolled since two patients consented simultaneously (median 10 cycles, range 1-25). One DLT occurred consisting of both, grade 3 nausea/vomiting and diarrhea not responding to treatment. This patient continued at 250 mg/m^2 (as allowed by protocol) and discontinued the treatment after seven cycles due to increased transaminase levels. The dose was further escalated to 400mg/m^2, and 11 patients were treated (median 13 cycles, range 0-21); as one subject was not evaluable for DLTs (due to insufficient number of doses received), hence the patient was replaced. One patient experienced a DLT (grade 3 transaminase increase, grade 2 bilirubin increase, and grade 3 rash with treatment interruption $>$ 7 days) which resolved completely. The patient continued the treatment at a reduced dose of 300mg/m^2.
Overall, 28 (100%) patients had at least one AE; the most common were diarrhea (93 %, n=26), abdominal pain (71%, n=20), vomiting (68%, n=19), nausea (61%, n-12), maculo-papular rash or other skin disorders (39%, n=11; and 43%, n=12 respectively). Among those assessed as related to bosutinib diarrhea (n=14, 50%), vomiting (n=11, 39%), and abdominal pain (n=10, 36%) were the most common (see Table 3 and Supplementary Table 6).

Table 3. Most frequent adverse events (frequency >10%)

Adverse Event Term	Gr. 1-2	Gr ≥ 3	Gr 1-2 related to bosutinib*	Gr. ≥ 3 related to bosutinib*
Diarrhea	23 (82%)	3 (11%)	12 (42%)	2 (7%)
Abdominal pain	19 (68%)	1 (4%)	9 (32%)	1 (4%)
Vomiting	16 (57%)	3 (11%)	9 (32%)	2 (7%)
Nausea	17 (61%)	0	11 (39%)	0
Fever	11 (39%)	1 (4%)	3 (11%)	0
Skin and subcutaneous tissue disorders	11 (39%)	1 (4%)	1 (4%)	1 (4%)
Rash maculo-papular	8 (29%)	3 (11%)	1 (4%)	2 (7%)
Headache	9 (32%)	1 (4%)	2 (7%)	0
Alanine aminotransferase increased	4 (14%)	5 (18%)	2 (7%)	4 (14%)
Fatigue	7 (25%)	1 (4%)	1 (4%)	0
Pain in extremity	7 (25%)	1 (4%)	1 (4%)	0
Constipation	7 (25%)	0	0	0
Gastrointestinal disorders	7 (25%)	0	2 (7%)	0
Anorexia	6 (21%)	0	1 (4%)	0
Creatinine increased	6 (21%)	0	2 (7%)	0
Infections and infestations	5 (18%)	1 (3%)	0	0
Metabolism and nutrition disorders	6 (21%)	0	0	0
Stomach pain	6 (21%)	0	3 (11%)	0
Aspartate aminotransferase increased	3 (11%)	2 (7%)	2 (7%)	0
Rhinitis infective	5 (18%)	0	0	0
Cough	4 (14%)	0	0	0
CPK increased	4 (14%)	0	0	0
Flatulence	4 (14%)	0	1 (4%)	0
General disorders - Other	4 (14%)	0	1 (4%)	0
Platelet count decreased	3 (11%)	1 (4%)	1 (4%)	1 (4%)
Rash acneiform	4 (14%)	0	0	0

* probably and definitely related based on the treating physician's judgment.

Excluding events ongoing at time of dataset lock, gastrointestinal AEs such as nausea, vomiting, diarrhea and abdominal pain had a median resolution time of 12 days; while skin disorders, such as rash, had a median resolution time of 29 days. Nevertheless, these AEs were still ongoing in 14 patients at the time of dataset lock, including some with prolonged episodes of diarrhea, nausea/vomiting or skin rash. Among grade 3 and 4 AEs, the most common were transaminase elevation (18%, n=5), maculo-papular rash (11%, n=3), vomiting (11%, n=3), and diarrhea (11%, n=3). Musculoskeletal toxicities, such as myalgia, pain at the extremities, and CPK elevation occurred in 3 (11%, 1 at

grade 3), 8 (29%, 0 at grade 3), and 4 (14%, 0 at grade 3) patients, respectively. No grade 5 AEs occurred. Laboratory and hematological abnormalities are summarized in Supplementary Table 7. No patients developed a clinically significant prolonged QTc during treatment (see supplementary table 8). No cases of arrhythmia were registered, nor abnormalities in cardiac function were detected at the echocardiograms performed at screening and every 12 months. Eleven patients had their dose level reduced due to AEs, and seven stopped the treatment due to intolerance.

3.3 Pharmacokinetics

386 samples were received from 27 patients and included in this analysis. The geometric mean AUCs at 300 mg/m^2, 350 mg/m^2 and 400 mg/m^2 were 2203 ng·hr/mL (range 1540 - 3070), 2516 ng·hr/mL (range 1850 - 4620) and 2661 ng·hr/mL (range 1470 - 3920), respectively. The geometric mean peak plasma concentrations at 300 mg/m^2, 350 mg/m^2, and 400 mg/m^2 were 188.5 ng/mL, 221.2 ng/mL, and 198.1 ng/mL, respectively and it was generally reached approximately 3 hours after the administration across all dose levels. The geomean trough concentrations at 300 mg/m^2, 350 mg/m^2 and 400 mg/m^2 were 46.58 ng/mL, 46.33 ng/mL, and 48.88 ng/mL, respectively. In 10 patients (33%) the capped dose of 600 mg/day was administered, five of which at the highest dose level of 400 mg/m^2/day. The target steady state exposure for ND patients (2370 (±20%) ng·hr/mL) was achieved at 300 mg/m^2/day, while for R/I patients the target exposure (3150 (±20%) ng·hr/mL) was achieved at 400 mg/m^2/day.

3.4 Efficacy

At data cut-off date, all patients had achieved and/or maintained CHR. Including patients already with the response at screening, at 6 months the cumulative incidence of first time achieving both MCyR and CCyR was 92.9% (95% CI: 69.8−98.5%), and it was maintained constant at 12 months. The cumulative incidence of first time achieving MMR was 36.6% (95% CI: 19.0−54.5%) and 47.8% (27.9−65.3%) and 6 and 12 months respectively. Considering only patients that achieved MCyR, CCyR and/or MMR for the first time on study (hence excluding patients with MCyR (n=16), CCyR (n=16) and MMR (n=4) at screening at all dose levels), the cumulative incidence of both MCyR and CCyR at 6 months was 83.3% (95%CI: 40.5-96.4), and it was maintained constant at following timepoints; while the cumulative incidence of MMR at 6 months was 26.09% (95%CI: 10.3-45.2) and increased to 39.13% (95%CI: 19.4-58.5) at 12 months to remain constant thereafter (Figure 1B). In this cohort, the median time to respond was 3 months, for

MCyR and CCyR; and 28 months for MMR, respectively. Five patients achieved MR4/MR4.5 responses while on treatment with bosutinib. All patients that achieved or entered the study in MCyR, CCyR and/or MMR maintained the response except two patients, who lost CCyR after 15 and 31 cycles, respectively. From a post-hoc analysis, no clear differences in the cumulative incidence of MMR or CCyR were observed across the dose levels (Supplementary Figure 2 and 3), but the study was not powered to detect them.

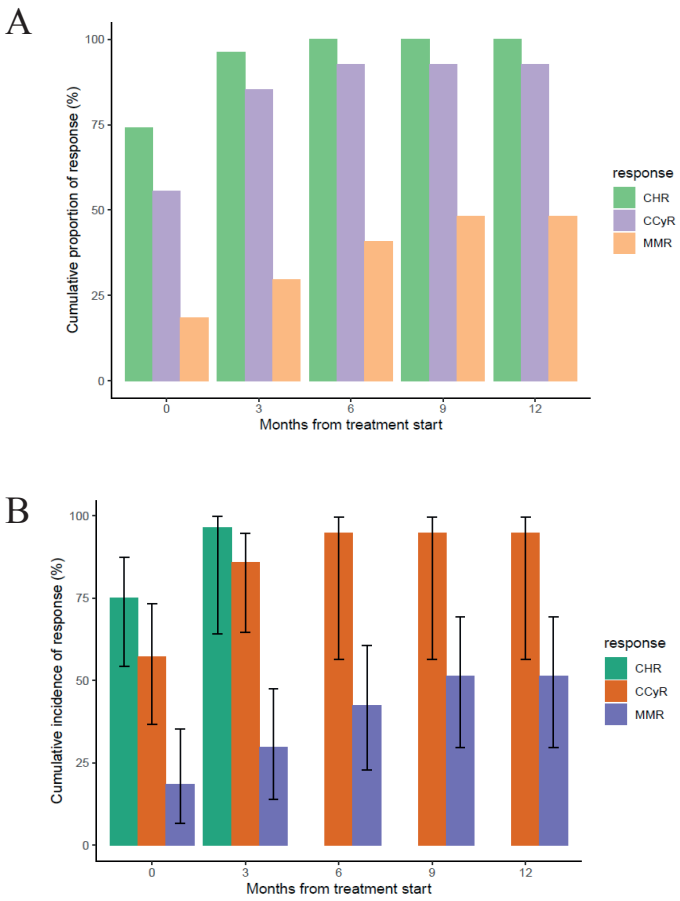


Figure 1. Proportions and cumulative incidence of patients with response**.**
A. Proportions of patients in Complete Hematologic Response (CHR, green bar), Complete Cytogenetic Response (CCyR, purple bar), and Major Molecular Response (MMR, orange bar) at study entrance, 3, 6, 9 and 12 months. As best response, the proportions of CHR, CCyR and MMR by end of treatment were 100%, 92.86%, and 50% respectively. **B.** Cumulative incidence of CHR (green bars), CCyR (orange bars) and MMR (purple bars). Patients with response at screening are shown as event at time zero. All patients achieved CHR by month 4, therefore the cumulative incidence for CHR at later time points is NA. No patients lost CHR.

The 12 and 24 months OS probabilities were 100% (95% CI: na); only one death occurred due to meningitis after HSCT, 15 months after the last dose of bosutinib, and 30 months after enrolment. EFS at one, two, and three years was 100% (95%CI: 100-100%), 95.65% (95%CI: 87.67-100%), 72.88% (95%CI: 49.32-100%), respectively (Figure 2) with four patients at risk afterwards.

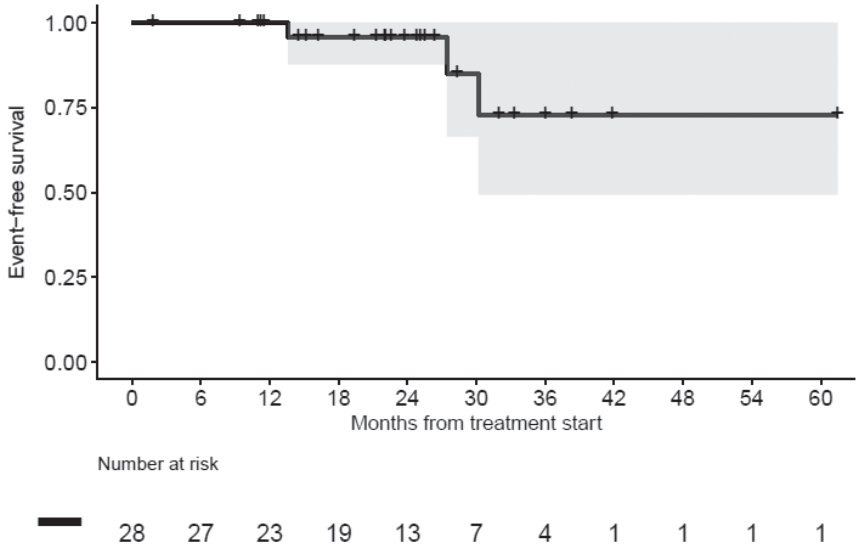


Figure 2. Event Free Survival (Kaplan-Meier method).
Events were defined as either: 1. Death due to any cause. 2. Transformation to accelerated phase or blastic phase at any time. 3. Loss of Complete Hematologic Response (CHR, as defined in supplementary), 4. Loss of Complete Cytogenetic Response (CCyR, as defined in supplementary), 5. Loss of Major Molecular Response (MMR, as defined in supplementary), 6. For patients not achieving a CHR: doubling of WBC at least 1 month apart with the second value > 20 x 10^9/L and maintained in subsequent assessments for at least 2 weeks. Only one patient died, one lost CCyR, and one lost both CCyR and MMR (counted as one event at the time of loss of CCyR).

At time of data cut-off, 8 patients stopped the treatment due to insufficient response per investigator judgment, of which two were treated at 300 mg/m^2, three at 350 mg/m^2, and three at 400 mg/m^2. As a post-hoc analysis, the cumulative incidence of treatment discontinuation is shown in Figure 3. Three of the patients which did not obtain sufficient response underwent HSCT, one of which after receiving further treatment with dasatinib. We did not record emergent mutations of T315I or V299L over the study period.

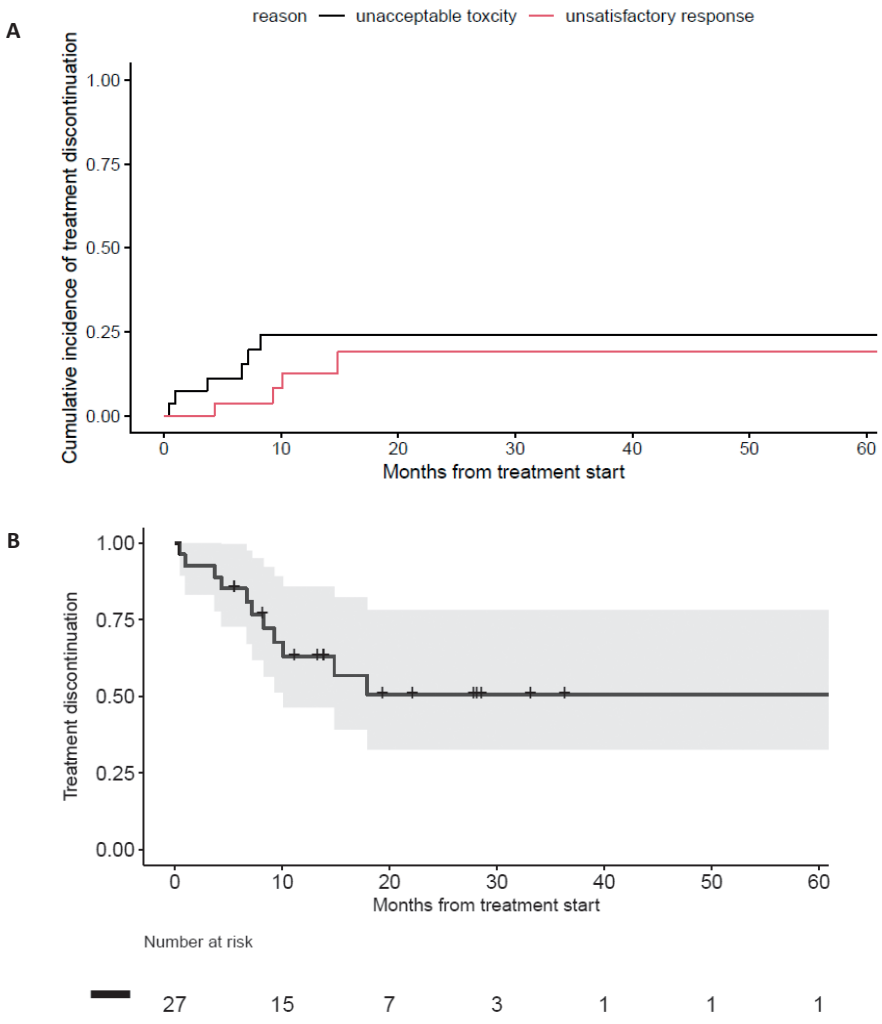


Figure 3. Cumulative incidence of treatment discontinuation.

Panel A: Cumulative incidence of treatment discontinuation as per investigator judgment in a competing risk setting. Discontinuation due to insufficient response is displayed in red and included loss of Complete Cytogenetic Response (CCyR) and/or Major Molecular Response (MMR) or failure to achieve CCyR and/or MMR. Discontinuation due to toxicities is displayed in black. Patients having a dose limiting toxicity discontinued the treatment within the first month (black line). **Panel B:** Cumulative incidence of treatment discontinuation due to all causes.

Discussion

In this first-in-child dose-finding study, bosutinib showed a tolerability profile consistent with the known safety profile in adults. Only two DLTs at different dose levels (350 and 400 mg/m^2) were reported. The RP2D for R/I patients was established at 400 mg/m^2 (max 600 mg/day). The RP2D for ND patients, extrapolated from safety and exposure data in R/I pediatric patients and based on the AUC of the recommended dose for ND adult patients, was established at 300 mg/m^2 (max 500 mg/day).

The most common adverse events with bosutinib were gastrointestinal toxicities, with almost all patients experiencing at least mild (grade 1 and 2) events, but rarely of grade 3 or higher (approximately 10% of the cases for diarrhea and vomiting), and usually resolving within approximately 2 weeks. Nevertheless, this frequency is higher when compared to imatinib and dasatinib, in which grade 3 and 4 gastrointestinal toxicities occurred in 2-5% of patients only.(26,27) Moreover, a small proportion of patients have bosutinib-related gastro-intestinal complaints that persist, and which may affect quality of life in these children. On the contrary, while muscle cramps, musculoskeletal pain, and myalgia are reported in 40% to 50% of patients treated with imatinib, these events were less common with bosutinib (~10-15%), particularly at grade 3 or higher.(26–28) Another regular adverse event observed in this trial was skin rash, which occurred in 40% (n=11) of the subjects, including grade 3 or 4 skin rash in 11% (n=3). Such frequencies are however comparable to published data for other TKIs used in children.(8,12,13) As observed in adult patients, the impact of bosutinib on cardiac function was negligible with no cases of clinically significant QTc prolongation, nor functional abnormalities based on echocardiography, confirming previous studies in adults, but longer follow-up may be needed to better assess cardiac side effects.(28) Seven patients discontinued bosutinib based on tolerability.

It remains to be established whether bosutinib might show a less toxic profile on longitudinal growth as demonstrated in murine models.(20,22) Currently, all TKIs approved in children for Ph+ CML show a negative impact on height, especially when started prior to puberty.(21,29) The potential benefit of bosutinib from this point of view will be better evaluable in our phase II cohort in ND patients, while the limited follow up, and more importantly the enrolment of pretreated subjects precludes a firm assessment in the R/I cohort.

In terms of PK, the AUC increased almost linearly with each tested dose level, even if 50% of the patients treated at 400 mg/m^2 actually received the capped maximum dose of 600 mg/day, rather than the BSA adjusted dose. This might suggest that the solubility in the GI tract and saturation of absorption was not reached in the

investigated dose ranges. Such phenomenon was observed in adults in a dose range of 500-600 mg per day, which was the reason for capping the maximum daily dose at 600 mg in our R/I cohort.(28,30) Nevertheless, a higher BSA-adjusted dose was necessary in younger children to achieve the target exposure comparable to exposure as defined in adult studies, whereas in older children the dose was capped as in adults if the BSA-adjusted dose was higher than 600 mg. These differences in pharmacokinetics might be influenced by a different absorption of the drug in younger children, who generally have an higher stomach pH compared to adults and less water in the gastro-intestinal tract.(31) In addition, although bosutinib was instructed to be administered following a meal, food intake was not standardized in our trial, while playing a role in its disposition. Indeed, bosutinib is a Biopharmaceutics Classification System (BCS) class II or IV drug, characterized by low permeability and low solubility, the latter being pH-dependent and increased by food intake, especially when rich in fat.(30)

In general, in adults, higher concentrations were associated with higher probability of response, likely reaching the plateau of exposure-efficacy at recommended doses in adult.(15) For example, in adults resistant to imatinib, it was suggested that individual bosutinib dose intensities of $\geq$ 350 mg/day were associated with an increased rate of MCyR.(24,28) In our study we did not identify a clear dose-efficacy relationship, which might be due to the limited sample size of each tested dose level or that participants are at or near the exposure-efficacy plateau.

In terms of preliminary efficacy, the cumulative incidence of CCyR and MMR with bosutinib appears comparable to the other published phase I/II trials with 2nd generation TKIs.(12,13) These figures are also similar to adult data from R/I patients, where the CCyR and MMR rates were respectively 80.6% and 70.5% at 1 year.(32) The main reasons to discontinue treatment in this study were attributable to intolerance and loss of response/insufficient response, which were approximately equally divided. It is worth noting though, that Ph+ CML in children is usually more aggressive than in adults, showing a higher tumor load and WBC at presentation, and potential differences in gene expression (e.g. downregulation of Rho pathways) and/or breakpoint distribution.(3,4,33)

Currently, two other 2nd generation TKIs are being tested in children. A dose finding trial of asciminib (targeting the ABL Myristoyl Pocket STAMP) in children is ongoing (NCT04925479).(34) In adults, the phase III ASCEMBL trial reported a MMR rate after six months of 25.5% with asciminib and 13.2% with bosutinib, and treatment discontinuation due to toxicities occurred in 6% and 21% cases respectively.

(35) Ponatinib is the other TKI under investigation in children. In adults, it proved effective, particularly in those carrying the T315I mutation, but at the expense of more frequent cardiovascular events than with other TKIs, although this may be dose dependent.(36,37) In children, a phase I/II trial is ongoing (NCT03934372), and for now data are mostly based on case reports and cancer registries.(38,39)

Since Ph+ CML is a very rare disease in children, one of the main limitations of this trial was the slow enrolment rate. It took six years to complete the enrolment in the phase I part, despite adding additional centers in North America since 2019, and finally recruiting in over 60 centers globally. The number of TKIs now approved for children reduces the number of eligible patients for dose-finding trials. To resolve this problem, it might be crucial to limit the number of dose-levels that need to be tested, and use PK-modelling to define the starting dose.

In conclusion initial data from the Phase 1 portion of this study indicates bosutinib is likely to be safe and effective in the R/I pediatric population. The phase II part of the trial, enrolling ND and R/I patients, is ongoing.

Acknowledgements

The authors would like to thank the ITCC consortium and the COG study group for providing the infrastructure and the collaborative environment to run early clinical trials in pediatric oncology, and Pfizer Inc. for providing funding and a clinical research collaboration to implement the study. Sponsorship for the study was provided by Erasmus MC/Sophia Children's Hospital, Department of Pediatrics, Rotterdam, The Netherlands. We thank all patients and their families for participating in the trial.

References

1. Suttorp M, Millot F. Treatment of pediatric chronic myeloid leukemia in the year 2010: use of tyrosine kinase inhibitors and stem-cell transplantation. Hematol Am Soc Hematol Educ Progr 2010; 2010: 368–76.
2. Ford M, Mauro M, Aftandilian C, Sakamoto KM, Hijiya N. Management of Chronic Myeloid Leukemia in Children and Young Adults. Curr Hematol Malig Rep 2022; 17: 121–6.
3. Youn M, Smith SM, Lee AG, et al. Comparison of the Transcriptomic Signatures in Pediatric and Adult CML. Cancers (Basel) 2021; 13. DOI:10.3390/CANCERS13246263.
4. Hijiya N, Suttorp M. How I treat chronic myeloid leukemia in children and adolescents. Blood 2019; 133: 2374–84.
5. Champagne MA, Capdeville R, Krailo M, et al. Imatinib mesylate (STI571) for treatment of children with Philadelphia chromosome-positive leukemia: results from a Children's Oncology Group phase 1 study. Blood 2004; 104: 2655–60.
6. Millot F, Guilhot J, Nelken B, et al. Imatinib mesylate is effective in children with chronic myelogenous leukemia in late chronic and advanced phase and in relapse after stem cell transplantation. Leukemia 2006; 20: 187–92.
7. Millot F, Baruchel A, Guilhot J, et al. Imatinib is effective in children with previously untreated chronic myelogenous leukemia in early chronic phase: results of the French national phase IV trial. J Clin Oncol 2011; 29: 2827–32.
8. Suttorp M, Schulze P, Glauche I, et al. Front-line imatinib treatment in children and adolescents with chronic myeloid leukemia: results from a phase III trial. Leuk 2018 327 2018; 32: 1657–69.
9. Tokarski JS, Newitt JA, Chang CYJ, et al. The Structure of Dasatinib (BMS-354825) Bound to Activated ABL Kinase Domain Elucidates Its Inhibitory Activity against Imatinib-Resistant ABL Mutants. Cancer Res 2006; 66: 5790–7.
10. de la Fuente J, Baruchel A, Biondi A, et al. Managing children with chronic myeloid leukaemia (CML). Br J Haematol 2014; 167: 33–47.
11. Zwaan CM, Rizzari C, Mechinaud F, et al. Dasatinib in children and adolescents with relapsed or refractory leukemia: Results of the CA180-018 phase I dose-escalation study of the Innovative Therapies for Children With Cancer Consortium. J Clin Oncol 2013; 31: 2460–8.
12. Gore L, Kearns PR, de Martino Lee ML, et al. Dasatinib in Pediatric Patients With Chronic Myeloid Leukemia in Chronic Phase: Results From a Phase II Trial. J Clin Oncol 2018; 36: 1330–8.
13. Hijiya N, Maschan A, Rizzari C, et al. Phase 2 study of nilotinib in pediatric patients with Philadelphia chromosome–positive chronic myeloid leukemia. Blood 2019; 134: 2036–45.
14. Hijiya N, Michel Zwaan C, Rizzari C, et al. Pharmacokinetics of nilotinib in pediatric patients with Philadelphia chromosome–positive chronic myeloid leukemia or acute lymphoblastic leukemia. Clin Cancer Res 2020; 26: 812–20.
15. Bosulif (bosutinib) - FDA Highlights of Prescribing Information. New York, NY. MA: Pfizer Inc. (accessed Jan 01 2023). https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/203341lbl.pdf.

16. Cortes JE, Apperley JF, Deangelo DJ, et al. Management of adverse events associated with bosutinib treatment of chronic-phase chronic myeloid leukemia: expert panel review. J Hematol Oncol 2018; 11. DOI:10.1186/S13045-018-0685-2.
17. Samis J, Lee P, Zimmerman D, Arceci RJ, Suttorp M, Hijiya N. Recognizing Endocrinopathies Associated With Tyrosine Kinase Inhibitor Therapy in Children With Chronic Myelogenous Leukemia. Pediatr Blood Cancer 2016; 63: 1332–8.
18. Patterson B, Samis J, Gore L, et al. PF416 growth rate and endocrine effects of dasatinib therapy observed in a retrospective analysis of a phase 2 clinical trial for pediatric patients with chronic myeloid leukemia in chronic phase (cml-cp). Hemasphere 2019; 3: 161.
19. Narayanan KR, Bansal D, Walia R, et al. Growth failure in children with chronic myeloid leukemia receiving imatinib is due to disruption of GH/IGF-1 axis. Pediatr Blood Cancer 2013; 60: 1148–53.
20. Ulmer A, Tabea Tauer J, Glauche I, Jung R, Suttorp M. TK inhibitor treatment disrupts growth hormone axis: clinical observations in children with CML and experimental data from a juvenile animal model. undefined 2013; 225: 120–6.
21. Bansal D, Shava U, Varma N, Trehan A, Marwaha RK. Imatinib has adverse effect on growth in children with chronic myeloid leukemia. Pediatr Blood Cancer 2012; 59: 481–4.
22. Tauer JT, Hofbauer LC, Jung R, Erben RG, Suttorp M. Micro-osmotic pumps for continuous release of the tyrosine kinase inhibitor bosutinib in juvenile rats and its impact on bone growth. Med Sci Monit Basic Res 2013; 19: 274–8.
23. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood 2013; 122: 872–84.
24. Janssen JM, Zwaan CM, Schellens JHM, Beijnen JH, Huitema ADR. Clinical trial simulations in paediatric oncology: A feasibility study from the Innovative Therapies for Children with Cancer Consortium. Eur J Cancer 2017; 85: 78–85.
25. El Edelbi R, Lindemalm S, Eksborg S. Estimation of body surface area in various childhood ages--validation of the Mosteller formula. Acta Paediatr 2012; 101: 540–4.
26. Sprycel (dasatinib). FDA Highlights Of Prescribing Information. New York, NY. MA: Pfizer Inc. (accessed Jan 01 2023). https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021986s7s8lbl.pdf
27. Gleevec (imatinib mesylate). FDA Highlights Of Prescribing Information. New York, NY. MA: Pfizer Inc. (accessed Jan 01 2023). https://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021588s024lbl.pdf
28. Cortes JE, Kantarjian HM, Brümmendorf TH, et al. Safety and efficacy of bosutinib (SKI-606) in chronic phase Philadelphia chromosome–positive chronic myeloid leukemia patients with resistance or intolerance to imatinib. Blood 2011; 118: 4567–76.
29. Hijiya N, Maschan A, Rizzari C, et al. A phase 2 study of nilotinib in pediatric patients with CML: long-term update on growth retardation and safety. Blood Adv 2021; 5: 2925–34.
30. Abbas R, Hsyu PH. Clinical Pharmacokinetics and Pharmacodynamics of Bosutinib. Clin Pharmacokinet 2016; 55: 1191–204.
31. Reinoso-Barbero F, Calvo C, Ruza F, López-Herce J, Bueno M, García S. Reference values of gastric intramucosal pH in children. Pediatr Anesth 1998; 8: 135–8.
32. Janssen JM, Dorlo TPC, Steeghs N, et al. Pharmacokinetic Targets for Therapeutic Drug Monitoring of Small Molecule Kinase Inhibitors in Pediatric Oncology. Clin Pharmacol Ther 2020; 108: 494–505.

33. Hochhaus A, Gambacorti-Passerini C, Abboud C, et al. Bosutinib for pretreated patients with chronic phase chronic myeloid leukemia: primary results of the phase 4 BYOND study. Leuk 2020 348 2020; 34: 2125–37.
34. Krumbholz M, Karl M, Tauer JT, et al. Genomic BCR-ABL1 breakpoints in pediatric chronic myeloid leukemia. Genes Chromosomes Cancer. 2012;51(11):1045-1053. doi:10.1002/gcc.21989
35. Hijiya N, Kapoor S, Descamps L, Bayar MA, Ramscar N. Trial in Progress: A Multicenter, Open-Label, Phase Ib/II Study to Determine the Dose and Safety of Asciminib in Pediatric Patients with Philadelphia Chromosome-Positive Chronic Myeloid Leukemia in Chronic Phase Treated with ≥1 Prior Tyrosine Kinase Inhibitor. Blood 2021; 138: 2561–2561.
36. Réa D, Mauro MJ, Boquimpani C, et al. A phase 3, open-label, randomized study of asciminib, a STAMP inhibitor, vs bosutinib in CML after 2 or more prior TKIs. Blood 2021; 138: 2031–41.
37. Cortes JE, Kim DW, Pinilla-Ibarz J, et al. Ponatinib efficacy and safety in Philadelphia chromosome–positive leukemia: final 5-year results of the phase 2 PACE trial. Blood 2018; 132: 393.
38. Cortes J, Apperley J, Lomaia E, et al. Ponatinib dose-ranging study in chronic-phase chronic myeloid leukemia: a randomized, open-label phase 2 clinical trial. Blood 2021; 138: 2042–50.
39. Rossoff J, Huynh V, Rau RE, et al. Experience with ponatinib in paediatric patients with leukaemia. Br J Haematol 2020; 189: 363–8.
40. Millot F, Suttorp M, Versluys AB, et al. Ponatinib in childhood Philadelphia chromosome-positive leukaemias: an international registry of childhood chronic myeloid leukaemia study. Eur J Cancer 2020; 136: 107–12.

Supplementary material

Supplementary Table 1. Inclusion/Exclusion Criteria Phase 1 (R/I patients only)

Inclusion Criterion	Specifications
Cytogenetic and molecular diagnosis of Philadelphia chromosome-positive Chronic Myelogenous Leukemia at either time of initial CML diagnosis or at time of study screening	Cytogenetics must be performed by chromosome banding analysis (CBA) of bone marrow cell metaphases, and requires at least 20 metaphases. Only if dividing marrow cells cannot be obtained, or if there is an insufficient number of metaphases, CBA can be substituted by interphase fluorescence in situ hybridization (I-FISH) of bone marrow or peripheral blood cells, using dual color dual fusion probes, that allow the detection of BCR-ABL+ nuclei; at least 200 nuclei should be counted. Qualitative RT-PCR should be performed on RNA extracted from freshly collected bone marrow or peripheral blood cells. It identifies the transcript type, either e14a2 or 13a2 (also known as b3a2 and b2a2), or much more rarely e19a2, or e1a2, indicating the BCR-ABL protein weight (P210, rarely P230 or P190).
Resistance (suboptimal response or failure, as defined by 2013 European Leukemia Net guidelines) or intolerance (with or without suboptimal response or failure) to at least one prior tyrosine kinase inhibitor (TKI)	The 2013 European LeukemiaNet guidelines will be used to define suboptimal response and failure to prior TKI therapy. Intolerance to prior TKI therapy will be determined by the treating investigator, but generally applies to patients who are unable to receive standard or reduced doses of a TKI due to significant drug-related toxicity and/or when the drug-related toxicity is not responding to appropriate medical management. Patients who enrol as a result of intolerance to prior TKI therapy may have any level of response to their prior therapy and still be eligible.
Age ≥1 and <18 years at day of attaining the informed consent.	
Lansky performance status ≥50% for patients ≤16 years of age, or Karnofsky scale ≥50% for patients >16 years of age (appendix 5).	
Adequate bone marrow function:	For second-line and third-line CP CML patients: Absolute neutrophil count >1000/mm3 (>1.0 x109/L); Platelets ≥75,000/mm3 (≥75 x109/L) without any platelet transfusions during the preceding 7 days. For fourth-line CP and all for all AP/BP CML patients: Absolute neutrophil count >500/mm3 (>0.5 x109/L); Platelets ≥50,000/mm3 (≥50 x109/L) without any platelet transfusions during the preceding 7 days.

Supplementary Table 1. Continued

Inclusion Criterion	Specifications
Adequate Renal Function	Subjects must have a calculated creatinine clearance (CrCl) ≥ 60 mL/min/1.73 m2, using the Schwartz formula to estimate GFR
Adequate liver function	AST/ALT ≤2.5 x upper limit normal (ULN) or ≤5 x ULN if attributable to disease involvement of the liver;
	Total bilirubin ≤1.5 x ULN unless the patient has documented Gilbert syndrome.
Recovered to Grade 0-1, or to baseline, from any acute toxicities of prior chemotherapy, immunotherapy, radiotherapy, differentiation therapy, or biologic therapy, with the exception of alopecia.	
Able to reliably swallow whole capsules, whole tablets; or drug added to a suitable foodstuff (from capsule contents, added to either apple sauce or yoghurt); or tablets and/or capsules dissolved in water as an oral syringe drinking solution, or tablets dissolved and administered by NG tube when needed.	
Serum/urine pregnancy test (for all girls ≥ age of menarche) negative at screening.	
Male and female patients of childbearing potential and at risk for pregnancy must agree to use a highly effective method of contraception throughout the study and for at least 30 days after the last dose of assigned treatment.	A patient is of childbearing potential if, in the opinion of the Investigator, he/she is biologically capable of having children and is sexually active.
Written informed consent of parent(s)/ legal guardian(s) and/or patients (when applicable depending on age and local law and regulations)	
Patients (including legally acceptable representative for minors where applicable) who are willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other study	
Exclusion Criterion	**Specifications**
Diagnosis of primary Ph+ acute lymphoblastic leukemia.	as defined by 2013 European Leukemia Net guidelines
In patients with AP/BP CML leptomeningeal leukemia, This assessment is not required for inclusion of CP CML patients.	defined as positive cytology on lumbar puncture (including both CNS2 and CNS3 status), or clinical symptoms or signs present.
Extramedullary disease only.	
Documented prior history of T315I or V299L BCR-ABL1 mutations (Note: BCR-ABL1 mutation testing will be performed at screening for a baseline assessment, but results are not used to determine eligibility.	This exclusion criterion is based on whether there is a known history of these mutations at the time of study entry. If these mutations become evident during the study the patient will go off study).

Supplementary Table 1. Continued

Exclusion Criterion	Specifications
Any prior treatment with a TKI within 7 days prior to starting bosutinib treatment, or other anti-tumor or anti-leukemia treatment (with the exception of hydroxyurea and/or anagrelide) within 14 days prior to start of bosutinib treatment.	
Prior growth factors or biologic agents within 7 days prior to bosutinib treatment.	
Use of strong or moderate CYP3A4 inhibitors and inducers (see Appendix 8) within 7 days prior and/or concomitant to bosutinib treatment	
Use of proton pump inhibitors (Ph-modifying agents) within 7 days prior and/or concomitant to bosutinib treatment.	
Prior radiotherapy within 3 months prior to bosutinib treatment.	
Allogeneic stem cell transplantation within 3 months prior to bosutinib treatment.	
Donor lymphocyte infusion (DLI) within 1 month prior to bosutinib treatment.	
Hereditary bone marrow failure disorder.	
Graft-versus-host disease (GVHD) within 60 days prior to bosutinib treatment.	
Major surgery within 14 days prior to bosutinib treatment (recovery from any previous surgery should be complete before day 1.	
History of clinically significant or uncontrolled cardiac disease	History of or active congestive heart failure; Clinically significant ventricular arrhythmia (such as ventricular tachycardia, ventricular fibrillation, or Torsades de pointes); Diagnosed or suspected congenital or acquired prolonged QT syndrome; History of prolonged QTc.
Prolonged QTc	>450 msec, average of triplicate ECGs
Need for medications known to prolong the QT interval.	
Pregnant and/or nursing women	
Uncorrected hypomagnesemia or hypokalemia due to potential effects on the QT interval.	
Left ventricular ejection fraction <50% or shortening fraction <28%.	
Recent or ongoing clinically significant gastrointestinal disorder that may interfere with the intake or absorption of the drug.	

Supplementary Table 1. Continued

Exclusion Criterion	Specifications
Evidence of serious active or uncontrolled bacterial, fungal or viral infection.	
Known history of hepatitis B (HBV), hepatitis C (HCV), or human immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome (AIDS)-related illness.	
Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the Investigator, would make the patient inappropriate for entry into this study.	

Supplementary Table 2. Amendment History

Protocol version number	Date of amendment	Type of amendment	Summary of amendment
Version 2.0	14-April-2016	Substantial	Adding HbsAg screening before treatment
Version 3.0	12-July-2018	Substantial amendment	- The definition of hematologic, and molecular response has been modified (Appendix 2) to add definitions of CHR for AP/BP CML and loss of CHR - Clarification on wash out period of TKIs before starting treatment - Clarification on screening of laboratory items allowing repeated sampling in case of abnormalities (especially for LFTs) - Addition of dosing instructions for younger children - Adding background pharmaceutical data about administration of alternative formulations for patients who are not able to swallow whole tablets or capsules - Updating approval status of bosutinib for newly diagnosed CML in adult patients and background section - Dexa scans eliminated for younger children < 4 years due to lack of normal values - Addition of clarification on DLT and SUSAR reporting requirements - Some lay-out changes to the schedule of events to better explain the timing of procedures - Administrative changes in e-mail addresses, adding a new statistician - Replacing Dr. Suttorp in the steering committee with Dr. Metzler - Revising the duration of treatment for phase I patients to 2 years from LPFV, and clarifying the follow-up period for patients after treatment discontinuation (detailed in table 4 and 5) - Revising the total trial duration and clarification on end of trial definition - Clarification on test results reporting as adverse events - Addition of exclusion criteria on pregnant and nursing women - Clarification on contraceptive methods - Addition of a palatability questionnaire

Supplementary Table 2. Continued

Protocol version number	Date of amendment	Type of amendment	Summary of amendment
Version 4.0	10-Oct-2019	Substantial amendment	- New approval for newly diagnosed adult CML patients was added - A cohort of newly diagnosed (ND) pediatric patients with CML in chronic phase was added in Phase 2 using the 300 mg/m2 once daily dose and the title was changed to refelect this - DL2 was changed to 400 mg/m2 (DL2B) instead of 350 mg/m2 (DL2A) based on PK results obtained in the DL1 cohort - The objectives of the Phase 1 and 2 were modified according to the new design of the study; i.e. safety and PK are the primary objectives in all cohorts, and response is considered a secondary objective - Inclusion/exclusion criteria for ND patients for phase 2 added - Based on the simulations from an updated population PK analysis using data from adult patients with CML or solid tumors and healthy volunteers, the target adult exposure at steady-state (AUCss) are now updated to 3150 ng•hr/mL for the dose of 500 mg/day, and 2270 ng•hr/mL for the dose of 400 mg/day. - Sample size has been updated to 60 patients in total - Updated guidelines for CYP3A inducers/ inhibitors and PPI concomitant medication at inclusion and during treatment - COG study number changed from AAML1621 to AAML1921 according to COG standard procedures, as the study will be active from 2019 in USA - Addition of the Bayesian analysis for futility and early stopping rules for toxicity for ND patients

Supplementary Table 3. Body surface to dose scheme.

	Actual Daily Dose in mg						
Body Surface Area (m^2)	**200mg/ m^2**	**250mg/ m^2**	**300mg/ m^2**	**350mg/ m^2**	**400mg/ m^2**	**450mg/ m^2**	**500mg/ m2**
0.3	50	75	100	100	125	125	150
0.4	75	100	125	150	150	175	200
0.5	100	125	150	175	200	225	250
0.6	125	150	175	200	250	275	300
0.7	150	175	200	250	275	325	350
0.8	150	200	250	275	325	350	400
0.9	175	225	275	325	350	400	450
1.0	200	250	300	350	400	450	500
1.1	225	275	325	375	450	500	550
1.2	250	300	350	425	475	550	600#
1.3	250	325	400	450	525	575	600#
1.4	275	350	425	500	550	600#	600#
1.5	300#	375	450	525	600#	600#	600#
1.6	300#	400#	475	550	600#	600#	600#
1.7	300#	400#	500#	600#	600#	600#	600#
1.8	300#	400#	500#	600#	600#	600#	600#
1.9	300#	400#	500#	600#	600#	600#	600#
2.0	300#	400#	500#	600#	600#	600#	600#
2.1	300#	400#	500#	600#	600#	600#	600#
2.2	300#	400#	500#	600#	600#	600#	600#
2.3	300#	400#	500#	600#	600#	600#	600#
2.4	300#	400#	500#	600#	600#	600#	600#

Note: dosages were rounded off to allow dosing with the available formulations. Please take into account that only tablets can be dissolved for administration through a NG tube, and that only capsules can be opened for sprinkling on food stuff, or a combination of tablets and capsules can be used for oral administration with a syringe. # Dosages do not exceed the maximum adult equivalent.

Supplementary Table 4. Endpoints

Endpoint	Outcome
Primary endpoints	• Incidence and severity of Dose-Limiting Toxicities (DLTs) assessed during the first 28 days of treatment.
	• PK parameters of bosutinib: Maximum observed plasma concentration (Cmax), time to Cmax (Tmax), area under the plasma concentration versus time curve from time zero to the dosing interval (AUCτ), pre-dose concentration (Ctrough) and apparent clearance (CL/F).
Secondary endpoints	• AEs, as characterized by type, frequency, severity (as graded using CTCAE version, v4.03), timing, seriousness, and relation to study therapy;
	• Laboratory abnormalities as characterized by type, frequency, severity and timing;
	• ECG and performance status abnormalities;
	• Overall cumulative disease response: complete hematologic response (CHR), major cytogenetic response (MCyR, defined as complete cytogenetic response [CCyR] plus partial cytogenetic response [PCyR]), CCyR, major molecular response (MMR) and deep molecular response (definitions in table 3 below).
Exploratory endpoints	• Parameters of bone metabolism and growth, including linear growth, bone age, bone mineral density of lumbar spine, physical signs of pubertal maturation (Tanner stage and testicular volume of boys), and hormones associated with growth and pubertal development (IGF-1, LH, FSH, estradiol for girls, and testosterone for boys) and a marker of bone formation and bone resorption (bone alkaline phosphatase and CTX).
	• Patient and/or caregiver-reported assessments of gastrointestinal symptoms, as measured by selected domains from the PedsQL Gastrointestinal Symptom Scale.
	• Patient and/or caregiver-reported assessment of the taste and ability to swallow the medicine, as measured by the Palatability Questionnaire for Bosutinib in patients aged 4-18 years of age.

Supplementary Table 5. Definitions of Hematologic, Cytogenetic, Molecular Response, and Event Free Survival

Response Type	Definition
Hematological Complete Response (CHR)	Chronic Phase (all criteria required) • WBC <10 x 109/L • Basophils <5% • No myelocytes, promyelocytes, myeloblasts in the differential • Platelet count <450 x 109/L • Spleen nonpalpable Accelerated/Blast Phase***(all criteria required) • WBC ≤ institutional upper limit of normal (ULN) • ANC ≥ 1.0 x 109 /L • No peripheral blood blasts or promyelocytes; • Peripheral blood myelocytes + metamyelocytes < 5%; • Basophils in peripheral blood < 5% • No evidence of extramedullary involvement • BM Blasts < 5%****
Loss of CHR	The appearance of any of the following, confirmed by a second determination ≥4 weeks later (unless associated with CML-related treatment discontinuation or due to other reason)) WBC count that rises to > 20.0 x 10^9/L Platelet count that rises to ≥ 600 x 10^9/L Appearance of extramedullary disease not present at baseline including hepatosplenomegaly by palpation or other extramedullary involvement proven by biopsy Appearance of ≥ 5% myelocytes + metamyelocytes in the peripheral blood or appearance of blasts or promyelocytes in the peripheral blood
Complete Cytogenetic Response (CCyR) *	• No Ph+ metaphases in at least 20 marrow cell metaphases analyzed by chromosome banding, or • < 1% BCR-ABL1–positive nuclei of at least 200 peripheral blood interphase nuclei analyzed by FISH
Partial Cytogenetic Response (PCyR)	1% to 35% Ph+ metaphases
Minor Cytogenetic Response (mCyR)	36% to 65% Ph+ metaphases
Minimal Cytogenetic Response (minCyR)	66% to 95% Ph+ metaphases
None Cytogenetic Response (noCyR)	>95% Ph+ metaphases
Loss of CCyR	At least one Ph+ metaphase from <100 metaphases analyzed confirmed by a second determination ≥4 weeks later (unless associated with CML-related treatment discontinuation or due to other reason)
Molecular Response (MR) ** 1	Either (i) detectable disease with ≤ 10% BCR-ABL1IS or (ii) undetectable disease in cDNA with a minimum number of ABL1 transcripts specified by the central laboratory
MR2	Either (i) detectable disease with ≤ 1% BCR-ABL1IS or (ii) undetectable disease in cDNA with a minimum number of ABL1 transcripts specified by the central laboratory

Supplementary Table 5. Continued

Response Type	Definition
MR3/Major Molecular Response (MMR)	Either (i) detectable disease with ≤ 0.1% BCR-ABL1IS or (ii) undetectable disease in cDNA with a minimum number of ABL1 transcripts specified by the central laboratory
Loss of MMR	Two assessments at least 4 weeks apart with at least 5x increase from lowest transcript level recorded.
MR4.0 (deep molecular response)	Either (i) detectable disease with ≤ 0.01% BCR-ABL1IS or (ii) undetectable disease in cDNA with a minimum number of ABL1 transcripts specified by the central laboratory.
MR4.5 (deep molecular response)	Either (i) detectable disease with ≤ 0.0032% BCR-ABL1IS or (ii) undetectable disease in cDNA with a minimum number of ABL1 transcripts specified by the central laboratory.
MR5.0 (deep molecular response)	Either (i) detectable disease with ≤ 0.001% BCR-ABL1IS or (ii) undetectable disease in cDNA with a minimum number of ABL1 transcripts specified by the central laboratory.
EFS	Time without occurrence of: 1. Death due to any cause. 2. Transformation to AP or BP at any time. 3. Loss of CHR (as defined above) 4. Loss of CCyR (as defined above) 5. For patients not achieving a CHR: doubling of WBC at least 1 month apart with the second value > 20 x 10^9/L and maintained in subsequent assessments for at least 2 weeks

Abbreviations: CHR, complete hematologic response; CCyR, complete cytogenetic response, PCyR, partial cytogenetic response; Ph+, Philadelphia Chromosome positive; MCyR, major cytogenetic response; minCyR, minor cytogenetic response; noCyR, no cytogenetic response; MR, molecular response; MMR, major molecular response; IS, international scale; EFS, event free survival.

*If bone marrow metaphases cannot be obtained or evaluated by chromosome banding analysis, the definition of CCyR (defined as <1% for FISH) may be based on interphase FISH of blood cells, provided that it is performed with BCR-ABL1 extrasignal, dual color, dual fusion, or in situ hybridization probes and at least 200 nuclei are scored. In this study, PCyR and CCyR are counted together and reported as MCyR.

**Molecular response is best assessed according to the International Scale (IS) as the ratio of BCR-ABL1 transcripts to ABL1 transcripts, or other internationally recognized control transcripts, and it is expressed and reported as BCR-ABL1% on a log scale, where 10%, 1%, 0.1%, 0.01%, 0.0032%, and 0.001% correspond to a decrease of 1, 2, 3, 4, 4.5, and 5 logs, respectively.

***If an on-treatment CBC is scheduled within 1 week from the last platelet transfusion or last dose of G-CSF, it should be considered “Not Evaluable” for hematological response assessment, and a CBC should be repeated at least 1 week from the last platelet transfusion or last dose of G-CSF received.

****CHR for AP/BP <5% BM blasts is only required when a BM differential is available.

Supplementary Table 6. List of Adverse Events

Adverse Event	N	(%)
Diarrhea	26	92.86
Abdominal pain	20	71.43
Vomiting	19	67.86
Nausea	17	60.71
Fever	12	42.86
Skin and subcutaneous tissue disorders - Other	12	42.86
Rash maculo-papular	11	39.29
Headache	10	35.71
Alanine aminotransferase increased	9	32.14
Fatigue	8	28.57
Pain in extremity	8	28.57
Constipation	7	25.00
Gastrointestinal disorders - Other	7	25.00
Anorexia	6	21.43
Creatinine increased	6	21.43
Infections and infestations - Other	6	21.43
Metabolism and nutrition disorders - Other	6	21.43
Stomach pain	6	21.43
Aspartate aminotransferase increased	5	17.86
Rhinitis infective	5	17.86
Cough	4	14.29
CPK increased	4	14.29
Flatulence	4	14.29
General disorders - Other	4	14.29
Platelet count decreased	4	14.29
Rash acneiform	4	14.29
Anemia	3	10.71
Blood and lymphatic system disorders - Other	3	10.71
Blood bilirubin increased	3	10.71
Cardiac disorders â€“ Other	3	10.71
Eye disorders - Other	3	10.71
Hypophosphatemia	3	10.71
Myalgia	3	10.71
Periorbital edema	3	10.71
Respiratory thoracic disorders - Other	3	10.71
Serum amylase increased	3	10.71
Upper respiratory infection	3	10.71
Urticaria	3	10.71

Supplementary Table 6. Continued

Adverse Event	N	(%)
Alkaline phosphatase increased	2	7.14
Alopecia	2	7.14
Dental caries	2	7.14
Dry eye	2	7.14
Dry skin	2	7.14
Ear pain	2	7.14
Hypocalcemia	2	7.14
Injury poisoning and procedural complications	2	7.14
Lipase increased	2	7.14
Musculoskeletal disorder - Other	2	7.14
Neck pain	2	7.14
Oral pain	2	7.14
Otitis externa	2	7.14
Otitis media	2	7.14
Pain	2	7.14
Palpitations	2	7.14
Pruritus	2	7.14
Reproductive system and breast disorders - Other	2	7.14
Urinary frequency	2	7.14
Vertigo	2	7.14
Weight loss	2	7.14
White blood cell decreased x	2	7.14
Agitation	1	3.57
Allergic rhinitis	1	3.57
Anal ulcer	1	3.57
Arthralgia	1	3.57
Avascular necrosis	1	3.57
Back pain	1	3.57
Blurred vision	1	3.57
Bone pain	1	3.57
Bruising	1	3.57
Catheter related infection	1	3.57
Chest pain - cardiac	1	3.57
Chills	1	3.57
Conjunctivitis	1	3.57
Cystitis noninfective	1	3.57
Dehydration	1	3.57
Delirium	1	3.57

Supplementary Table 6. Continued

Adverse Event	N	(%)
Depression	1	3.57
Dizziness	1	3.57
Dysphagia	1	3.57
Edema face	1	3.57
Edema limbs	1	3.57
Epistaxis	1	3.57
Eye infection	1	3.57
Hematuria	1	3.57
Hepatobiliary disorders - Other	1	3.57
Hypercalcemia	1	3.57
Hyperkalemia	1	3.57
Hypoalbuminemia	1	3.57
Hypoglycemia	1	3.57
Hypokalemia	1	3.57
Hypomagnesemia	1	3.57
Hyponatremia	1	3.57
Hypothyroidism	1	3.57
Infusion site extravasation	1	3.57
Insomnia	1	3.57
Investigations - Other	1	3.57
Lethargy	1	3.57
Lung infection	1	3.57
Lymphocyte count decreased	1	3.57
Lymphocyte count increased	1	3.57
Malaise	1	3.57
Nasal congestion	1	3.57
Neutrophil count decreased	1	3.57
Non-cardiac chest pain	1	3.57
Oral dysesthesia	1	3.57
Proteinuria	1	3.57
Pulmonary hypertension	1	3.57
Rectal hemorrhage	1	3.57
Renal and urinary disorders - Other	1	3.57
Restlessness	1	3.57
Sinus tachycardia	1	3.57
Skin hyperpigmentation	1	3.57
Skin ulceration	1	3.57
Sneezing	1	3.57

Supplementary Table 6. Continued

Adverse Event	N	(%)
Sore throat	1	3.57
Syncope	1	3.57
Tremor	1	3.57
Urinary incontinence	1	3.57

[a]Highest toxicity grade per patient, counted only once per patient

ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; WBC, white blood cell

Supplementary Table 7. List of Chemistry and Hematologic Laboratory Tests Abnormalities

7a. Chemistry Tests

Grade	0	1	2	3	4	Total
Albumin	26	4	0	0	0	30
Alkaline phosphatase	28	2	0	0	0	30
ALT	13	11	1	4	1	30
Amylase	18	7	3	1	0	29
AST	9	17	1	3	0	30
Bilirubin (Total)	24	4	2	0	0	30
Creatinine	21	9	0	0	0	30
Glucose	10	20	0	0	0	30
Lipase	25	2	0	2	0	29
Magnesium	22	7	0	0	0	29
Phosphorous	19	8	2	0	0	29
Potassium	23	6	1	0	0	30
Sodium	24	6	0	0	0	30
total calcium	29	1	0	0	0	30
Uric acid	22	5	0	0	1	28

7b. Hematologic Tests

Grades	0	1	2	3	4	Total
Hemoglobin	12	14	4	0	0	30
Platelet count	19	8	1	2	0	30
White blood cell count	15	10	4	1	0	30

Supplementary Table 8. QTc measurements per patient

Patient	Sex	Age*	Time Point	QTc (ms)
1	Female	17	SCR	394.33
1	Female	17	C1D14	392.67
1	Female	17	C1D14 timepoint2	417.33
1	Female	17	C1D15	386.00
1	Female	17	C2	402.00
1	Female	17	C3	390.67
1	Female	17	C4	395.33
1	Female	17	EOT	399.67
2	Male	9	SCR	404.00
2	Male	9	C1D14	390.00
2	Male	9	C1D14 timepoint2	387.00
2	Male	9	C1D15	387.00
2	Male	9	C2	394.67
2	Male	9	C3	382.00
2	Male	9	C4	388.67
2	Male	9	EOT	405.00
3	Male	4	SCR	343.00
3	Male	4	C1D14	361.00
3	Male	4	C1D14 timepoint2	337.67
3	Male	4	C1D15	372.00
3	Male	4	C2	337.00
3	Male	4	C3	342.33
3	Male	4	C4	363.00
3	Male	4	EOT	352.33
4	Male	11	SCR	421.33
4	Male	11	C1D14	401.33
4	Male	11	C1D14 timepoint2	409.33
4	Male	11	C1D15	391.67
4	Male	11	C2	391.67
4	Male	11	C3	391.67
4	Male	11	C4	397.00
4	Male	11	EOT	414.67
5	Male	1	SCR	337.00
5	Male	1	C1D14	355.33
5	Male	1	C1D14 timepoint2	339.67
5	Male	1	C1D15	360.67
5	Male	1	C2	351.33
5	Male	1	C3	357.00

Supplementary Table 8. Continued

Patient	Sex	Age*	Time Point	QTc (ms)
5	Male	1	C4	360.00
5	Male	1	EOT	365.00
6	Male	8	SCR	385.00
6	Male	8	C1D14	410.67
6	Male	8	C1D14 timepoint2	411.00
6	Male	8	C1D15	401.67
6	Male	8	C2	410.00
6	Male	8	C3	414.33
6	Male	8	C4	407.00
7	Female	5	SCR	407.67
8	Female	6	SCR	389.00
8	Female	6	C1D14	389.33
8	Female	6	C1D14 timepoint2	389.00
8	Female	6	C1D15	400.67
8	Female	6	C2	393.00
8	Female	6	C3	416.00
8	Female	6	C4	384.00
9	Female	11	SCR	365.00
9	Female	11	C1D14	403.00
9	Female	11	C1D14 timepoint2	406.00
9	Female	11	C1D15	401.67
9	Female	11	C2	405.67
9	Female	11	C3	413.00
9	Female	11	C4	413.00
9	Female	11	EOT	409.67
10	Female	11	SCR	403.67
10	Female	11	C1D14	396.00
10	Female	11	C1D14 timepoint2	408.00
10	Female	11	C1D15	404.00
10	Female	11	C2	392.67
10	Female	11	C3	397.00
10	Female	11	C4	395.67
10	Female	11	EOT	394.33
11	Female	12	SCR	398.00
11	Female	12	C1D14	381.33
11	Female	12	C1D15	390.67
11	Female	12	C2	388.00
11	Female	12	C3	394.67

Supplementary Table 8. Continued

Patient	Sex	Age*	Time Point	QTc (ms)
11	Female	12	C4	400.33
12	Male	12	SCR	423.00
12	Male	12	C1D14	386.33
12	Male	12	C1D14 timepoint2	395.00
12	Male	12	C1D15	399.67
12	Male	12	C2	398.00
12	Male	12	C3	408.67
12	Male	12	EOT	400.33
13	Male	7	SCR	367.00
13	Male	7	C1D14	357.67
13	Male	7	C1D14 timepoint2	362.33
13	Male	7	C1D15	372.67
13	Male	7	C2	381.33
13	Male	7	C3	379.33
13	Male	7	C4	366.00
13	Male	7	EOT	385.67
14	Female	16	SCR	391.67
14	Female	16	C1D14	382.33
14	Female	16	C1D14 timepoint2	387.67
14	Female	16	C2	382.00
14	Female	16	Unscheduled Event	381.00
14	Female	16	Unscheduled Event	387.00
14	Female	16	C3	384.33
14	Female	16	C4	379.67
15	Male	17	SCR	393.67
15	Male	17	C1D14	378.67
15	Male	17	C1D14 timepoint2	394.00
15	Male	17	C2	375.33
15	Male	17	C3	397.00
15	Male	17	C4	385.67
15	Male	17	EOT	384.00
16	Female	4	SCR	395.00
16	Female	4	C1D14	383.67
16	Female	4	C1D14 timepoint2	365.00
16	Female	4	Unscheduled Event	378.33
16	Female	4	Unscheduled Event	365.00
16	Female	4	C1D15	353.00
16	Female	4	Unscheduled Event	353.00

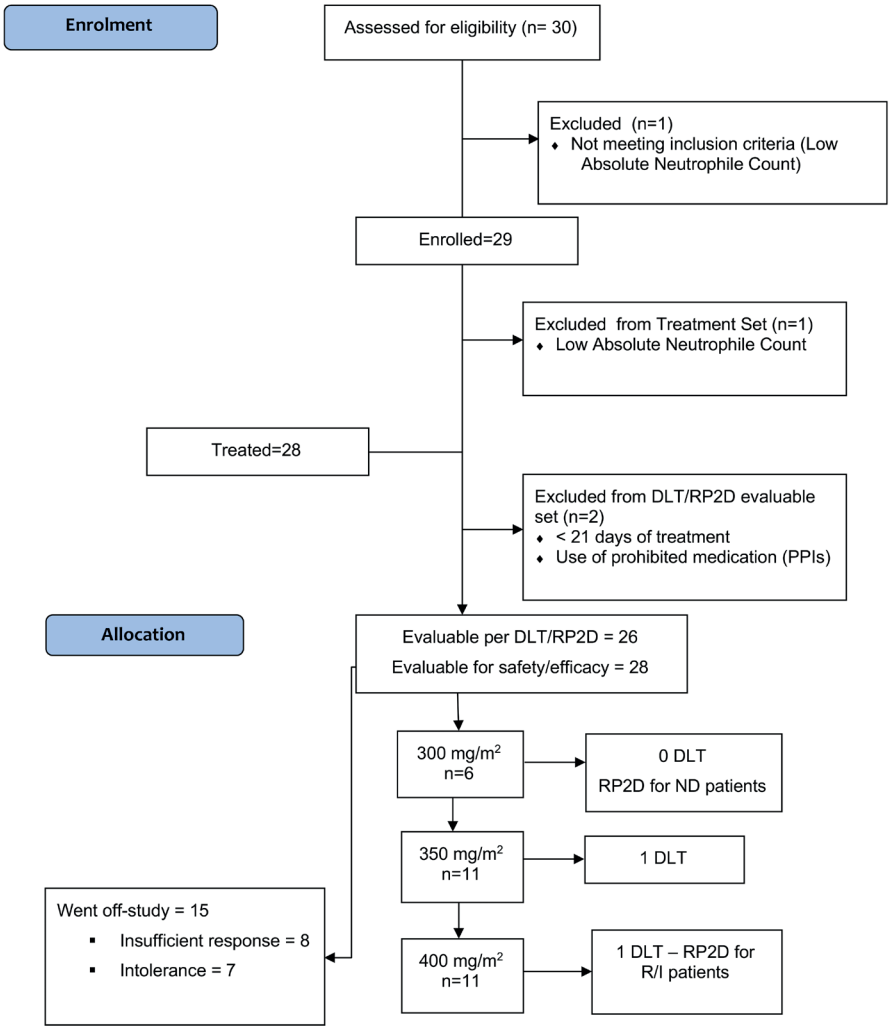
Supplementary Table 8. Continued

Patient	Sex	Age*	Time Point	QTc (ms)
16	Female	4	C2	350.67
16	Female	4	C3	377.33
16	Female	4	C4	383.67
17	Male	14	SCR	376.00
17	Male	14	C1D14	367.33
17	Male	14	C1D14 timepoint2	369.67
17	Male	14	C2	386.67
17	Male	14	C3	378.67
17	Male	14	C4	389.33
17	Male	14	EOT	267.00
18	Male	13	SCR	402.33
18	Male	13	C1D14	385.67
18	Male	13	C1D14 timepoint2	395.00
18	Male	13	C2	382.33
18	Male	13	C3	390.00
18	Male	13	C4	387.33
19	Female	17	SCR	425.00
19	Female	17	C1D14	412.33
19	Female	17	C2	404.00
19	Female	17	C3	400.00
19	Female	17	C4	401.67
19	Female	17	C1D14 timepoint2	404.00
20	Male	8	SCR	400.67
20	Male	8	Unscheduled Event	358.00
20	Male	8	Unscheduled Event	373.00
20	Male	8	C1D14	400.00
20	Male	8	C1D14 timepoint2	401.00
20	Male	8	C2	391.00
20	Male	8	C3	391.00
20	Male	8	C4	393.00
20	Male	8	EOT	388.33
21	Female	17	SCR	391.00
21	Female	17	C1D14	393.33
21	Female	17	C1D14 timepoint2	386.33
21	Female	17	C2	396.00
21	Female	17	C3	372.67
21	Female	17	C4	377.33
21	Female	17	EOT	269.33

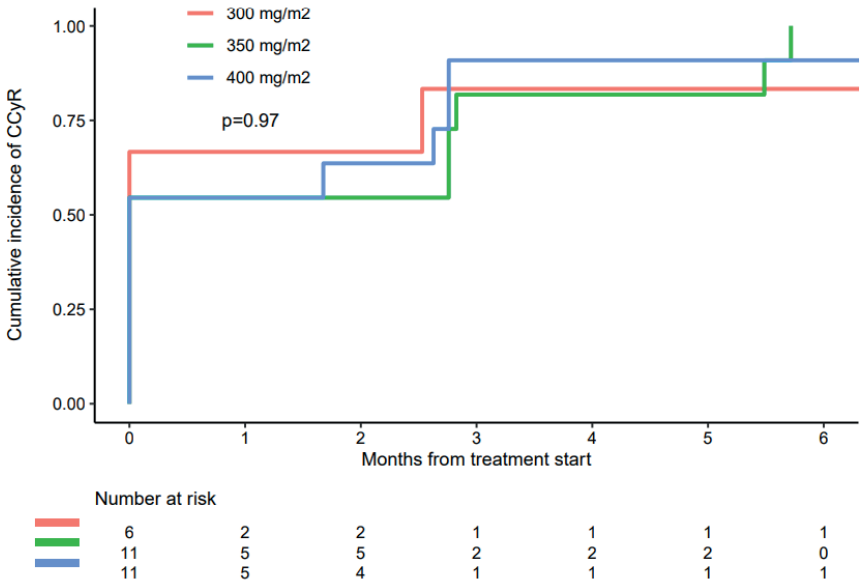
Supplementary Table 8. Continued

Patient	Sex	Age*	Time Point	QTc (ms)
22	Female	15	SCR	408.33
22	Female	15	C2	402.67
22	Female	15	C3	399.00
22	Female	15	C3 timepoint2	417.00
22	Female	15	Unscheduled Event	417.00
22	Female	15	C4	389.00
23	Male	13	SCR	386.67
23	Male	13	C1D14	398.00
23	Male	13	C1D14 timepoint2	418.00
23	Male	13	C2	397.67
23	Male	13	C3	408.67
23	Male	13	C4	396.67
23	Male	13	EOT	379.00
24	Male	16	SCR	407.00
24	Male	16	C1D14	396.67
24	Male	16	C1D14 timepoint2	419.33
24	Male	16	C1D15	390.33
24	Male	16	C2	388.67
24	Male	16	C3	392.33
24	Male	16	C4	400.00
25	Female	16	C1D14	411.00
26	Male	15	EOT	390.00
27	Male	10	SCR	385.33
27	Male	10	C1D14	383.00
27	Male	10	C1D14 timepoint2	385.67
27	Male	10	C2	371.00
27	Male	10	C3	385.33
27	Male	10	C4	383.33
28	Male	6	SCR	398.00
28	Male	6	C1D14	399.67
28	Male	6	C1D14 timepoint2	407.00
28	Male	6	C1D15	392.33
28	Male	6	C2	391.00
28	Male	6	C3	408.33

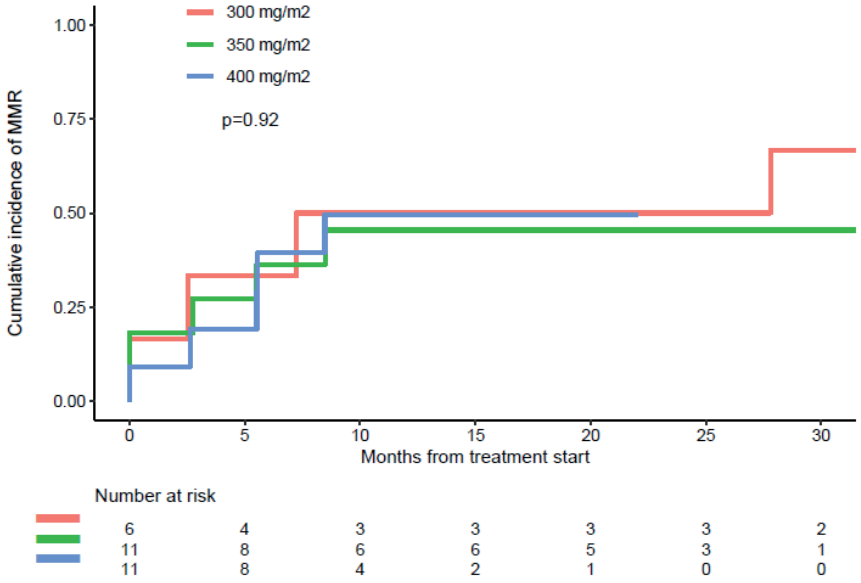
C: cycle, D: day, EOT: End of Treatment,
*Age is reported at time of enrolment



Supplementary Figure 1. Patients Enrolment and Allocation



Supplementary Figure 2. Cumulative incidence of Complete Cytogenetic Response (CCyR) per dose level. Subjects entering the study already in CCyR are displayed as event at time zero, respectively 2, 3 and 4 patients at 300, 350 and 400 mg/m^2. No difference in response was shown.



Supplementary Figure 3. Cumulative incidence of Major Molecular Response (MMR) per dose level. Subjects entering the study already in MMR are displayed as event at time zero, respectively 2, 3 and 4 patients at 300, 350 and 400 mg/m^2. No difference in response was shown.

Chapter 7

General discussion

This thesis is focused on new therapeutic options for pediatric leukemia, and provides the results of two clinical trials developed in the context of Pediatric Investigational Plans (PIPs), conducted with the final aim to inform the Regulatory Authority (RA) to obtain approval of inotuzumab ozogamicin and bosutinib for pediatric ALL and CML respectively. This is relevant as the majority of drugs we use in pediatric oncology today are not approved for the indication, and sometimes not even for use in children. (1) Many drugs that physicians commonly prescribe for infants and children are not officially available for their treatment and were mainly developed by extrapolation from adult data. This may lead to harm in children, as summarized by EMA in 2004. (EMEA/126327/2004)

In the last two decades the development of new agents for hematologic malignancies has dramatically improved. The ALL field is experiencing the quickest and biggest changes, with the growing role of immunotherapy in the form of targeted antibodies as well as CAR T-cell therapy. These new approaches aim to improve not only the outcome of specific groups of patients in terms of disease control, but also in terms of toxicity compared to the standard chemotherapy regimens. This exciting time for the new agents opens the door to a possible 'chemo-free' future, at least for some of the hematological malignancies. An example is the newly developed combination of steroids, blinatumomab and TKIs for induction of Ph-chromosome positive ALL in adults.(2,3) CML can be considered in this sense the pioneer of chemo-free treatment, with the use of the TKIs as targeted therapy.

Although the field is rapidly developing, pediatric patients benefit of these new treatments with years of delay compared to adults. As a an example, InO was approved for adults with relapsed or refractory BCP-ALL in 2017, while at the time of writing, it is not yet approved for children.

7.1 The role of early phase clinical trials in pediatric cancer drug approvals

Approval of new agents for hematological malignancies in pediatrics lags behind adults due to different reasons. Early phase clinical trials in pediatric oncology are always a challenge, mostly due to the rarity of the diseases, and therefore the slow recruitment of the patients.

Many efforts have been made in the recent past to provide the deserved attention to the development of new agents for pediatric cancers. The United States and European Union regulations require the pharmaceutical industry to submit pediatric plans for product development. With the recent RACE for Children Act implemented in North-America, pediatric investigation is required if a drug intended for the

treatment of an adult cancer is directed at a molecular target that is considered relevant in a pediatric cancer. This regulation results in a target-based and not disease-based waiver system, as is still the case in Europe.(4) The FDA also published a pediatric target list to facilitate this process.(5) However, competing agents of the same class in rare populations present a challenge, as well as a large number of pediatric plans for rare cancers. Prioritization and international collaboration is required to avoid delays in testing the most promising new agents for children with cancer. This is achieved via the Pediatric Forums organized by Accelerate, where all stakeholders review the current evidence and try to set the most urgent priorities.(6) As the Pediatric Investigational Plans are meant to be flexible, adaptations when new evidence emerges is possible and needs to be endorsed.(7)

To perform the ITCC-059 trial (InO) and the ITCC-054 trial (bosutinib), an innovative collaboration between pharma and academy has been implemented, under the name of 'clinical research collaboration'. Within this collaboration, the company (Pfizer Inc.) provided the funding and the investigational drug, while the academic partner acted as Sponsor for the intent-to-file clinical trials, which represents an exception in the pediatric landscape. These registration trials have been developed within the European collaborative consortium for early clinical trials, ITCC, created in 2005 to facilitate early drug development in children with cancer.

The role of the Academia in registration studies could help accelerating the approval of new agents for pediatric use. Academic clinical trials consortia have collaborated for decades in phase 3 clinical trials evaluating improvements in conventional poly-chemotherapeutic treatment regimes, which led to the current outcome rates for many children with cancer.(8) Although academic consortia have long-standing expertise to conduct robust clinical trials, standard procedures and resources necessary to marketing authorization applications still needs to be improved, as highlighted by a "fit for filing" working group from the Accelerate platform.(9) Identifying the essential documents and resources needed to run a fit for filing trial (i.e. data management plan, an adequate database, quality control measures) are the primary steps every academic sponsor needs to fulfil for an intent to file trial. (9) The clinical research collaboration method used for the ITCC-054 and ITCC-059 studies warrants the possibility for the Academia to guide the first use in pediatrics and to lead additional studies (i.e. pharmacodynamics), relevant for the future uses of the drug.

It may be argued that early phase clinical trials in pediatrics provide largely a repetition of data already known from trials in adults. Our experience gained form the trials

presented in this thesis provides some examples of good reasons to continue to pursue the way of separate phase 1 and 2 clinical trials in children.

1. Pediatric cancers differ from their adult forms. In the InO ITCC-059 trial (*Chapter 3 and 4*) we could demonstrate the efficacy of InO with an ORR of 81.5% in multiple relapsed ALL, in much more heavily pretreated patients compared to the patients treated in the adult trial.(10) While in the adult trial, 70% of the patients had a first relapse and only 16% had been previously transplanted, in the ITCC-059 trial phase 1 and 2 almost 50% of the patients had previously been transplanted and had a median of 2 prior lines of treatment (range 2-7). Genetic characteristics and prognosis of ALL in children and adults show great differences. ALL has an OS in children of >90%, while in patients who are older than 60 years still have poor survival, typically under 20% at 5 years, unless novel innovative combinations on InO and blina are used.(11,12) In general in adults with ALL there is a low prevalence of genetic subtypes associated with favorable outcome (i.e. *ETV6-RUNX1,* hyperdiploid) and a concomitant elevated incidence of subtypes associated with poor outcome (i.e. *BCR-ABL1*, *KMT2A* rearranged). (13)

   The specific differences in genetics related to age also led to the collection of data on the response to InO in infants with ALL (not included in the trial), known for carrying a *KMT2A*-rearrangement in about 75% of cases, and having a dismal prognosis, especially at relapse.(14) Hence to collect data about response to InO in this group was relevant, given the general intrinsic chemotherapy resistance, but also the possibly lower CD22 expression associated with immature leukemias arrested in the pro-B cell stage. In line with this, *KMT2A*-r leukemias have shown a lower average CD22 antigen density, and a proportion of patients with sub-populations of blasts lacking CD22 expression. However, CD22 expression did not to correlate with response in our small retrospective cohort. In addition, we showed that InO can be safely used in infants with a valuable ORR of 46.6% in these patients with a dismal prognosis (*Chapter 5*).

   Also, CML in children has different characteristics in terms of disease presentation (higher proportion of patients exhibiting a larger spleen size and higher leukocyte and platelets counts)(15) and response to treatment (a higher proportion of children fails to achieve optimal early response to imatinib)(16), supporting the need of clinical trials with TKIs in children (*Chapter 6*).

2. Specific toxicities can be experienced in the pediatric age. There is evidence that long-term exposure to imatinib, but also to other TKI as dasatinib, results

in growth impairment and dysregulation of bone metabolism, especially in prepubertal children.(17) Pre-clinical data indicated that this toxicity may not be observed or be less prominent with bosutinib.(18) A clinical trial in children is of primary importance to answer this specific question relevant to the pediatric population, that can be exposed for a very long time (or even life-long) to the TKI treatment. Therefore a long-term follow-up conducted in a registration study is of primary importance in the pediatric population.

Another example is the hepatotoxicity and specifically the risk of veno-occlusive disease (VOD) related to InO. Despite the higher incidence of VOD generally reported in the pediatric population (19) and the larger proportion of patients previously transplanted in our cohort, the incidence of VOD in our trial (phase 1A and 2 combined) was comparable to the adult data (general incidence 17% in pediatrics versus 14% in adults; 26% versus 22.8% post-HSCT, in children and adults respectively).(10)

3. Metabolism and pharmacokinetics of drugs in adult and children is not the same.
Drug disposition is influenced by physiologic changes during growth, affecting drug absorption and metabolism, and hence PK needs to be tested specifically in pediatric patients, especially in those under 2 years of age.(20) In addition oral administration in children may be a challenge, and different formulations are needed to realize a successful intake.

In the bosutinib trial different formulations of the drug were tested, to guarantee administration via naso-gastric tubes, or using a syringe, or with content of capsules sprinkled on food stuff. Data of bioequivalence of these formulations have been captured prior to starting the trial (*Chapter 6*). In addition, we found that a higher dose compared to the approved adult dose adjusted for body surface area (BSA) was necessary in our pediatric population to achieve the target exposure defined in adult studies. This different pharmacokinetics might be explained by a different enteral solubility and different absorption of the drug in children. Children have generally an higher stomach pH compared to adults and less water in the gastro-intestinal tract, which probably explains the different absorption and exposure profile of bosutinib in children and adults, together with a possibly higher liver metabolism excretion. Indeed, bosutinib is a Biopharmaceutics Classification System (BCS) class IV drug, characterized by low permeability and low solubility, the latter being pH-dependent and increased by food intake.(21) A final analysis of the pediatric PK data may provide more insight and is in progress.

In conclusion pediatric clinical trials generate valuable knowledge, that can be translated in safer and more effective drug administration in children. The Academia can still gain a more relevant guidance role in the trials necessary to the marketing authorization of new medicines for children. This role becomes essential when new agents are incorporated in backbones therapies, used within in cooperative groups able to maximizing patients inclusion in trials. When single agent activity is not expected to be superior to existing therapies, integration in backbone treatment should be accelerated with the help of Academia.

7.2 Treatment landscape for relapsed and refractory BCP-ALL (*Chapter 3,4 and 5)*

Immunotherapy in the form of targeted antibodies, as well as CAR T-cell therapy, represents the most promising future direction for hematologic malignancies.

CD19 targeting therapies: blinatumomab and CAR T cells

Blinatumomab is a bi-specific antibody that directs CD3-positive effector T cells to CD19-positive target cells. It has already achieved a label for pediatric use: it is approved by the FDA for the treatment of children with relapsed or refractory (R/R) BCP-ALL, or in first or second complete remission (CR) with persisting positive minimal residual disease (MRD). In Europe, it has not yet an approval for the front-line use, while it is approved for patients with high-risk first relapse as consolidation therapy. In the single-agent trial in overt relapse, neurological toxicity (experienced by 24% of patients) and cytokine release syndrome (CRS, 11% of patients) were noticed as common and specific toxicities, the latter as a result of high tumor burden. In that first pediatric trial including patients with bone marrow blasts $\geq$25%, the ORR at the RP2D was 39%, with 52% of those patients being MRD negative.(22) In minimal residual disease setting the drug was very well tolerated and also more efficacious. Two randomized trials have highlighted the superiority of blinatumomab as post-reinduction consolidation treatment for first relapse ALL compared to chemotherapy. This was due both to enhanced anti-leukemic activity as well as to lower toxicity than chemotherapy, especially inducing less hematologic toxicity and infections. Based on these results, blinatumomab seems to be best positioned in the MRD setting, hence in post-induction consolidation therapy.(23, 24)

Anti-CD19 CAR T cells are modified T cells that express a CAR construct after being transduced by a viral vector. Tisagenlecleucel has been approved in the US and Europe for the treatment of R/R CD19 expressing BCP-ALL in children and young

adults up to age 25 years. The phase I-II Eliana trial yielded encouraging results with a CR rate of 81%, with 59% of responders remaining relapse-free at 12 months.(25) Nevertheless, recently published EFS at 3 years for patients included in the Eliana trial was 44% with most events occurring within the first 2 years.(26) High rates of toxicities have complicated this approach in overt disease setting with 80%-90% of patients experiencing CRS, up to 40% experiencing neurologic toxicity, and 20%-40% requiring intensive care unit level–care. Reducing tumor burden at time of product infusion has dramatically diminished the incidence of these complications. The duration of remission may vary depending on disease burden, the type of construct used and exposure to fludarabine.(27, 28) The latter was found to be correlated to leukemia-free survival and CD19-positive relapse.(28)

In October 2021, another autologous anti-CD19 CAR T-cell therapy (KTE-X19; brexucabtagene autoleucel or Tecartus®) was granted accelerated approval by the FDA for adult patients with R/R BCP-ALL, but this product is not yet approved for children.(29)

Two of the major concerns regarding long-term outcome after CAR T-cell therapy are the loss of CART cells, leading to CD19 positive relapses, and the antigen escape leading to CD19 negative relapses. In the latter case, a second infusion of anti-CD19 CAR T-cells or blinatumomab are no longer valuable options. There are ongoing trials testing dual CART (CD19x22), aiming to avoid this possible mechanism of relapse.(30) CD19+ relapses, accounting for the majority of relapses (up to 78%), frequently involve short persistence CAR T-cells. Immune responses are thought to be responsible for rejection of CAR T-cells, especially against the fragment domains derived from mouse monoclonal antibodies. Humanization of CAR components could avoid immune-mediated rejection.(31) Fresh products derived from decentralized manufacturing, could possibly also improve CAR T-cell persistence, and facilitate a larger access to this therapy. (32)

As both blinatumomab and CART-cell therapy target CD19, the option to target another antigen, such as CD22, is of major importance in case of CD19 negative relapses, but also considering possible tandem therapy against multiple targets.

InO

InO represents an additional important new option in the BCP-ALL treatment landscape. It targets CD22, therefore it differs substantially from blinatumomab and tisagenlecleucel. It is an antibody coniugated to chemotherapy (calicheamicin), therefore a targeted delivery of chemotherapy rather than immunotherapy. InO was approved for adults in 2017 with R/R CD22-positive BCP-ALL after the results

of the INO-VATE phase 3 study, which showed a complete remission rate of 80.7% in the InO arm compared to 29.4% in the standard intensive chemotherapy control arm.(10) The long-term follow-up showed also a benefit in survival with InO (2-year OS rates of 22.8% and 10.0%, respectively, HR 0.75; 1-sided P = .01). Our ITCC-059 trial is the phase 1-2 'intent to file' trial designed to collect pediatric data in R/R ALL to pursue the pediatric label for this drug. Simultaneously, the Children's Oncology Group (COG) performed additional studies on InO in pediatric ALL, the AALL1621 phase 2 study, using the adult RP2D. AALL1621 demonstrated an ORR of 58.6% in children and young adults with R/R CD22-positive BCP-ALL, and is proceeding with InO combined with chemotherapy in a manner to complement the ITCC-059 study. (33)

In adults, InO has been already studied in different contexts and combination strategies. InO was successfully combined with the mini–hyper-CVD chemotherapy treatment (mini-HCVD: cyclophosphamide and dexamethasone at 50% dose reduction, no anthracycline, methotrexate at 75% dose reduction, and cytarabine at 0.5 g/m2 × 4 doses), given in a single dose on day 3 at the approved full dose of 1.8 (or lower to 1.3 mg/m2) for cycle 1, followed by 1.3 to 1.0 mg/m2 for subsequent cycles. The results were encouraging in first salvage setting (median OS 17 months compared with 9 months in historical series treated with InO or blinatumomab). (34) The combination have been tested also in newly diagnosed older patients with (Ph)-negative pre-B-cell ALL. In order to decrease the risk of VOD, the protocol was amended to give InO in fractionated doses and the dose was lowered (0.6 mg/m^2 and 0.3 mg/m^2 in cycle 1; 0.3 mg/m^2 on day 2 and 8 of cycles 2-4). In addition, 4 cycles of Blina were given following 4 cycles of mini-HCVD plus InO. Maintenance consisted of 12 cycles of chemotherapy and 4 cycles of Blina. Mini-hyper-CVD plus InO, with or without blinatumomab, in older adults with newly diagnosed Ph-negative ALL resulted in an overall response rate of 98% and a 5-year OS rate of 47%.(12) In adult younger patients (18-59 years) with newly diagnosed Ph- BCP ALL, hyper-CVAD with sequential blinatumomab, with or without InO (0.3 mg/m^2 day 1-8 added to the 2 cycles of Methotrexate/Ara-C and to 2 cycles of blinatumomab consolidation), was tested as front-line treatment. For the entire cohort, the 3-year OS rate was 84%, and outcomes of the patients treated in the InO arm were particularly promising. (35) InO has also been tested in the subset of adult patients with R/R Ph+ ALL, a group of patients with a particular dismal prognosis, with encouraging improvement in responses and rates of HSCT, although not in terms of OS. TKIs in combination with InO and/or blina may also be worth studying to enhance response rates and

durability of response.(36) A recent study in patients with R/R ALL or CML in lymphoid blast phase reported that the combination of InO and bosutinib was well tolerated, with demonstrable clinical activity.(37) In a similar way, ponatinib and blinatumomab have been study as a chemo-free regimen for newly diagnosed or relapsed adult patients with Ph+-ALL, showing high rates of complete molecular response.(3)
The poorer outcome in the adult setting allows piloting different strategies in a larger population with a dismal prognosis. In pediatric ALL innovations in the front-line setting have to face the OS of about 90% currently reached with the poly-chemotherapy regimens. Nevertheless the existing pediatric regimens will need to change significantly to replace toxic elements with the newer agents.

Ongoing trials in pediatric ALL

As a start, InO and blinatumomab are currently being introduced in front-line treatment for children with newly diagnosed ALL, as single-agent cycles during consolidation therapy (NCT03959085, NCT03911128, NCT03914625, NCT03643276 – trials details below). It could be argued that especially InO demonstrated its activity as single agent induction treatment reaching ORR above 80% in the relapse setting, in patients with bulky disease. This would qualify InO as a good option as induction therapy, rather than consolidation. Nevertheless, considering that 98% of the patients achieve a first morphological complete remission with the current 3 or 4 drugs induction regimens, radical changes in this setting are to date still difficult to embrace. Different randomized studies are running in parallel in the US and Europe by multiple study groups. A phase 3 randomized trial of InO for newly diagnosed high-risk CD22-positive BCP-ALL (AALL1732 study; NCT03959085) is ongoing in the US, which tests if adding InO to standard post-induction chemotherapy maintains or improves outcomes. In Europe, the 'ALL together' group (NCT03911128) is testing InO as additional consolidation treatment in a randomized fashion within the intermediate-high risk patient group; while blinatumomab is included in the study for patients with Down Syndrome, who have poor tolerance to chemotherapy. The Cassiopeia trial (NCT03876769) is testing Tisasagenlecleucel in first line high-risk BCP-ALL with MRD $\geq$ 0.01% at end of consolidation. The COG NCT03914625/AALL1731 trial and the BFM (Berlin-Frankfurt-Münster)/AIEOP (Associazione Italiana Emato-oncologia pediatrica) NCT03643276 trial include randomized questions of blinatumomab during consolidation in front-line. In addition, blinatumomab showed improved results in infants with *KMT2A*-r ALL (NCT03643276).(38)
InO is also continuing to be studied in the R/R BCP-ALL setting, following the attempts

made in adult ALL, in combination with chemotherapy. An additional phase 1B cohort within the ITCC-059 has tested the combination of InO with a modified R3 reinduction regimen, and has been recently completed. The study showed comparable results as in the single agent part of the study, not providing evidence of further improvement with the addition of chemotherapy, and showing that the liver toxicity was a limiting factor and required dexamethasone dose reduction and prohibited the addition of asparaginase to the combination regimen.(39) Finally, the trial is currently including a cohort of patients with very high-risk first relapse ALL, who will receive single agent InO (NTR57360), followed by consolidation with SCT or CAR T-cells once in CR. The COG group is also testing InO combined with chemotherapy in a manner to complement the ITCC-059 study (NCT02981628). The future plan will be to further test InO against regular reinduction therapy in first relapse BCP-ALL within the European IntReALL group, with the aim to show whether InO may be more efficacious and less toxic in that setting, than regular 4 drug reinduction chemotherapy. Mechanism of resistance to InO still needs to be fully understood and potential synergistic mechanism with other new agents, such as venetoclax as suggested in murine models(40), are the future field to be explored.

Even if satisfying results have been reached with the current chemotherapy regimens for pediatric ALL, especially in front-line induction treatment, new agents, such as InO, should be seen as a valuable strategy to reduce the morbidity and possibly mortality in this setting.

Future perspectives in pediatric BCP-ALL

With multiple options of novel therapies (blinatumomab, InO, CAR T-cells) available for R/R B-ALL, the best combination or sequence of treatment for a relapse or refractory state and for newly diagnosed patients remains one of the most important unanswered question at the present stage.

In general, if the patient has a high disease burden, InO would be preferred, given its response rates in high burden of disease compared with blinatumomab.(22, 41) CAR T-cell therapy has a good response rate, even with high disease burden, but at expenses of very high toxicity in terms of cytokine release syndrome and neurotoxicity and less durable response rates. Therefore CAR T- cells are now generally used after a disease debulking and always after lymphodepletion treatment. Blinatumomab seems a very good option as a consolidative treatment or in case of persistent MRD positivity, given the high rate of MRD-negativity after treatment and the low toxicity rate in this setting.(23, 24) InO and/or blina are not considered stand-alone therapies

and an allo-SCT is recommended in the pediatric setting to consolidate the response and as a definitive curative option. In this regard, CAR T-cell therapy could be seen as an alternative option, that not necessarily requires an allo-SCT as subsequent treatment: CAR T-cells are certainly a preferable option in previously transplanted patients, that are not suitable candidates for a second allo-SCT. In addition CAR T-cells infusion does not require that a deep remission is achieved, whereas a low or negative MRD is required to proceed to allo-SCT. It is important to identify in the near future those patients in whom CAR T-cell therapy can replace allo-SCT and those in whom it could represent a more effective bridge to transplantation. It is an advantage that patients, who are able to maintain remission with stand-alone CART cells therapy, avoid the long-term toxicity caused by allo-SCT. Nevertheless, about 50% of patients will relapse after remission achieved with CAR T cells.(42) In addition a number of patients experience a loss of B-cell aplasia or rising MRD, before a relapse. Whether these patients could benefit of an early allo-SCT before relapse, represent another open question. Longer follow-up of patients treated with CAR T-cell therapy will help in the coming years answering these questions.

Which is the best sequence of treatment in this context? This question has yet to be answered. Data regarding the safety profile of allo-SCT after InO and blina have been investigated both in adults and children. It is important to remark the risk of VOD after HSCT following InO. In the pediatric population, risk factors for developing post-HSCT VOD are not yet supported by statistical evidence due to the limited sample size studied. In our trial cohort (*chapter 4*) the only significant risk factor found was the time elapsed between the last InO dose and HSCT. Whether an interim maintenance therapy or a cycle of blina between InO and HSCT help in reducing the incidence (about 25%) of VOD/SOS after HSCT post-InO needs to be studied. In adult trials other risk factors associated with increased VOD/SOS risk after HSCT in InO-treated patients could be identified: conditioning regimens containing dual alkylators, and bilirubin levels at or above the upper limit of normal range at the last measurement before conditioning therapy. An age $\geq$55 years and the number of treatment cycles received were also identified as risk factors but did not remain significant in the multivariate analysis.(43) Nevertheless this is reflected in the current InO label.

Another question is whether blinatumomab and InO are suitable bridging therapies before CAR T-cells, given their potential impact on T-cell quality, or by reducing the antigen load (CD19 in case of blinatumomab). Prior therapy with blinatumomab, directed to CD19 as the available CAR T-cell products, has been associated with higher rates of CAR T-cell failure.(44) Nevertheless a recent report showed that

only patients with no response to blinatumomab had an inferior event free survival after subsequent CAR T-cell therapy. It is yet unclear whether this is a direct effect of blinatumomab, or it is caused by selection bias for resistant leukemias.(27) The most common CAR T-cell products are CD19-directed, therefore not competing on the same target antigen of InO. A possible expected effect on CAR T efficacy may be mediated by the reduction of CD19+ positive B cells, both leukemic and native, following InO treatment. It was previously reported that marrow CD19+ antigen load of less than 15% can be correlated with suboptimal expansion and persistence of CAR T-cells.(45) Nevertheless a recent retrospective study showed similar response rate and OS/EFS for patients treated with InO prior to CAR T-cells therapy compared to published data of patients treated with CAR T-cells without prior InO exposure. (46) Multiple factors should be considered to choose the best suitable therapy: presence/absence of target antigen (CD19 and CD22), prior exposure to one of these agents, disease burden (MRD only or high disease burden), comorbid conditions that might be exacerbated by a particular immune-targeting approach and indication for allo-SCT. The decision for the clinicians remains a challenge in absence of evidence-based prioritizations. Clinical trials remain the best way to provide answers to the clinical practice, but very long lasting studies are probably not anymore the best option in this era of rapidly changing treatment paradigms. Smaller studies addressing key questions to shape the next era of treatment protocols may be the most suitable approach.

7.3 Treatment landscape for CML *(Chapter 6)*

TKIs are the gold standard treatment for pediatric CML, as first and second-line therapy, and they could be considered as a third-line option, before proceeding to an allo-SCT for those patients that have insufficient response or intolerance to multiple TKIs.(47) In the current landscape, there are already three TKIs approved for pediatric use by FDA and EMA: the first generation imatinib, approved since 2003, and the second generation dasatinib and nilotinib, approved in 2017 and 2018 respectively. TKIs have a much broader use than only CML, even in pediatric hemato-oncologic diseases: Ph+ ALL has become a clearly separate entity since the introduction of TKIs, additionally TKIs are also being studied in other *ABL*-class fusion within the BCP-ALL group or for T-ALL. The third generation TKI ponatinib works through additional targets that can be valuable also against a variety of myeloproliferative disorders and solid tumors.(48) Focusing on CML, some important improvements in TKI treatment can still be pursued.

Children have a longer life expectancy and therefore the necessity of a longer exposure to TKI therapy. Nevertheless there are no data on the long-term efficacy of TKI therapy beyond 15-20 years.(15) Treatment discontinuation must be preferred above long-life treatment with TKI in young patients, but this option is still under clinical investigation in pediatric age, and in the literature there are only small series reported.(49, 50) To discontinue TKI treatment, a deep prolonged first remission is required, at least 2 years of sustained deep molecular remission according to the European LeukemiaNet 2020 guidelines for adults.(51) These criteria are met only by a minority of patients, namely about 11% and 29.7% of adult patients after 5 and 10 years of treatment with imatinib and 20.9% and 48.6% with nilotinib in front-line.(52) The proportion of pediatric patients eligible for treatment-free remission (TFR) are expected to be similar, or lower, considering the more aggressive clinical and biological features of CML in children than in adults.(15) Therefore an insufficient proportion of patients could be able to discontinue treatment. Even when discontinued under safe criteria, 50% of the patients are expected to experience a reactivation of the disease. Luckily they show again sensitivity after TKI resumption.(50) Data in children will become available through a TKI stopping study currently ongoing within COG (NCT03817398).

To improve the proportion of patients eligible to TFR, second generation TKI can be considered as first choice to achieve a faster and deeper remission in a larger proportion of patients, if the costs do not represent an issue.(47) In addition, side effects of TKIs remain a relevant aspect, especially in pediatric patients expected to continue the therapy potentially for decades.

Bosutinib

Bosutinib is a second generation TKI, approved since 2017 for adult patients with newly diagnosed (or resistant/intolerant) CML. As one third of the pediatric patients with CML has an unsatisfactory response or is intolerant to the first generation TKI imatinib, second generation TKIs including bosutinib can be a valuable option to improve efficacy or tolerance. For these patients dasatinib QD and nilotinib BID are approved, but the latter requires administrations under fasting conditions and twice daily, which is a less attractive option in pediatric patients. Compliance to therapy is essential for effectiveness of TKI, and this can be especially difficult in adolescents. Imatinib, dasatinib and nilotinib showed growth impairment in pediatric patients, while preclinical data indicated that this toxicity may not be observed or be less prominent with bosutinib.(53) Bosutinib might represent an alternative in pediatric

patients not responding or intolerant to the other TKIs, with a different safety profile (more gastrointestinal and less musculoskeletal toxicity), as shown in *chapter 6*. In addition, if the data on preservation of bone growth observed in animal models are confirmed in the clinical setting, bosutinib might have an advantage for children with CML (especially if naïve to treatment), as it might avoid impairment of longitudinal growth, particularly in those diagnosed at prepubertal age.(18) This however requires longer follow-up of newly-diagnosed patients recruited in our ITCC-054 study. As mentioned above, second generation TKI are also relevant to try to increase the proportion of patients eligible for treatment-free remission, and bosutinib could contribute to this aim.

Future perspectives in pediatric CML

If a patient fails treatment with second generation TKIs, allo-SCT is still considered as a valuable option. Nevertheless post-SCT morbidity and mortality are significantly high.(54) New options are becoming available and could further reduce the number of patients in need of an allo-SCT. The third generation TKI ponatinib is under investigation in children and showed activity against the *T315I* mutation, which causes resistance to first- and second-generation TKIs (NCT03934372). Compassionate use series with ponatinib in children, showed a good tolerability profile in 14 children, without arterial thrombotic events events.(48, 55) Asciminib is a BCR-ABL inhibitor specifically targeting the ABL Myristoyl Pocket (STAMP) recently approved for the treatment of adult patients.(56) It works by binding to a different binding site to inhibit the constitutively active BCR-ABL1 kinase, and hence it may also be studied in combination with TKI to evaluate the possible synergistic effect, as currently under investigation in a trial in adults combining asciminib with imatinib (NCT03578367). A randomized trial of adult patients with CML-CP previously treated with $\geq$2 TKI showed a superior efficacy of asciminib compared with that of bosutinib (based on major molecular response rate at 6 months).(56) The study suggests that asciminib as rescue treatment after failing other TKIs is better than bosutinib.

Although TKIs have changed the landscape in CML, patients are not cured with TKI therapy alone, with allo-SCT remaining the only proven curative treatment to date. The more recent goal of CML treatment to induce treatment-free remission after durable deep molecular response, open the way to a curative option for CML patients without allo-SCT. To achieve this goal, it is necessary to understand the mechanism leading to relapse in half of the patients discontinuing TKI when in deep molecular remission. The results of STOP studies in children are urgently needed,

as they usually tolerate SCT better than older adults, for whom TKIs are the best option. Moreover, a long follow-up in adults who stopped TKIs will also be helpful for strategies in children.(57)

Despite imatinib effectively inhibits BCR-ABL kinase CML LSCs, it does not induce cell death in vitro, suggesting LSCs are resistant to TKI treatment.(58) Possible targetable pathways required by CML LSCs for survival are leading to possible combination treatment of TKI with non-TKI drugs such as interferon and JAK2 inhibitors. Emerging data suggests that also the combination of TKIs with BCL-2 inhibitors can enhance cytotoxicity and thus eradicate CML LSCs (59). In addition, checkpoint inhibitors could be an option in combination with TKI based on preclinical data and clinical data showing correlation between the expression of CD86 (the CTLA-4 ligand) and the risk of recurrence after TKI discontinuation.(60)

Additional attempts, especially relevant to the pediatric population, in order to reduce the long-term side effects of TKIs are under investigation. A study on TKI de-escalation demonstrated its feasibility, possibly proving that patients in deep molecular response could be treated with lower dosages with the same efficacy and less side effects.(61) An Italian study including pediatric patients is testing intermittent treatment (3 weeks on- 1 week off), in order to reduce side effects of TKI.(62)

In conclusion, longer follow-up data on TKI use, but also from STOP trials in adult and children are awaited. Especially for children, data on quality of life with a life-long therapy are of highly relevant, as well as a good transition in the adult care with adequate follow-up data.

7.4 Conclusion

Pediatric clinical trials demonstrate to generate valuable knowledge, that can be translated in safer and more effective guidelines of drug administration in children. Nevertheless approvals of new agents for pediatric hemato-oncologic diseases lags behind adults, showing that current incentives for pediatric drug development are still insufficiently attractive for pharmaceutical companies.(63) Further development of the academic networks, including sponsorship capabilities, for early phase studies in children with cancer is needed to accommodate the growing number of ‘intent to file’ studies, plus development of pediatric study teams that can deliver such studies. (9) Smaller studies addressing key questions should guide the choices to shape the next era of treatment protocols, in this era of rapidly changing landscape.

Starting from the knowledge generated from the ITCC-054 and ITCC-059 clinical trials, further steps in the treatment of pediatric patients with leukemia have been made.

InO represent a valuable option as re-induction treatment in relapsed and refractory ALL (C*hapter 3 and 4*), including very young patients (*Chapter 5*). The most important questions to answer through the ongoing or upcoming clinical trials include the possibility of combination treatment of InO with chemotherapy, or with new agents that could improve its efficacy . In addition, the role of InO in the treatment of first relapse ALL and also its positioning in front-line treatment are of primary importance. Bosutinib is a valid option for patients resistant or intolerant to other TKI (*chapter 6*), and its role in newly-diagnosed patients and especially on bone growth will be probably answered by the ongoing cohort of the phase 2 of the ITCC-054 study. Results of the STOP study from COG are very much awaited and treatment free remission for a larger proportion of patients will be the next step in the CML treatment field.
Hopefully InO and bosutinib will be approved soon for pediatric use, based on the data generated by the ITCC-054 and ITCC-059 trials, presented in this thesis.

References

1. `t Jong GW, Vulto AG, de Hoog M, Schimmel KJM, Tibboel D, van den Anker JN. Unapproved and Off-Label Use of Drugs in a Children's Hospital. New England Journal of Medicine. 2000;343(15):1125-.
2. Foà R, Bassan R, Vitale A, Elia L, Piciocchi A, Puzzolo MC, et al. Dasatinib-Blinatumomab for Ph-Positive Acute Lymphoblastic Leukemia in Adults. N Engl J Med. 2020;383(17):1613-23.
3. Jabbour E, Short NJ, Jain N, Huang X, Montalban-Bravo G, Banerjee P, et al. Ponatinib and blinatumomab for Philadelphia chromosome-positive acute lymphoblastic leukaemia: a US, single-centre, single-arm, phase 2 trial. Lancet Haematol. 2023;10(1):e24-e34.
4. Pearson AD, Heenen D, Kearns PR, Goeres A, Marshall LV, Blanc P, et al. 10-year report on the European Paediatric Regulation and its impact on new drugs for children's cancers. Lancet Oncol. 2018;19(3):285-7.
5. List UFaDAPMT. https://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/OCE/ucm544641.htm. Accessed January 30, 2023.
6. Pearson ADJ, Scobie N, Norga K, Ligas F, Chiodin D, Burke A, et al. ACCELERATE and European Medicine Agency Paediatric Strategy Forum for medicinal product development for mature B-cell malignancies in children. Eur J Cancer. 2019;110:74-85.
7. Karres D, Lesa G, Ligas F, Benchetrit S, Galluzzo S, Van Malderen K, et al. European regulatory strategy for supporting childhood cancer therapy developments. Eur J Cancer. 2022;177:25-9.
8. Gatta G, Botta L, Rossi S, Aareleid T, Bielska-Lasota M, Clavel J, et al. Childhood cancer survival in Europe 1999-2007: results of EUROCARE-5--a population-based study. Lancet Oncol. 2014;15(1):35-47.
9. Wilde BD, Barry E, Fox E, Karres D, Kieran M, Manlay J, et al. The Critical Role of Academic Clinical Trials in Pediatric Cancer Drug Approvals: Design, Conduct, and Fit for Purpose Data for Positive Regulatory Decisions. Journal of Clinical Oncology. 2022;40(29):3456.
10. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab Ozogamicin versus Standard Therapy for Acute Lymphoblastic Leukemia. N Engl J Med. 2016;375(8):740-53.
11. O'Dwyer KM, Liesveld JL. Philadelphia chromosome negative B-cell acute lymphoblastic leukemia in older adults: Current treatment and novel therapies. Best Pract Res Clin Haematol. 2017;30(3):184-92.
12. Short NJ, Kantarjian H, Ravandi F, Huang X, Jain N, Kadia TM, et al. Updated Results from a Phase II Study of Mini-Hyper-CVD Plus Inotuzumab Ozogamicin, with or without Blinatumomab, in Older Adults with Newly Diagnosed Philadelphia Chromosome-Negative B-Cell Acute Lymphoblastic Leukemia. Blood. 2021;138(Supplement 1):3400-.
13. Roberts KG. Genetics and prognosis of ALL in children vs adults. Hematology Am Soc Hematol Educ Program. 2018;2018(1):137-45.
14. Pieters R, De Lorenzo P, Ancliffe P, Aversa LA, Brethon B, Biondi A, et al. Outcome of Infants Younger Than 1 Year With Acute Lymphoblastic Leukemia Treated With the Interfant-06 Protocol: Results From an International Phase III Randomized Study. J Clin Oncol. 2019;37(25):2246-56.

15. Hijiya N, Schultz KR, Metzler M, Millot F, Suttorp M. Pediatric chronic myeloid leukemia is a unique disease that requires a different approach. Blood. 2016;127(4):392-9.
16. Millot F, Guilhot J, Baruchel A, Petit A, Bertrand Y, Mazingue F, et al. Impact of early molecular response in children with chronic myeloid leukemia treated in the French Glivec phase 4 study. Blood. 2014;124(15):2408-10.
17. Samis J, Lee P, Zimmerman D, Arceci RJ, Suttorp M, Hijiya N. Recognizing Endocrinopathies Associated With Tyrosine Kinase Inhibitor Therapy in Children With Chronic Myelogenous Leukemia. Pediatr Blood Cancer. 2016;63(8):1332-8.
18. Tauer JT, Hofbauer LC, Jung R, Erben RG, Suttorp M. Micro-osmotic pumps for continuous release of the tyrosine kinase inhibitor bosutinib in juvenile rats and its impact on bone growth. Med Sci Monit Basic Res. 2013;19:274-8.
19. Corbacioglu S, Carreras E, Ansari M, Balduzzi A, Cesaro S, Dalle JH, et al. Diagnosis and severity criteria for sinusoidal obstruction syndrome/veno-occlusive disease in pediatric patients: a new classification from the European society for blood and marrow transplantation. Bone Marrow Transplant. 2018;53(2):138-45.
20. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental Pharmacology — Drug Disposition, Action, and Therapy in Infants and Children. New England Journal of Medicine. 2003;349(12):1157-67.
21. Abbas R, Hsyu PH. Clinical Pharmacokinetics and Pharmacodynamics of Bosutinib. Clin Pharmacokinet. 2016;55(10):1191-204.
22. von Stackelberg A, Locatelli F, Zugmaier G, Handgretinger R, Trippett TM, Rizzari C, et al. Phase I/Phase II Study of Blinatumomab in Pediatric Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia. J Clin Oncol. 2016;34(36):4381-9.
23. Brown PA, Ji L, Xu X, Devidas M, Hogan LE, Borowitz MJ, et al. Effect of Postreinduction Therapy Consolidation With Blinatumomab vs Chemotherapy on Disease-Free Survival in Children, Adolescents, and Young Adults With First Relapse of B-Cell Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. JAMA. 2021;325(9):833-42.
24. Locatelli F, Zugmaier G, Rizzari C, Morris JD, Gruhn B, Klingebiel T, et al. Effect of Blinatumomab vs Chemotherapy on Event-Free Survival Among Children With High-risk First-Relapse B-Cell Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. JAMA. 2021;325(9):843-54.
25. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018;378(5):439-48.
26. Laetsch TW, Maude SL, Rives S, Hiramatsu H, Bittencourt H, Bader P, et al. Three-Year Update of Tisagenlecleucel in Pediatric and Young Adult Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia in the ELIANA Trial. J Clin Oncol. 2022:Jco2200642.
27. Myers RM, Taraseviciute A, Steinberg SM, Lamble AJ, Sheppard J, Yates B, et al. Blinatumomab Nonresponse and High-Disease Burden Are Associated With Inferior Outcomes After CD19-CAR for B-ALL. J Clin Oncol. 2022;40(9):932-44.
28. Dekker L, Calkoen FG, Jiang Y, Blok H, Veldkamp SR, De Koning C, et al. Fludarabine exposure predicts outcome after CD19 CAR T-cell therapy in children and young adults with acute leukemia. Blood Adv. 2022;6(7):1969-76.

29. Shah BD, Bishop MR, Oluwole OO, Logan AC, Baer MR, Donnellan WB, et al. KTE-X19 anti-CD19 CAR T-cell therapy in adult relapsed/refractory acute lymphoblastic leukemia: ZUMA-3 phase 1 results. Blood. 2021;138(1):11-22.

30. Cordoba S, Onuoha S, Thomas S, Pignataro DS, Hough R, Ghorashian S, et al. CAR T cells with dual targeting of CD19 and CD22 in pediatric and young adult patients with relapsed or refractory B cell acute lymphoblastic leukemia: a phase 1 trial. Nature Medicine. 2021;27(10):1797-805.

31. Myers RM, Li Y, Leahy AB, Barrett DM, Teachey DT, Callahan C, et al. Humanized CD19-Targeted Chimeric Antigen Receptor (CAR) T Cells in CAR-Naive and CAR-Exposed Children and Young Adults With Relapsed or Refractory Acute Lymphoblastic Leukemia. Journal of Clinical Oncology. 2021;39(27):3044-55.

32. Pearson AD, Rossig C, Mackall C, Shah NN, Baruchel A, Reaman G, et al. Paediatric Strategy Forum for medicinal product development of chimeric antigen receptor T-cells in children and adolescents with cancer: ACCELERATE in collaboration with the European Medicines Agency with participation of the Food and Drug Administration. Eur J Cancer. 2022;160:112-33.

33. O'Brien MM, Ji L, Shah NN, Rheingold SR, Bhojwani D, Yuan CM, et al. Phase II Trial of Inotuzumab Ozogamicin in Children and Adolescents With Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia: Children's Oncology Group Protocol AALL1621. J Clin Oncol. 2022;40(9):956-67.

34. Jabbour E, Ravandi F, Kebriaei P, Huang X, Short NJ, Thomas D, et al. Salvage Chemoimmunotherapy With Inotuzumab Ozogamicin Combined With Mini-Hyper-CVD for Patients With Relapsed or Refractory Philadelphia Chromosome-Negative Acute Lymphoblastic Leukemia: A Phase 2 Clinical Trial. JAMA Oncol. 2018;4(2):230-4.

35. Short N, Jabbour E, Ravandi F, Yilmaz M, Kadia TM, Thompson PA, et al. The Addition of Inotuzumab Ozogamicin to Hyper-CVAD Plus Blinatumomab Further Improves Outcomes in Patients with Newly Diagnosed B-Cell Acute Lymphoblastic Leukemia: Updated Results from a Phase II Study. Blood. 2022;140(Supplement 1):8966-8.

36. Stock W, Martinelli G, Stelljes M, DeAngelo DJ, Gökbuget N, Advani AS, et al. Efficacy of inotuzumab ozogamicin in patients with Philadelphia chromosome-positive relapsed/refractory acute lymphoblastic leukemia. Cancer. 2021;127(6):905-13.

37. Jain N, Maiti A, Ravandi F, Konopleva M, Daver N, Kadia T, et al. Inotuzumab ozogamicin with bosutinib for relapsed or refractory Philadelphia chromosome positive acute lymphoblastic leukemia or lymphoid blast phase of chronic myeloid leukemia. Am J Hematol. 2021;96(8):1000-7.

38. van der Sluis I, Lorenzo P, Kotecha R, Attarbaschi A, Escherich G, Nysom K, et al. A Phase 2 Study to Test the Feasibility, Safety and Efficacy of the Addition of Blinatumomab to the Interfant06 Backbone in Infants with Newly Diagnosed KMT2A -Rearranged Acute Lymphoblastic Leukemia. a Collaborative Study of the Interfant Network. Blood. 2021;138:361-.

39. Pennesi E, Brivio E, Ammerlaan ACJ, Jiang Y, van der Velden VHJ, Beverloo B, et al. Inotuzumab Ozogamicin (InO) Combined with UKALL-R3 Modified Chemotherapy in Pediatric Patients with B-Cell Precursor CD22+ Acute Lymphoblastic Leukemia (BCP-ALL) - Results from the ITCC-059 Phase 1B Trial. Blood. 2022;140(Supplement 1):8976-8.

40. Kirchhoff H, Karsli U, Schoenherr C, Battmer K, Erschow S, Talbot SR, et al. Venetoclax and dexamethasone synergize with inotuzumab ozogamicin-induced DNA damage signaling in B-lineage ALL. Blood. 2021;137(19):2657-61.

41. Pennesi E, Michels N, Brivio E, van der Velden VHJ, Jiang Y, Thano A, et al. Inotuzumab ozogamicin as single agent in pediatric patients with relapsed and refractory acute lymphoblastic leukemia: results from a phase II trial. Leukemia. 2022;36(6):1516-24.

42. Pasquini MC, Hu ZH, Curran K, Laetsch T, Locke F, Rouce R, et al. Real-world evidence of tisagenlecleucel for pediatric acute lymphoblastic leukemia and non-Hodgkin lymphoma. Blood Adv. 2020;4(21):5414-24.

43. Kantarjian HM, DeAngelo DJ, Stelljes M, Liedtke M, Stock W, Gökbuget N, et al. Inotuzumab ozogamicin versus standard of care in relapsed or refractory acute lymphoblastic leukemia: Final report and long-term survival follow-up from the randomized, phase 3 INO-VATE study. Cancer. 2019;125(14):2474-87.

44. Pillai V, Muralidharan K, Meng W, Bagashev A, Oldridge DA, Rosenthal J, et al. CAR T-cell therapy is effective for CD19-dim B-lymphoblastic leukemia but is impacted by prior blinatumomab therapy. Blood Adv. 2019;3(22):3539-49.

45. Gardner RA, Finney O, Annesley C, Brakke H, Summers C, Leger K, et al. Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. Blood. 2017;129(25):3322-31.

46. Ceolin V, Brivio E, van Tinteren H, Rheingold SR, Leahy A, Vormoor B, et al. Outcome of chimeric antigen receptor T-cell therapy following treatment with inotuzumab ozogamicin in children with relapsed or refractory acute lymphoblastic leukemia. Leukemia. 2022.

47. Hijiya N, Suttorp M. How I treat chronic myeloid leukemia in children and adolescents. Blood. 2019;133(22):2374-84.

48. Rossoff J, Huynh V, Rau RE, Macy ME, Sulis ML, Schultz KR, et al. Experience with ponatinib in paediatric patients with leukaemia. British Journal of Haematology. 2020;189(2):363-8.

49. Giona F, Saglio G, Moleti ML, Piciocchi A, Rea M, Nanni M, et al. Treatment-free remission after imatinib discontinuation is possible in paediatric patients with chronic myeloid leukaemia. Br J Haematol. 2015;168(2):305-8.

50. Shima H, Kada A, Tanizawa A, Sato I, Tono C, Ito M, et al. Discontinuation of tyrosine kinase inhibitors in pediatric chronic myeloid leukemia. Pediatr Blood Cancer. 2022;69(8):e29699.

51. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966-84.

52. Kantarjian HM, Hughes TP, Larson RA, Kim DW, Issaragrisil S, le Coutre P, et al. Long-term outcomes with frontline nilotinib versus imatinib in newly diagnosed chronic myeloid leukemia in chronic phase: ENESTnd 10-year analysis. Leukemia. 2021;35(2):440-53.

53. Patterson B SJ, Gore L, Zwaan C.M., Sacchi M, Sy O, Hijiya N. GROWTH RATE AND ENDOCRINE EFFECTS OF DASATINIB THERAPY OBSERVED IN A RETROSPECTIVE ANALYSIS OF A PHASE 2 CLINICAL TRIAL FOR PEDIATRIC PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP). Hemasphere. 2019;3:161.

54. Bhatia S. Long-term health impacts of hematopoietic stem cell transplantation inform recommendations for follow-up. Expert Rev Hematol. 2011;4(4):437-52; quiz 53-4.

55. Millot F, Suttorp M, Versluys AB, Kalwak K, Nelken B, Ducassou S, et al. Ponatinib in childhood Philadelphia chromosome-positive leukaemias: an international registry of childhood chronic myeloid leukaemia study. Eur J Cancer. 2020;136:107-12.

56. Réa D, Mauro MJ, Boquimpani C, Minami Y, Lomaia E, Voloshin S, et al. A phase 3, open-label, randomized study of asciminib, a STAMP inhibitor, vs bosutinib in CML after 2 or more prior TKIs. Blood. 2021;138(21):2031-41.

57. Saussele S, Richter J, Guilhot J, Gruber FX, Hjorth-Hansen H, Almeida A, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. Lancet Oncol. 2018;19(6):747-57.

58. Chomel JC, Bonnet ML, Sorel N, Bertrand A, Meunier MC, Fichelson S, et al. Leukemic stem cell persistence in chronic myeloid leukemia patients with sustained undetectable molecular residual disease. Blood. 2011;118(13):3657-60.

59. Mu H, Zhu X, Jia H, Zhou L, Liu H. Combination Therapies in Chronic Myeloid Leukemia for Potential Treatment-Free Remission: Focus on Leukemia Stem Cells and Immune Modulation. Frontiers in Oncology. 2021;11.

60. Schütz C, Inselmann S, Saussele S, Dietz CT, Müller MC, Eigendorff E, et al. Expression of the CTLA-4 ligand CD86 on plasmacytoid dendritic cells (pDC) predicts risk of disease recurrence after treatment discontinuation in CML. Leukemia. 2018;32(4):1054.

61. Clark RE, Polydoros F, Apperley JF, Milojkovic D, Pocock C, Smith G, et al. De-escalation of tyrosine kinase inhibitor dose in patients with chronic myeloid leukaemia with stable major molecular response (DESTINY): an interim analysis of a non-randomised, phase 2 trial. Lancet Haematol. 2017;4(7):e310-e6.

62. Giona F, Malaspina F, Putti MC, Ladogana S, Mura R, Burnelli R, et al. Results and outcome of intermittent imatinib (ON/OFF schedule) in children and adolescents with chronic myeloid leukaemia. British Journal of Haematology. 2020;188(6):e101-e5.

63. de Rojas T, Kearns P, Blanc P, Skolnik J, Fox E, Knox L, et al. Changing incentives to ACCELERATE drug development for paediatric cancer. Cancer Med. 2023.

Chapter 8

Summary

Summary

This thesis is focused on new therapeutic options for pediatric acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML). We provide the results of two 'first-in-child' clinical trials, developed in the context of Pediatric Investigational Plans (PIPs), with the final aim to inform the Regulatory Authority and obtain approval of inotuzumab ozogamicin (InO) and bosutinib for pediatric use.

The majority of drugs we use in pediatric oncology are not approved for the indication, and sometimes not even for use in children. In fact, development and approval of new agents for pediatric cancers lags behind adult development. New regulations in Europe and U.S. are enacted to ensure commitment of companies to develop drugs in children, but current incentives are still insufficiently attractive for pharmaceutical companies. Clinical trials in children, especially early phase 1-2 studies, are challenging due to the small number of patients with cancer and relapsed or refractory disease.

Chapter 2 provides an overview of the current state of the art of targeted therapies for children with leukemia and lymphoma, including clinical trials ongoing in the pediatric population. The studies are mostly initiated within the European Innovative Therapies for Children with Cancer (ITCC) group and the North-American Children's Oncology Group (COG). Immunotherapy in the form of targeted antibodies (as well as CAR T-cell therapy) represents at present the most promising future direction for hematologic malignancies. InO (anti-CD22) and blinatumomab (anti-CD19) are already being tested in front-line treatment for newly diagnosed ALL. Whether similar results may be obtained with other (conjugated) antibodies for other subsets of leukemia is still under investigation. With the focus on immunotherapy the urge to develop targeted agents is somewhat diminished. However, *BCR-ABL* inhibitors have an established role in CML and Ph+-ALL, as well as *ALK* inhibitors in ALCL. The role of other targeted therapies (i.e. MEK-inhibitors, FLT3 inhibitors, menin inhibitors and venetoclax) needs to be studied further in order to assess their added value. Molecular profiling and international tumor boards to facilitate allocation of patients to the best suitable target-based trial or therapy is of primary importance and in leukemia need to take surface marker expression and potentially also drug response profiling into account.

The subsequent chapters present the results of two phase 1-2 trials developed within the ITCC consortium and sponsored by Erasmus MC and performed in close collaboration with the Princess Máxima Center since its opening in 2018.

In ***chapter 3 and 4*** the results of the international phase I and II clinical trial of single-

agent InO (ITCC-059), a CD22 directed antibody conjugated to calicheamicin, in relapsed and refractory (R/R) pediatric ALL patients are discussed. InO studied as single agent for adult patients with R/R BCP-ALL demonstrated clearly superiority compared to standard of care chemotherapy. The ITCC-059 trial, presented in this thesis, has provided evidence of efficacy and safety of the conjugated anti-CD22 antibody in the pediatric population. At first, we established the recommended phase II dose (RP2D) of InO as single agent in a fractionated schedule, which resulted the same dose recommended in adults (1.8 mg/m2 in course 1). The phase I was followed by a phase II cohort to determine the ORR of single-agent InO and to collect additional safety and pharmacokinetic data at the RP2D. The combined response data from 40 patients treated at the RP2D (13 in phase I and 27 in phase II) showed an ORR of 82.5%, and 81.8% of the responders were MRD negative. These results are especially relevant considering that heavily pretreated patients enrolled, with half of the cases already transplanted. InO was generally well tolerated, with a low incidence of infections during treatment (17.8%). Sinusoidal obstruction syndrome (SOS) was the most serious adverse event, as in adults, although mostly occurring after subsequent HSCT and only occasionally while on treatment. The ITCC-059 study is still ongoing, testing InO as single agent in very high risk first relapse ALL; while InO tested in combination with standard reinduction therapy in R/R pediatric ALL did not provide evidence of further improvement in terms of efficacy. The liver toxicity was a limiting factor and required dexamethasone dose reduction and prohibited the addition of asparaginase to the combination regimen.

In ***chapter 5*** the use of InO in infants with R/R ALL was investigated. Infants (children < 1 year of age) are generally not eligible for treatment in clinical trials, as well as in the ITCC-059 for InO, which was a first-in child-trial. In addition, no data on InO in infant ALL was published before this study. Therefore, to collect real world data about safety and efficacy of InO in this specific ALL population with a dismal prognosis, we performed a retrospective collection of data in infants treated with InO on a compassionate use basis. Specific questions were considered relevant in these very young patients: the management of drug administration in this category of patients with very low body weight and low Body Surface Area (BSA) is always challenging; CD22 expression is lower in *KMT2A*-r ALL (75% of infant ALL carry this rearrangement) and when experiencing relapse these patients face a very poor prognosis with event free survival rates as low as 20%. Within the limitation of the retrospective nature of the study, InO appeared to be generally well tolerated and active. The median dose used was close to the adult recommended

dose based on body surface area (BSA), despite the relatively high BSA in infants compared to body weight. This finding suggested that infants might be treated at the same BSA-based RP2D established in the pediatric Phase I study of InO (ITCC-059) without facing excessive toxicity. The median percentage of $CD22^{+}$ blasts was 72% (range 40-100%, n=9), lower than in the general pediatric population. Seven patients (47%) achieved complete remission, less than in the pediatric R/R ALL cohort, but the efficacy of InO seems still valuable in these patients with a dismal prognosis. Toxicity reported was in line with adult and pediatric data, justifying further evaluation of InO in this subgroup of ALL.

In ***chapter 6,*** the preliminary results of the phase I part of the ITCC-054 trial in children with CML treated with bosutinib are presented and focused on pharmacokinetics and safety in the subgroup of resistant and intolerant (R/I) patients. These patients have an unmet medical need and require new treatment options that improve efficacy and provide a better tolerability profile compared to currently available TKIs, especially regarding growth. A deeper or more rapid molecular response may be beneficial in children who could ideally aim to stop TKIs to achieve treatment-free remission. In addition, if the data on preservation of bone growth observed in animal models are confirmed in the clinical setting, bosutinib might have an advantage, as it might avoid impairment of longitudinal growth, particularly in those patients diagnosed at prepubertal age. (10). The RP2D of Bosutinib for pediatric R/I CML patients was established at 400 mg/m^2 QD (max 600 mg/day), based on safety data and on target exposure as defined in adults. A higher BSA-adjusted dose was necessary to achieve the target exposure comparable to exposure as defined in adult studies, probably related to the different disposition of the drug in younger children, due to its low permeability and low solubility profile. The RP2D of bosutinib for pediatric newly diagnosed CML patients was extrapolated from the safety and PK data in R/I children and target exposure as derived from adults, and established as 300 mg/m2 (max 500 mg/day). Bosutinib showed an efficacy comparable to published data for other second generation TKIs approved in children with CML (cumulative incidence of CCyR at six months, excluding patients with CCyR at screening, 83.3% (95% CI: 40.5−96.4%)). Gastro-intestinal adverse events are the most commonly reported side effects, while the effect on bone growth will be better characterized in patients naïve to TKIs and with a longer follow up currently enrolled in the phase II part of the study.

In conclusion, InO represents a valuable option as re-induction treatment in relapsed and refractory pediatric ALL (C*hapter 3 and 4*), including very young patients (*Chapter 5*). Bosutinib is a valid option for CML patients resistant or intolerant to other TKI (C*hapter 6*), and its role in newly-diagnosed patients and especially on bone growth will be probably answered by the ongoing cohort of the phase II of the ITCC-054 study. Hopefully InO and bosutinib will be approved soon for pediatric use, based on the favorable benefit-risk data generated by the ITCC-054 and ITCC-059 trials, presented in this thesis.

A continuous effort to improve timely initiation of early-phase studies for approval of new agents in pediatric oncology is necessary. The partnership between collaborative groups in Europe and North-America (as promoted by the ACCELERATE platform, harmonization of regulatory incentives on both sides of the Atlantic Ocean and further development of the academic networks, including sponsorship capabilities, is needed to accommodate the regulatory requirements associated with 'intent to file' studies sponsored by academia.

Samenvatting

Dit proefschrift is gericht op nieuwe therapeutische opties voor pediatrische acute lymfatische leukemie (ALL) en chronische myeloïde leukemie (CML). We presenteren de resultaten van twee 'first-in-child' klinische onderzoeken, ontwikkeld binnen twee Pediatric Investigational Plans (PIP's), met als uiteindelijk doel de Regelgevende Instantie te informeren en goedkeuring te verkrijgen voor inotuzumab ozogamicine (InO) en bosutinib voor pediatrische patiënten.

De meeste geneesmiddelen die we in de kinderoncologie gebruiken, zijn niet goedgekeurd voor de indicatie, en soms zelfs niet voor gebruik bij kinderen. In feite loopt de ontwikkeling en goedkeuring van nieuwe geneesmiddelen voor kinderkanker achter op de ontwikkeling bij volwassenen. In Europa en de VS wordt aan nieuwe regelgeving gewerkt om ervoor te zorgen dat bedrijven zich inzetten om geneesmiddelen voor kinderen te ontwikkelen, maar de huidige stimulansen zijn nog steeds onvoldoende aantrekkelijk voor farmaceutische bedrijven. Klinische onderzoeken bij kinderen, met name vroege fase 1-2-onderzoeken, zijn een uitdaging vanwege het kleine aantal patiënten met kanker, vooral met recidiverende of refractaire ziekte.

Hoofdstuk 2 geeft een overzicht van de huidige stand van zaken op het gebied van gerichte therapieën voor kinderen met leukemie en lymfoom, inclusief lopende klinische studies bij pediatrische patiënten. De onderzoeken worden meestal geïnitieerd binnen de European Innovative Therapies for Children with Cancer (ITCC)-groep en de North-American Children's Oncology Group (COG). Immunotherapie in de vorm van gerichte antilichamen (evenals CAR T-celtherapie) vertegenwoordigt de meest veelbelovende toekomstige richting voor hematologische maligniteiten. InO (anti-CD22) en blinatumomab (anti-CD19) worden al getest als eerstelijnsbehandeling voor nieuw gediagnosticeerde ALL. Er wordt nog onderzocht of vergelijkbare resultaten kunnen worden verkregen met andere (geconjugeerde) antilichamen voor andere subgroepen van leukemie. Met de focus op immunotherapie is de drang om gerichte middelen te ontwikkelen wat afgenomen. BCR-ABL-remmers spelen echter een belangrijke rol bij CML en Ph+-ALL, evenals ALK-remmers bij ALCL. De rol van andere gerichte therapieën (d.w.z. MEK-remmers, FLT3-remmers, menin-remmers en venetoclax) moet verder worden onderzocht om hun toegevoegde waarde te beoordelen. Moleculaire profilering en internationale tumorboards zijn van groot belang om de toewijzing van patiënten aan de meest geschikte studie of therapie te vergemakkelijken, en bij leukemie moet rekening worden gehouden met expressie van oppervlaktemarkers en mogelijk ook met profilering van geneesmiddelrespons.

De volgende hoofdstukken presenteren de resultaten van twee fase 1-2-onderzoeken die zijn ontwikkeld binnen het ITCC-consortium, zijn gesponsord door het Erasmus MC, en die sinds de opening in 2018 in nauwe samenwerking met het Prinses Máxima Centrum zijn uitgevoerd.

In ***hoofdstuk 3 en 4*** worden de resultaten besproken van de internationale fase 1-2 klinische studie van single-agent InO (ITCC-059), een anti-CD22 antilichaam geconjugeerd aan calicheamicine, bij pediatrische patiënten met recidiverende en refractaire (R/R) ALL. InO, bestudeerd als monotherapie voor volwassen patiënten met R/R BCP-ALL, vertoonde duidelijk superioriteit in vergelijking met standaard chemotherapie.(6) De ITCC-059-studie, gepresenteerd in dit proefschrift, heeft bewijs geleverd voor de werkzaamheid en veiligheid van het geconjugeerde antilichaam in de pediatrische populatie. In eerste instantie hebben we de aanbevolen fase II-dosis (RP2D) van InO als monotherapie vastgesteld in een gefractioneerd schema, wat resulteerde in dezelfde aanbevolen dosis als bij volwassenen (1,8 mg/m^2 in kuur 1). Het fase I cohort werd gevolgd door een fase II-cohort om de activiteit van single-agent InO te bepalen en om aanvullende veiligheids- en farmacokinetische gegevens te verzamelen. Van de 40 patiënten die met de RP2D werden behandeld (13 in fase I en 27 in fase II) werd in 82,5% respons gezien; 81,8% van de patiënten die reageerden werd MRD-negatief. Deze resultaten zijn vooral relevant gezien het feit dat zwaar voorbehandelde patiënten deelnamen, waarvan de helft van de patiënten al was getransplanteerd. InO werd over het algemeen goed verdragen, met een lage incidentie van infecties tijdens de behandeling (17,8%). Sinusoïdaal obstructiesyndroom (SOS) was de ernstigste bijwerking, net als bij volwassenen, hoewel het meestal optrad na op de behandeling met InO volgende transplantatie, en slechts af en toe tijdens de behandeling met InO. De ITCC-059-studie is nog aan de gang en test InO als monotherapie bij zeer hoog risico eerste recidief ALL; het toevoegen van InO aan standaard chemotherapie bij pediatrische R/R leukemie leidde niet tot een betere respons.(7) Levertoxiciteit was een beperkende factor; de dosering van dexamethason diende daardoor te worden verlaagd en asparaginase kon niet aan het combinatieregime worden toegevoegd.(7)

In ***hoofdstuk 5*** is het gebruik van InO bij zuigelingen met R/R ALL onderzocht. Zuigelingen (kinderen $<$ 1 jaar oud) komen over het algemeen niet in aanmerking voor behandeling in klinische onderzoeken, evenals in de ITCC-059 voor InO, wat een eerste studie in kinderen was. Bovendien werden vóór deze studie geen gegevens over InO bij zuigelingen met ALL gepubliceerd. Om de veiligheid en werkzaamheid van InO in deze specifieke ALL-populatie (met een sombere prognose) te beschrijven

hebben we retrospectief gegevens verzameld over zuigelingen die met InO werden behandeld op basis van 'compassionate use'. Specifieke vragen werden als relevant beschouwd bij deze zeer jonge patiënten: medicijntoediening bij deze categorie patiënten met een zeer laag lichaamsgewicht en een laag lichaamsoppervlak (BSA) is altijd een uitdaging; CD22-expressie is lager in KMT2A-r ALL (75% van de baby's met ALL draagt deze herschikking) en bij recidiverende ziekte hebben deze patiënten een zeer slechte prognose met ziektevrije overlevingspercentages van slechts 20%. Binnen de beperking en van het retrospectieve karakter van de studie leek InO over het algemeen goed verdragen en werkzaam te zijn. De mediane dosis die werd gebruikt lag dicht bij de aanbevolen dosis voor volwassenen op basis van het lichaamsoppervlak (BSA), ondanks het relatief hoge BSA bij zuigelingen in vergelijking met het lichaamsgewicht. Deze bevinding suggereerde dat baby's zouden kunnen worden behandeld met dezelfde op BSA gebaseerde RP2D die is vastgesteld in de pediatrische fase I-studie van InO (ITCC-059), zonder overmatige toxiciteit. Het mediane percentage CD22+-blasten was 72% (spreiding 40-100%, n=9), lager dan bij de algemene pediatrische populatie. Zeven patiënten (47%) bereikten volledige remissie, minder dan in het pediatrische R/R ALL-cohort, maar InO lijkt nog steeds waardevol bij deze patiënten met een sombere prognose. De gerapporteerde toxiciteit was in overeenstemming met de gegevens voor volwassenen en kinderen, wat verdere evaluatie van InO in deze subgroep van ALL rechtvaardigt.

In ***hoofdstuk 6*** worden de voorlopige resultaten van het fase I-deel van de ITCC-054 studie bij kinderen met CML die behandeld worden met bosutinib gepresenteerd. We richtten ons daarbij op farmacokinetiek en veiligheid in de subgroep van patiënten met resistentie en intolerantie (R/I) voor andere (tyrosine kinase inhibitors) TKI's. Deze patiënten hebben behoefte aan nieuwe geneesmiddelen die beter werken en minder bijwerkingen hebben in vergelijking met de momenteel beschikbare TKI's, vooral met betrekking tot de groei. In het streven naar behandelingsvrije remissie is het bereiken van een snellere of diepere moleculaire respons mogelijk gunstig. Als het behoud van botgroei dat werd waargenomen in diermodellen wordt bevestigd in de klinische setting, zou bosutinib een voordeel kunnen hebben omdat het verslechtering van de lengtegroei zou kunnen voorkomen, vooral bij patiënten die op prepuberale leeftijd werden gediagnosticeerd. De RP2D van bosutinib voor pediatrische R/I CML-patiënten werd vastgesteld op 400 mg/m2 eenmaal daags (maximum 600 mg/dag), gebaseerd op veiligheidsgegevens en doelblootstelling bij volwassenen. Een hogere BSA-aangepaste dosis was nodig om de in volwassenen bepaalde doelblootstelling te bereiken. Waarschijnlijk dit was gerelateerd aan een afwijkend metabolisme

van het geneesmiddel bij jongere kinderen, vanwege de lage permeabiliteit en het lage oplosbaarheidsprofiel van bosutinib. De RP2D van bosutinib voor pediatrische nieuw gediagnosticeerde CML-patiënten werd geëxtrapoleerd uit de veiligheidsgegevens en farmacokinetiek bij R/I-kinderen en doelblootstelling in volwassenen, en vastgesteld op 300 mg/m2 (max. 500 mg/dag). De werkzaamheid van bosutinib was vergelijkbaar met andere TKI's van de tweede generatie die zijn goedgekeurd bij kinderen met CML (cumulatieve incidentie van complete cytogenetic response - CCyR na zes maanden, exclusief patiënten met CCyR bij screening, 83,3% (95% BI: 40,5-96,4%)). Gastro-intestinale bijwerkingen zijn het meest gemeld. Het effect op de botgroei kan beter worden bestudeerd met een langere follow-up en in patiënten die nog niet eerder TKI's hebben gehad, zoals de patiënten die momenteel deelnemen aan het fase II-deel van het onderzoek.

Concluderend vertegenwoordigt InO een waardevolle optie als herinductiebehandeling bij pediatrische patiënten met R/R_ALL (*hoofdstuk 3 en 4*), inclusief zeer jonge patiënten (*hoofdstuk 5*). Bosutinib is een goede optie voor CML-patiënten die resistent of intolerant zijn voor andere TKI (*hoofdstuk 6*). De rol van bosutinib bij nieuw gediagnosticeerde patiënten en de effecten er op de botgroei zullen waarschijnlijk met het lopende cohort van de fase II van de ITCC-054 studie duidelijk worden. Hopelijk worden InO en bosutinib binnenkort goedgekeurd voor pediatrisch gebruik, op basis van de gunstige risico-bateanalyse uit de ITCC-054- en ITCC-059-onderzoeken, gepresenteerd in dit proefschrift. Een voortdurende inspanning is noodzakelijk om de tijdige start van vroege fase-onderzoeken voor de goedkeuring van nieuwe middelen in de pediatrische oncologie te verbeteren. De samenwerking tussen groepen in Europa en Noord-Amerika (zoals gepromoot door het ACCELERATE-platform), harmonisatie van regelgevende prikkels aan beide zijden van de Atlantische Oceaan en verdere ontwikkeling van de academische netwerken is nodig om tegemoet te komen aan de wettelijke vereisten die verband houden met 'intent to file'-onderzoeken.

Appendices

List of affiliations of co-authors

List of publications

About the author

PhD portfolio

List of affiliations of co-authors

A.C.J.	Ammerlaan	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
A.	Baruchel	Hématologie-Immunologie Pédiatrique, Hôpital Universitaire Robert Debré (APHP) and Université de Paris, France
F.J.	Bautista Sirvent	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands; Department of Pediatric Oncology and Hematology, Hospital Niño Jesús, Madrid, Spain;
A.	Beishuizen	Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands
Y.	Bertrand	Institute of Pediatric Hematology and Oncology, Civil Hospital of Lyon, Claude Bernard University, Lyon, France
B.H.	Beverloo	Department of Clinical Genetics, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands;
B.	Bielorai	Department of Pediatric Hematology-Oncology, The Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Ramat-Gan, Israel
N.	Bodmer	Department Oncology, University Children's Hospital Zürich, Zürich, Switzerland;
J.P.	Bourquin	Department of Oncology and Children's Research Center, University Children's Hospital Zurich, Switzerland;
E.H.	Breese	Division of Oncology, Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center; Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH,USA
B.	Brethon	Department of Pediatric Hematology, Hôpital Robert-Debré, APHP, Paris, France
P.A.	Brown	Departments of Oncology and Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
B.	Bruno	Pediatric Hematology, Hôpital Jeanne de Flandre, CHRU de Lille, Lille, France;
A.	Bukowinski	UPMC Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, USA;
C.	Chantrain	Division of Pediatric Hematology Oncology, CHC-Clinique du MontLégia - Liège, Belgium;
Y.	Chen	Pfizer Global Product Development, San Diego, CA, USA
C.	Chen-Santel	Department of Pediatrics, Division of Oncology and Hematology, Charité – Universitätsmedizin Berlin, Germany; and Universitätsmedizin Rostock, Kinder- und Jugendklinik, Germany
A.	Contet	Department of Oncology and Hematology, Children's Hospital, CHRU Nancy, Vandoeuvre Lès Nancy, France
T.	Cooper	Aflac Cancer and Blood Disorders Center/Children's Healthcare of Atlanta/Emory University, Atlanta, GA, USA
S.	Cooper	Johns Hopkins University/Sidney Kimmel Cancer Center, Baltimore, USA;

L.	Dalla-Pozza	Cancer Centre for Children, The Children's Hospital at Westmead, Westmead, Australia; Cancer Centre for Children, The Childrens' Hospital at Westmead, Westmead, Australia Cancer Centre for Children, The Childrens' Hospital at Westmead, Westmead, Australia
M.	den Boer	Princess Máxima Center for Pediatric Oncology, The Oncode Institute, Utrecht; Department of Pediatric Oncology, Erasmus MC Sophia Children's Hospital, Rotterdam, The Netherlands
C.	Díaz-de-Heredia	Department of Pediatric Hematology and Oncology, University Hospital Vall d'Hebron, Barcelona, Spain
S.	Elitzur	Pediatric Hematology –Oncology, Schneider Children's Medical Center of Israel, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
G.	Englster	St Anna Children's Hospital, Medical University of Vienna, Vienna, Austria
W.	Fritschle	Hematologics inc. in Seattle, Washington, USA
L.	Gore	University of Colorado School of Medicine and Center for Cancer and Blood Disorders, Children's Hospital Colorado, Aurora, USA
T.A.	Gruber	Department of Oncology, St Jude Children's Research Hospital, Memphis, TN, USA;
N.	Heerema	Nationwide Children's Hospital in Columbus, Ohio, USA
N.	Hijiya	Columbia University Irving Medical Center, New York, NY, USA
R.	Hoogendijk	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
C.	Hudson	Hematologics inc. in Seattle, Washington, USA
A.D.R.	Huitema	Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands;The Netherlands Cancer Institute, Amsterdam, the Netherlands; University Medical Center Utrecht, Utrecht, the Netherlands;
Y.	Jiang	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
K.M.	Kállay	Pediatric Hematology and Stem Cell Transplantation Department, Central Hospital of Southern Pest – National Institute of Hematology and Infectious Diseases, Budapest, Hungary;
M.	Kato	Department of Pediatric Hematology and Oncology Research, National Research Institute for Child Health and Development, Tokyo, Japan;
E.A.	Kolb	Nemours Center for Cancer and Blood Disorders, Nemours/ Alfred I DuPont Hospital for Children, Wilmington, Delaware
K.	Kowalski	Pfizer Inc, La Jolla, CA, USA;
J.	Krystal	The Steven and Alexandra Cohen Children's Medical Center of New York, New York, USA
M.	Kutny	Children's Hospital of Alabama, Birmingham, Alabama, USA
L.	Kuttschreuter	Pfizer R&D Limited, Surrey, United Kindom;

D.	Lancaster	The Royal Marsden NHS Foundation Trust, London, , United Kingdom;
C.K.	Li	Department of Paediatrics, The Chinese University of Hong Kong, Hong Kong SAR, China
F.	Locatelli	Department of Pediatric Hematology/Oncology and Cell and Gene Therapy, IRCCS Ospedale Pediatrico Bambino Gesù, Sapienza, University of Rome, Italy
M.	Lopez-Yurda	Netherlands Cancer Institute
A.	Malone	Children's Health Ireland at Crumlin, Dublin, Ireland
I.	Markova	R.M. Gorbacheva Memorial Institute of Children Oncology, Hematology and Transplantation, Pavlov University, Saint-Petersburg, Russia
S.L.	Maude	Division of Oncology, Department of Pediatrics, Center for Childhood Cancer Research, Children's Hospital of Philadelphia and Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.
M.	Metzler	Universitätsklinikum Erlangen, Erlangen, Germany;
N.	Michels	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
J.	Motwani	Birmingham Children's Hospital, Birmingham, United Kingdom;
L.	Murillo-Sanjuán	Hospital Universitari Vall d'Hebron, Barcelona, Spain;
F.J.	Mussai	Institute for Immunology and Immunotherapy, Cancer Research UK Birmingham Centre, The University of Birmingham, Birmingham, United Kingdom
K.	Nysom	Department of Paediatrics and Adolescent Medicine, Juliane Marie Centre, Rigshospitalet, Copenhagen, Denmark
I.	Øra	Department of Pediatric Oncology and Hematology, Childhood Cancer Research Unit, Lund University Hospital, Lund, Sweden; and Pediatric Oncology and Hematology, Karolinska Hospital, Stockholm, Sweden
E.	Pennesi	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
A.	Petit	Department of pediatric Hematology and Oncology, Hopital Armand Trousseau, APHP, Sorbonne Université, Paris, France
R.	Pieters	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
G.	Plat	Service d'Hématologie-Immunologie-Oncologie, Hôpital des Enfants, CHU Toulouse, Toulouse, France;
J.	Pollard	Dana-Farber Cancer Institute/Boston Children's Cancer and Blood Disorders Center, Boston, Massachusetts, USA
L.A.J.	Rammeloo	VU University Medical Center, Amsterdam, The Netherlands
M.	Redell	Baylor College of Medicine/Texas Children's Cancer Center, Houston, Texas, USA
D.	Reinhardt	Department of Pediatric Oncology, Essen University Hospital, Essen, Germany;

F.	Rialland	Service Onco-Hématologie Pédiatrique, Hôpital Mère-Enfant, Nantes University Hospital, Nantes, France
S.	Rives	Pediatric Oncology and Hematology Department, Hospital Sant Joan de Déu de Barcelona, Barcelona; Institut de Recerca Sant Joan de Déu, Barcelona; Spain
C.	Rizzari	Pediatric Hematology-Oncology Unit, Department of Pediatrics, MBBM Foundation, ASST Monza, University of Milano-Bicocca, Monza, Italy;
C.	Rossig	Department of Pediatric Hematology and Oncology, University Children´s Hospital Münster, Germany
M.E.	Roth	University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA;
K.	Schmiegelow	Department of Pediatrics and Adolescent Medicine, Rigshospitalet Copenhagen University Hospital; Institute of Clinical Medicine, Faculty of Health, University of Copenhagen, Denmark.
L.	Šrámková	Department of Paediatric Haematology and Oncology, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic
B.	Sleight	Pfizer Inc., Groton, Connecticut, USA
J.	Starý	Department of Pediatric Hematology and Oncology, University Hospital Motol, Prague, Czech Republic;
A.	Thano	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
I.M.	van der Sluis	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
V.H.J.	van der Velden	Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands
H.	van Tinteren	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
L.	Vinti	Department of Pediatric Hematology/Oncology and Cell and Gene Therapy, IRCCS Ospedale Pediatrico Bambino Gesù, Sapienza, University of Rome, Italy
A.	Viqueira	Pfizer SLU, Madrid, Spain;
A.	von Stackelberg	Charité-Children's Hospital Berlin, Berlin
J.	Vormoor	Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands; University Medical Center, Utrecht, the Netherlands; Newcastle University, Newcastle, UK
B.	Vormoor-Burger	Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands
M.E.	Willemse	Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands
S.	Zarnegar-Lumley	Vanderbilt University, Nashville, Tennesssee, USA
H.	Zhao	Pfizer (China) R&D Co., Ltd., Shanghai, China;
C.M.	Zwaan	Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands; Pediatric Oncology, Erasmus MC-Sophia Children's Hospital, Rotterdam, Netherlands

List of publications

In this thesis

Brivio E, Baruchel A, Beishuizen A, Bourquin JP, Brown PA, Cooper T, Gore L, Kolb EA, Locatelli F, Maude SL, Mussai FJ, Vormoor-Bürger B, Vormoor J, von Stackelberg A, Zwaan CM *Targeted inhibitors and antibody immunotherapies: Novel therapies for paediatric leukaemia and lymphoma.* Eur J Cancer. 2022 Mar;164:1-17.

Brivio E, Locatelli F, Lopez-Yurda M, Malone A, Díaz-de-Heredia C, Bielorai B, Rossig C, van der Velden VHJ, Ammerlaan ACJ, Thano A, van der Sluis IM, den Boer ML, Chen Y, Sleight B, Brethon B, Nysom K, Šrámková L, Øra, Vinti L, Chen-Santel C, Zwaan CM. *A phase 1 study of inotuzumab ozogamicin in pediatric relapsed/refractory acute lymphoblastic leukemia (ITCC-059 study)* Blood. 2021 Mar 25;137(12):1582-1590.

Brivio E, Chantrain CF, Gruber TA, Thano A, Rialland-Battisti F, Contet A, Elitzur S, Dalla-Pozza L, Kállay KM, Li CK, Kato M, Markova I, Schmiegelow K, Bodmer N, Breese EH, Hoogendijk R, Pieters R, Zwaan CM. *Inotuzumab Ozogamicin in Infants and Young Children with Relapsed or Refractory Acute Lymphoblastic Leukemia: a case series*. Br J Haematol. 2021 Jun;193(6):1172-1177

Pennesi E, Michels N, **Brivio E***,* van der Velden VHJ, Jiang Y, Thano A, Ammerlaan AJC, Boer JM, Beverloo HB, Sleight B, Chen Y, Vormoor-Bürger B, Rives S, Bielorai B, Rössig C, Petit A, Rizzari C, Engstler G, Starý J, Bautista Sirvent FJ, Chen-Santel C, Bruno B, Bertrand Y, Rialland F, Plat G, Reinhardt D, Vinti L, Von Stackelberg A, Locatelli F, Zwaan CM *Inotuzumab ozogamicin as single agent in pediatric patients with relapsed and refractory acute lymphoblastic leukemia: results from a phase II trial.* Leukemia. 2022 Jun;36(6):1516-1524.

Brivio E*, Pennesi E*, Willemse ME, Huitema ADR, Jiang Y, van Tinteren H, van der Velden VHJ, Beverloo BH, den Boer ML, Rammeloo LAJ, Hudson C, Heerema N, Kowalski K, Zhao H, Kuttschreuter L, Bautista Sirvent FJ, Bukowinski A, Rizzari C, Pollard J, Murillo-Sanjuán L, Kutny M, Zarnegar-Lumley S, Redell M, Cooper S, Bertrand Y, Petit A, Krystal J, Metzler M, Lancaster D, Bourquin JP, Motwani J, van der Sluis IM, Locatelli F, Roth ME, Hijiya N, Zwaan CM *Bosutinib in Resistant and Intolerant Pediatric Patients with Chronic Phase Chronic Myeloid Leukemia: Results from the Phase I part of Study ITCC054/COG AAML1921.* Submitted to JCO * contributed equally

Other publications

Ceolin V*, **Brivio E***, van Tinteren H, Rheingold SR, Leahy A, Vormoor B, O'Brien MM, Rubinstein JD, Kalwak K, De Moerloose B, Jacoby E, Bader P, López-Duarte M, Goemans BF, Locatelli F, Hoogerbrugge P, Calkoen FG*, Zwaan CM* *Outcome of chimeric antigen receptor T-cell therapy following treatment with inotuzumab ozogamicin in children with relapsed or refractory acute lymphoblastic leukemia.* Leukemia. 2023 Jan;37(1):53-60. * contributed equally

Ilan U*, **Brivio E***, Algeri M, Balduzzi A, Gonzalez-Vincent M, Locatelli F, Zwaan CM, Baruchel A, Lindemans C, Bautista F *The development of new agents for post-hematopoietic stem cell transplantation non-infectious complications in children*. J.Clin. Med. 2023 , 12 (6), 2149 contributed equally

Brivio E, Cossio A, Borra D, Silvestri D, Prunotto G, Colombini A, Verna M, Rizzari C, Biondi A, Conter V, Valsecchi MG, Balduzzi A *Osteonecrosis in pediatric acute lymphoblastic leukemia: incidence, risk factors, radiological patterns and evolution in a single-center cohort.* Br J Haematol. 2022 Jun;197(5):602-608

Pearson ADJ, Zwaan CM, Kolb EA, Karres D, Guillot J, Kim SY, Marshall L, Tasian SK, Smith M, Cooper T, Adamson PC, Barry E, Benettaib B, Binlich F, Borgman A, **Brivio E**, Capdeville R, Delgado D, et al *Paediatric Strategy Forum for Medicinal Product Development for Acute Myeloid Leukaemia in Children and Adolescents.* European Journal of Cancer 136 (2020) 116-129

Brivio E, Verna M, Migliorino GM, Foresti S, Zucchini N and Rovelli A. *Isavuconazole-Induced Acute Liver Failure in a Paediatric Patient With Invasive Aspergillosis*. Pediatr Infect Dis J,38 (10), 1035-1037, Oct 2019

Brivio E, Zwaan M.C. *ALK inhibition in two emblematic cases of pediatric inflammatory myofibroblastic tumor: efficacy and side effects.* Pediatr Blood Cancer. 2019;66:e27645.

Balduzzi A, **Brivio E** , Rovelli A, Rizzari C, Gasperini S, Melzi ML, Conter V, Biondi A *Lessons After the Early Management of the COVID-19 Outbreak in a Pediatric Transplant and Hemato-Oncology Center Embedded Within a COVID-19 Dedicated Hospital in Lombardia, Italy.* Bone Marrow Transplant. 2020 Apr 20;1-6

Navarrete M, Rossi E, **Brivio E**, Carrillo JM, Bonilla M, Vasquez R, Peña A, Fu L, Martinez R, Espinoza CM, Lacayo LF, Rodriguez H, Batista R, Barr R, Howard SC, Ribeiro RC, Masera G, Biondi A, Conter V, Valsecchi MG. *Treatment of childhood acute lymphoblastic leukemia in central America: A lower-middle income countries experience*. Pediatr Blood Cancer. 2014 May;61(5):803

Conter V., Polillo R., **Brivio E**, Faravelli A., Masera G The role of twinning programs and telemedicine in the development of pediatric oncology in developing countries. Book chapter in "Pediatric Hematology-Oncology in Countries with limited resources: a practical manual" - Springer Clinical Medicine 2013

About the author

Erica Brivio was born in Monza (Italy) on 21 April 1987. After completing her secondary education at Liceo B. Zucchi in Monza (Italy) in 2006, she studied Medicine at the University of Milano Bicocca. In 2010, she went to Hospital Infantil La Mascota, Managua - Nicaragua, for a 3-months clinical internship in pediatric and hematology-oncology. She wrote her thesis on 'Treatment of childhood acute lymphoblastic leukemia in central America' focusing on reduced intensity chemotherapy options for lower-middle income countries, under the supervision of dr. V. Conter. In 2013, after obtaining her medical degree cum laude, she started her clinical training in Pediatrics at Ospedale San Gerardo, Monza (Prof. A. Biondi). During her training in Pediatrics, she went for a 6-months traineeship in Phase I-II clinical trials in pediatric hematology-oncology, at the Erasmus Medical Center in Rotterdam, under the supervision of Prof. C.M. Zwaan. During the internship, the research projects of this thesis were started. After completing her training in Pediatrics in 2018, Erica decided to move to the Netherlands to pursue the research projects previously started, formally beginning her PhD research at the Erasmus MC in Rotterdam. In 2020 she obtained the Dutch license to practice as a Pediatrician and started her clinical fellowship in Pediatric Oncology at the Princess Máxima Center for Pediatric Oncology in Utrecht. At the beginning of 2023 she was selected for a rotation at the European Medicines Agency within the Accelerate program, to promote development of new agents in pediatric oncology.

Erica lives in Utrecht with Alessandro and their two children, Anita and Vittorio.

PhD Portfolio

PhD training	Year
Courses	
EORTC course: Workshop on Methods in Clinical Cancer Research (MCCR)	2019
BROK (Basiscursus Regelgeving Klinisch Onderzoek) - GCP-MWO-BROK examen	2021
Master Course pediatric Oncology - module Neuro-oncology	2020
Master Course pediatric Oncology - module Solid tumors	2021
Master Course pediatric Oncology - module Hemato-oncology	2022
Master Course pediatric Oncology - module General aspects	2021
LEGEND - Leukemia gene discovery by data sharing mining and collaboration Scientific integrity	2022 2023
Seminars and congresses	
Webinar “Everything you always wanted to know about developing drugs for children” - ACCELERATE	2021
New Agents in Leukemia/Lymphoma meeting - ITCC Hematological malignancies committees	2021
63rd ASH American Society of Hematology Annual meeting and exposition	2021
Presentations at international conferences and meetings	
Interim meeting of the Resistant Disease Committee of the International BFM Study Group	2020
62nd ASH American Society of Hematology Annual meeting and exposition	2020
New agents in leukemia and lymphoma meeting	2020- 2023
Biennial childhood leukemia and lymphoma symposium	2021
SIOP Europe - 2nd annual meeting	2021
SIOP Europe - 3rd annual meeting	2022
Preceptorship Pediatric and Adolescent ALL - Princess Máxima Centrum	2021
Supervising	
Keuzecursus kinderoncologie (Tutoring bachelor’s students)	2021
Other	
Peer reviewing of articles for scientific journals	2020-2023

Acknowledgements

Firstly I would like to thank all the patients and their families taking part in these studies. With their participation they are making the difference for many others.

Prof.dr. Zwaan. Thank you, Michel, for guiding me in these exciting projects. These years of work together have shaped the kind of doctor I would like to be. I have always admired your ability to foresee what is coming next for our patients. I can definitely say that ours was a life-changing encounter, for me and my family. Thank you for encouraging and supporting me in becoming a Dutch speaking doctor. It has not been easy, but it was worth it.

Dr. Vincent van der Velden en Prof.dr. M. den Boer. Dear Vincent, thank you for our fruitful meetings, you could always give a clear answer to my doubts. I have enjoyed learning from you. Dear Monique, thank you for your helpful feedback on my manuscripts and on my thesis, it helped in developing a more concise and scientific writing.

I would like to thank the members of the defense committee: Dr. Anita Rijneveld, Prof.dr. Saskia de Wildt, Prof.dr. Arjan van de Loosdrecht, for taking the time to read my thesis and providing their suggestions. Thank you to Prof.dr. Marry van den Heuvel-Eibrink, Prof.dr. Peter Hoogerbrugge and Prof.dr. Adriana Balduzzi for willing to take part in the large committee.

Phase 1-2 trials can exist only through the work of many. I am not able to mention all, but I am thankful to everyone. Thank you to Anneke Ammerlaan and Marieke Willemse, who did an incredible organizational work for the ITCC-059 and 054 studies. Dear Anneke, I am happy I could learn from your accuracy and knowledge. Dear Marieke, I have found a special affinity with you since our first months together in Rotterdam, we have learnt a lot together in the last five years. Thank you Edoardo for sharing with me all the efforts on these studies. Without your work the projects would have not been finalized yet.

Thank you to the statisticians who have worked on the trials during the years, Harm van Tinteren, Marta Lopez, Adriana Thano and Yilin Jiang. Thank you for the long sessions necessary to analyze the data. I have learned a lot from you all. A special thanks to Adriana, for being my support in preparation of the first manuscript of this thesis and for hosting me at her (child-free) home for my first important oral presentation at ASH 2020, during the pandemic. I enjoyed Utrecht a lot when you were here.

Thank you Nieke, Mireille, Shivani, Yen, all the central data managers for the huge work done to achieve the best completeness of the data.

Thank you to the Trial and Data Centrum of the Prinses Máxima, to all the people working and collaborating there, it is a privilege to work in such a well organized system.

Thank you to all the participating Institutions around Europe and USA. Thank you to all the experienced colleagues, local principal investigators and co-authors of the papers, for the fruitful discussions on clinical cases, for helping in taking difficult decisions for the future of the studies and for giving important feedback to the manuscripts. It has been a great opportunity working together.

Thank you Raoull for your work on the InO in infants project. This rather small project gave us the possibility to do everything ourselves, and I am proud of that. Thank you Naomi for the months spent preparing our manuscript, our different expertise and point of view complemented each other.

Thank you to my colleagues at the Prinses Màxima Center. Especially thanks to the hematology and SCT department, where I could feel again part of the team, despite the time spent in the research and some additional difficulties due to the nuances of the language. Thank you Josef, Prof.dr. Josef Vormoor, for letting me realize that adapting to a new system and language does require some time, for everyone. Our conversations have been reassuring and motivating. Thank you to all patients I met after my new start as a Dutch doctor, especially those I followed as main treating physician: I do have special memories of them all.

Thanks to my former colleagues in Monza, Clinica pediatrica Ospedale San Gerardo, where my interest in pediatric oncology started. Thanks to the good advice of Prof. dr. Carmelo Rizzari I went for an internship in Rotterdam, where the projects of this dissertation started. Thank you for foreseeing all the opportunities that could come from such an experience. Prof.dr. Adriana Balduzzi, thank you Adriana for the projects we have shared in these years, even if I was so far away. Thank you to Prof.dr. Biondi for still being in contact and supportive over the years.

Thank you Marialuisa, pioneer among the Italian pediatricians in Utrecht. Meeting you was a beautiful surprise, thank you for all your good advices on my scientific and clinical carreer in the Netherlands.

Thanks to my Italian friends, still my best friends, for letting me feel their warmth when I go home. A special thanks to Francesca and Silvia, friends of a lifetime, for reminding me that I am not so different from my 16-year-old-self, in all the possible positive ways.

Thank you Uri and Federica for willing to be my paranymphs. Uri, when I met you at the Màxima, I could immediately feel the Mediterranean warmth coming along with you. We couldn’t avoid being friends. Thank you to you and your beautiful family for the fun we have together. Thank you Federica for being such a careful and supportive friend since our arrival in Utrecht. You and Davide have been our safe Italian home in the Netherlands. You are a natural part of the family, especially to Anita.

Thank you Luca and Maddalena because you are always on our side, even in the distance.

Grazie alla mia mamma, perché c'è molto di lei in tutto quello che faccio. Grazie per avermi insegnato il valore dell’essere indipendenti e la bellezza di appassionarsi a ciò che si fa. Grazie papà per aver reso la nostra casa una piccola biblioteca, inizia tutto da lì, e grazie per esserci sempre per me. Hoping to become such a good parent as you both have been.

Dear Alessandro, without you, all this would have never happened. When I was skeptical, you have been ready for our new life. I would have never done it without you. Anita and Vittorio are making it perfect.