



Irinotecan and temozolomide in combination with dasatinib and rapamycin versus irinotecan and temozolomide for patients with relapsed or refractory neuroblastoma (RIST-rNB-2011): a multicentre, open-label, randomised, controlled, phase 2 trial



Selim Corbacioglu, Holger Lode, Susanne Ellinger, Florian Zeman, Meinolf Suttrop, Gabriele Escherich, Konrad Bochennek, Bernd Gruhn, Peter Lang, Marius Rohde, Klaus Michael Debatin, Daniel Steinbach, Andreas Beilken, Ruth Ladenstein, Rainer Spachtholz, Peter Heiss, Dirk Hellwig, Anja Tröger, Michael Koller, Karin Menhart, Markus J Riemenschneider, Saida Zoubaa, Silke Kietz, Marcus Jakob, Gunhild Sommer, Tilman Heise, Patrick Hundsdoerfer, Ingrid Kühnle, Dagmar Dilloo, Stefan Schönberger, Georg Schwabe, Irene von Luetichau, Norbert Graf, Paul-Gerhardt Schlegel, Michael Frühwald, Norbert Jorch, Michael Paulussen, Dominik T Schneider, Markus Metzler, Alfred Leipold, Michaela Nathrath, Thomas Imschweiler, Holger Christiansen, Irene Schmid, Roman Crazzolara, Naghmeh Niktooreh, Gunnar Cario, Joerg Faber, Martin Demmert, Florian Babor, Birgit Fröhlich, Stefan Bielack, Toralf Bernig, Johann Greil, Angelika Eggert, Thorsten Simon, Juergen Foell

Summary

Background Neuroblastoma is the most common extracranial solid tumour in children. Relapsed or refractory neuroblastoma is associated with a poor outcome. We assessed the combination of irinotecan–temozolomide and dasatinib–rapamycin (RIST) in patients with relapsed or refractory neuroblastoma.

Methods The multicentre, open-label, randomised, controlled, phase 2, RIST-rNB-2011 trial recruited from 40 paediatric oncology centres in Germany and Austria. Patients aged 1–25 years with high-risk relapsed (defined as recurrence of all stage IV and MYCN amplification stages, after response to treatment) or refractory (progressive disease during primary treatment) neuroblastoma, with Lansky and Karnofsky performance status at least 50%, were assigned (1:1) to RIST (RIST group) or irinotecan–temozolomide (control group) by block randomisation, stratified by MYCN status. We compared RIST (oral rapamycin [loading 3 mg/m² on day 1, maintenance 1 mg/m² on days 2–4] and oral dasatinib [2 mg/kg per day] for 4 days with 3 days off, followed by intravenous irinotecan [50 mg/m² per day] and oral temozolomide [150 mg/m² per day] for 5 days with 2 days off; one course each of rapamycin–dasatinib and irinotecan–temozolomide for four cycles over 8 weeks, then two courses of rapamycin–dasatinib followed by one course of irinotecan–temozolomide for 12 weeks) with irinotecan–temozolomide alone (with identical dosing as experimental group). The primary endpoint of progression-free survival was analysed in all eligible patients who received at least one course of therapy. The safety population consisted of all patients who received at least one course of therapy and had at least one post-baseline safety assessment. This trial is registered at ClinicalTrials.gov, NCT01467986, and is closed to accrual.

Findings Between Aug 26, 2013, and Sept 21, 2020, 129 patients were randomly assigned to the RIST group (n=63) or control group (n=66). Median age was 5·4 years (IQR 3·7–8·1). 124 patients (78 [63%] male and 46 [37%] female) were included in the efficacy analysis. At a median follow-up of 72 months (IQR 31–88), the median progression-free survival was 11 months (95% CI 7–17) in the RIST group and 5 months (2–8) in the control group (hazard ratio 0·62, one-sided 90% CI 0·81; p=0·019). Median progression-free survival in patients with amplified MYCN (n=48) was 6 months (95% CI 4–24) in the RIST group versus 2 months (2–5) in the control group (HR 0·45 [95% CI 0·24–0·84], p=0·012); median progression-free survival in patients without amplified MYCN (n=76) was 14 months (95% CI 9–7) in the RIST group versus 8 months (4–15) in the control group (HR 0·84 [95% CI 0·51–1·38], p=0·49). The most common grade 3 or worse adverse events were neutropenia (54 [81%] of 67 patients given RIST vs 49 [82%] of 60 patients given control), thrombocytopenia (45 [67%] vs 41 [68%]), and anaemia (39 [58%] vs 38 [63%]). Nine serious treatment-related adverse events were reported (five patients given control and four patients given RIST). There were no treatment-related deaths in the control group and one in the RIST group (multiorgan failure).

Interpretation RIST-rNB-2011 demonstrated that targeting of MYCN-amplified relapsed or refractory neuroblastoma with a pathway-directed metronomic combination of a multikinase inhibitor and an mTOR inhibitor can improve progression-free survival and overall survival. This exclusive efficacy in MYCN-amplified, relapsed neuroblastoma warrants further investigation in the first-line setting.

Funding Deutsche Krebshilfe.

Lancet Oncol 2024; 25: 922–32

University Medical Center
Regensburg, Regensburg,
Germany

(S Corbacioglu MD PhD,
S Ellinger MSc, F Zeman MSc,
R Spachtholz MSc, P Heiss MD,
D Hellwig MD PhD,
A Tröger MD PhD, M Koller PhD,
K Menhart MD,

M J Riemenschneider MD,
S Zoubaa MD, S Kietz MD,
M Jakob MD, G Sommer PhD,
T Heise PhD, J Foell MD PhD);
University Medicine

Greifswald, Greifswald,
Germany (H Lode MD PhD);
Medical Faculty, Technical

University Dresden, Dresden,
Germany (M Suttrop MD PhD);
University Medical Center

Hamburg-Eppendorf,
Hamburg, Germany
(G Escherich MD); University

Medical Center Frankfurt,
Frankfurt am Main, Germany
(K Bochennek MD); University

Medical Center Jena, Jena,
Germany (B Gruhn MD);
University Medical Center

Tuebingen, Tuebingen,
Germany (P Lang MD);
University Medical Center

Giessen, Giessen, Germany
(M Rohde MD); University
Medical Center Ulm, Ulm,

Germany (K M Debatin MD PhD,
D Steinbach MD); University
Medical Center Hannover,

Hannover, Germany
(A Beilken MD); St Anna
Children's Cancer Research

Institute, University Medical
Center, Vienna, Austria
(R Ladenstein MD); Helios

Klinikum Berlin-Buch, Berlin,
Germany (P Hundsdoerfer MD);

Copyright © 2024 The Author(s). Published by Elsevier Ltd. This is an Open Access article under the CC BY 4.0 license.

Introduction

Neuroblastoma is the most common extracranial solid tumour in children. *MYCN* is amplified in approximately 25% of neuroblastomas and remains an indicator of poor prognosis. Over half of the patients with high-risk neuroblastoma relapse despite intensive multimodal therapy, and most relapses occur within 2 years of diagnosis.¹ Further, 10–20% are refractory to first-line chemotherapy. With a 4-year progression-free survival and overall survival for patients with relapsed high-risk neuroblastoma of 6% and 20%, respectively,² and a median progression-free survival of 6.4 months,³ high-risk neuroblastoma remains one of the most dismal diseases of childhood cancer. Irinotecan and temozolomide demonstrated activity in relapsed and refractory neuroblastoma^{4,5} and are currently key components of combination therapies.^{6,7} mTORs have been shown to have activity in neuroblastoma. In preclinical studies, the mTOR inhibitor rapamycin acted synergistically with tyrosine kinase inhibitors, inducing radiosensitisation and chemosensitisation,^{8,9} predominantly in *MYCN*-amplified neuroblastoma.^{10–13} Since induction of transitory resistance with continuous treatment through activation of surrogate pathways was observed,¹⁴ a metronomic approach was chosen for synergism, chemosensitisation, and avoidance of resistance.

The drug combination of irinotecan–temozolomide with dasatinib–rapamycin (RIST) is aligned in a metronomic, pathway-directed design in which the multikinase inhibitor dasatinib is added to expand targeting to surrogate non-receptor tyrosine kinases of the SRC-kinase family.

In a pilot series of 21 patients with relapsed or refractory neuroblastoma treated with RIST, 12 (57%) patients had a

complete response and overall survival after a median follow-up of 148 weeks was 43% (nine patients).¹⁵

In the RIST-rNB-2011 trial, we aimed to assess the benefit of the pathway-directed drugs rapamycin–dasatinib combined with irinotecan–temozolomide in a metronomic design.

Methods

Study design and participants

RIST-rNB-2011 is an investigator-initiated, multicentre, open-label, randomised, controlled, phase 2 trial conducted in 40 paediatric oncology centres in Germany and Austria (appendix pp 4–7).

Children, adolescents, and young adults aged 1–25 years (performance status at least 50% according to Lansky and Karnofsky) with high-risk (International Neuroblastoma Staging System stage IV disease and all *MYCN*-amplified stages) relapsed (defined as recurrence after response to treatment; including multiple relapses, without limitations on previous treatment) neuroblastoma after intensive, multimodal high-risk therapy or refractory neuroblastoma (progression during primary treatment), who had confirmatory histology of neuroblastoma with or without evidence of neuroblastoma cells in the bone marrow, were enrolled after patients or legal guardians provided written, informed consent. Relapsed patients who received irinotecan or temozolomide, or both, before enrolment in this trial were not explicitly excluded. Full details on eligibility criteria are in the appendix (pp 9–10).

The trial was approved by the institutional review board of the principal investigator in Regensburg, Germany. The trial was designed and overseen by a steering committee. The protocol, performed in accordance with

University Medical Center Göttingen, Göttingen, Germany (I Kühnle MD); University Medical Center Bonn, Bonn, Germany (D Dilloo MD); University Medical Center Essen, Essen, Germany (S Schönberger MD, N Niktoreh MD); Carl-Thieme Clinic Cottbus, Cottbus, Germany (G Schwabe MD); Technical University of Munich, Munich, Germany (I von Lüttichau MD); Saarland University, Homburg, Homburg, Germany (N Graf MD); University Medical Center Würzburg, Würzburg, Germany (P-G Schlegel MD); University Medical Center Augsburg, Augsburg, Germany (M Frühwald MD PhD); University Medical Center Bielefeld, Bielefeld, Germany (N Jorch MD); University Medical Center Witten/Herdecke, Datteln, Germany (M Paulussen MD); University Medical Center Witten/Herdecke, Dortmund, Germany (D T Schneider MD); University Medical Center Erlangen, Erlangen, Germany (M Metzler MD); Medical Center Karlsruhe, Karlsruhe, Germany (A Leipold MD); Klinikum Kassel, Kassel, Germany (M Nathrath MD); Helios Hospital Krefeld, Krefeld, Germany (T Immschweiler MD); University Medical Center Leipzig, Leipzig, Germany (H Christiansen MD); Ludwig Maximilians University Munich, Munich, Germany (I Schmid MD); University Medical Center Innsbruck, Innsbruck, Austria (R Crazzolara MD); University Medical Center Kiel, Kiel, Germany (G Cario MD); University Medical Center Mainz, Mainz, Germany (J Faber MD); University Medical Center Lübeck, Lübeck, Germany (M Demmert MD); University Medical Center Düsseldorf, Düsseldorf, Germany (F Babor MD); University Medical Center Muenster, Muenster, Germany (B Fröhlich MD); University Medical Center Stuttgart, Stuttgart, Germany (S Bielack MD); University Medical Center Halle, Halle, Germany (T Bernig MD); University Medical Center Heidelberg, Heidelberg, Germany (J Greil MD); Charité, University Medical Center

Research in context

Evidence before this study

We searched PubMed, the Library of Congress, and the National Library of Medicine for original research articles published before Aug 26, 2012, in any language that addressed molecular or pathway-directed treatment of relapsed or refractory neuroblastoma or childhood sarcoma, using the keywords “neuroblastoma”, “sarcoma”, “relapsed”, “refractory”, “kinase inhibitor”, “mTOR inhibitor”, and “metronomic”, excluding case reports and small single-centre case series. Some studies combining irinotecan and temozolomide reported feasibility and improvements in outcomes, but in summary, the outcome of relapsed or refractory neuroblastoma remained poor.

Added value of this study

The RIST rNB-2011 trial has shown in a large, randomised, patient population and with over 6 years of follow-up that targeting of *MYCN*-amplified relapsed or refractory

neuroblastoma, which has historically been associated with the worst outcomes, with a pathway-directed metronomic combination of a multikinase inhibitor and rapamycin could improve progression-free survival and overall survival.

Implications of all the available evidence

Efficacy analyses of future treatment options in neuroblastoma should be segregated by *MYCN* amplification. Incorporating pathway-directed treatment in neuroblastoma based on *MYCN* amplification into first-line therapy might be an appropriate approach that could improve progression-free survival while reducing chemotherapy-related toxicities. Cellular therapy options, such as a haploidentical stem-cell transplantation, as shown in this study, or chimeric antigen receptor T-cell therapy could be promising options for consolidation if a robust minimal residual remission can be achieved beforehand.

Berlin, Berlin, Germany
(A Eggert MD PhD); University
Medical Center Cologne,
Cologne, Germany
(T Simon MD); Department of
Pediatric Hematology,
Oncology, and Stem Cell
Transplantation, University
Medical Center Regensburg,
Regensburg, Germany
(S Corbacioglu)

Correspondence to:
Dr Selim Corbacioglu,
Department of Pediatric
Hematology, Oncology, and
Stem Cell Transplantation,
University Medical Center
Regensburg, 93053 Regensburg,
Germany
selim.corbacioglu@mac.com

See Online for appendix

For the trial protocol see http://www.ukr.de/fileadmin/UKR/2-medicin-pflege/kliniken_institute_abteilungen/paediatrische_haematologie_onkologie_und_stammzelltransplantation/THELANCETONCOLOGY-D-23-01524_Clinical_Trial_Protocol.pdf

the principles of the Declaration of Helsinki, was approved by the respective scientific ethics committees at each institution. The trial protocol has been published online.

Randomisation and masking

Patients were randomly assigned (1:1) to RIST (RIST group) or irinotecan–temozolomide (control group) via computer-based randomisation with sequence generation defined by block randomisation, with a block size of six, stratified to ensure equal distribution of *MYCN* amplification status, as the predictive marker for worst outcome and based on the preclinical evidence of *MYCN* amplification as a target for rapamycin–dasatinib. No further evidence was available for other stratification factors to affect outcome. As the trial was open-label, participants, families, and medical staff assigning therapy were aware of the randomisation outcome. However, centralised radiology review was masked. All trial-related staff were not involved in the randomisation besides administrative tasks, such as assignment to the trial groups.

Procedures

RIST had a metronomic design consisting of courses, cycles, and phases (appendix pp 11–12). The RIST group consisted of rapamycin–dasatinib and irinotecan–temozolomide courses. In rapamycin–dasatinib courses, the patients received oral rapamycin (loading 3 mg/m² on day 1, maintenance 1 mg/m² on days 2–4) and oral dasatinib (2 mg/kg per day, maximum 140 mg per day) for 4 days, followed by 3 days off. On day 5, rapamycin serum concentration was determined and adjusted in consecutive courses between 3 ng/mL to 10 ng/mL. The irinotecan–temozolomide courses consist of oral temozolomide (150 mg/m² per day) and intravenous irinotecan (50 mg/m² per day) over 5 days followed by 2 days off. In phase 1, four cycles consisting of one rapamycin–dasatinib and irinotecan–temozolomide course (8 weeks) were applied, followed by phase 2 with four cycles, consisting of two rapamycin–dasatinib and one irinotecan–temozolomide course per cycle (12 weeks). Phase 2 with additional rapamycin–dasatinib courses was implemented to reduce the chemotherapy-related toxicity, thereby avoiding treatment delay due to adverse effects and allowing for haematological recovery, while maintaining the targeted treatment. Total treatment duration was 20 weeks. The control group consisted of eight irinotecan–temozolomide courses. Patients in remission after phase 2 were eligible for consolidation therapy according to the investigators' preferences. Patients refractory to treatment in the control group could crossover to RIST. Based on individual or investigator decision, patients could leave the trial or were switched to the respective treatment and were followed up as observational

patients. No modifications to the trial design or drug dosages were made throughout the study, but dose reductions or interruptions were permitted. Race and ethnicity data were not collected. Biological sex data were collected from medical records. The modified International Neuroblastoma Response Criteria were used to assess overall response.¹⁶ For response assessment, serum markers, such as lactic dehydrogenase and neuron-specific enolase, urine catecholamine metabolites, MRI, and bone marrow morphology were assessed at baseline, at the end of phase 1 and at the end of phase 2, as well as every 3 months for up to 2 years and then biannually. Nuclear medicine imaging was required at baseline, at the end of phase 1 and at the end of phase 2, as well as at the end of the 1-year follow-up, thereafter only if clinically indicated. If tumour progression was detected at the end of phase 1 and at the end of phase 2, the date of progression was set at a predefined timepoint instead of the day of the actual assessment.¹⁷ All response assessments were locally and centrally reviewed. Adverse events were assessed at each follow-up visit and assessed with Common Terminology Criteria for Adverse Events version 3.0. For quality-of-life assessments (QoL) Lansky and Karnofsky scores were used, with rankings from 100 to 0, where 100 reflects 'perfect' health and 0 is death. Assessments were scheduled at the end of phase 1 and 2, and consecutively every three months for one year.

Outcomes

The primary endpoint was progression-free survival, defined as time from the date of randomisation to the date of progression, of death of any cause, or of last visit. Patients with no progression at last disease evaluation or patients lost to follow-up were classified as censored at last visit and were confirmed to be progression-free. Secondary endpoints were overall survival (defined as time to death of any cause, in which surviving patients were censored at last visit), response (defined according to the International Neuroblastoma Risk Group criteria with stable disease or above) to the investigational treatment in the RIST group, duration of best response, safety, prognostic relevance of the International Neuroblastoma Risk Group criteria laboratory parameters on event-free survival (defined as the time of duration of stable disease, complete response, and partial response), and QoL.

Statistical analysis

To detect a hazard ratio (HR) of 0.58 with a power of 1–β=80% at a one-sided α significance level of 0.10, which was expected to be of high clinical relevance, a minimum of 90 patients were required to be randomised to yield the necessary number of 63 progression-free survival events. The formulas used in the estimations were based on the assumptions of uniform accrual over

time, and exponentially distributed event times. With an estimated drop-out rate of 20%, the target enrolment was 130 patients. The main analyses for the primary and secondary efficacy endpoints (progression-free survival and overall survival) were performed according to the intention-to-treat principle in all eligible patients who entered the study and received at least one course of therapy. A patient had to complete a course of therapy to be included in the analysis. The per-protocol population consisted of the intention-to-treat population without major protocol violations. The primary analysis was repeated in the per-protocol population as a prespecified sensitivity analysis to assess the robustness of the results. The safety population consisted of all patients who received at least one course of therapy and had at least one post-baseline safety assessment. For the primary and secondary endpoints, progression-free survival and overall survival distribution and median progression-free survival and overall survival times were estimated using the Kaplan–Meier plots. A Cox proportional hazards model with *MYCN* status and treatment group as covariates was applied to test the null hypothesis of a treatment HR of 1. For the HR, a point estimate and a

one-sided 90% (according to the trial design) was provided. The median follow-up time was calculated using the reverse Kaplan–Meier method. As a post-hoc analysis of the primary endpoint, a two-sided 95% CI was added to show the clinical relevance and statistical certainty of the study result to the conventional two-sided α of 5%. *MYCN*-based subgroup analyses, including the overall survival analyses, were post-hoc analyses, including a post-hoc defined multivariable Cox proportional hazards model including treatment, *MYCN* status and the interaction term treatment**MYCN* as well as disease status at trial inclusion, time from diagnosis to first relapse, sex, age, surgery, histology, and CNS recurrence as covariates to adjust for known predictors of progression-free survival. The proportional hazards assumption was tested with the Schoenfeld residuals. Two post-hoc sensitivity analyses, which were not defined in the statistical analysis plan, were additionally performed. In patients with treatment delays due to adverse events, the average time between two cycles was added to the multivariable model to assess the impact on outcome. In patients with missing assessments at the predefined visits, sensitivity analyses were performed by

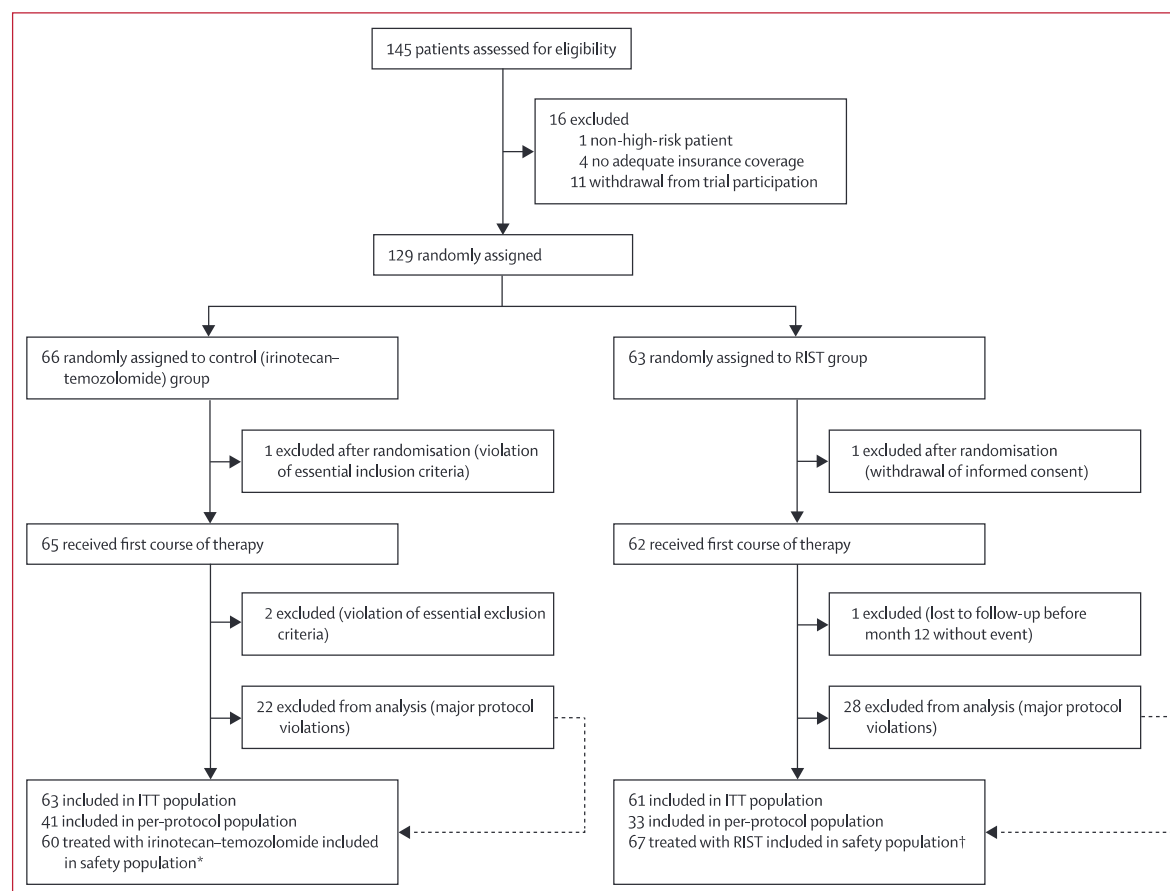


Figure 1: Trial profile

ITT=intention-to-treat. RIST=irinotecan-temozolomide and dasatinib-rapamycin. *Six patients refused result of randomisation and received RIST therapy.

†One patient refused result of randomisation and received irinotecan-temozolomide therapy.

	Control group (n=63)	RIST group (n=61)
Sex		
Female	25 (40%)	21 (34%)
Male	38 (60%)	40 (66%)
Age at diagnosis, years		
Median (IQR)	3.5 (2.3–4.9)	3.3 (1.9–5.4)
Age group, years		
<1	3 (5%)	4 (7%)
1 to <1.5	2 (3%)	5 (8%)
1.5 to <5	44 (70%)	36 (59%)
≥5	14 (22%)	16 (26%)
Age at enrolment, years		
Median (IQR)	5.3 (3.8–7.5)	5.6 (3.7–8.6)
Age group, years		
<1	0	0
1 to <1.5	1 (2%)	2 (3%)
1.5 to <5	28 (44%)	25 (41%)
≥5	34 (54%)	34 (56%)
MYCN status		
Not amplified	39 (62%)	37 (61%)
Amplified	24 (38%)	24 (39%)
Time from diagnosis to first relapse, months		
≤18	17 (35%)	12 (24%)
>18	32 (65%)	38 (76%)
International Neuroblastoma Staging System stage at diagnosis		
2A	1 (2%)	0
3	3 (5%)	3 (5%)
4	59 (94%)	57 (93%)
Unknown	0	1 (2%)
International Neuroblastoma Risk Group at diagnosis		
Intermediate risk	4 (6%)	5 (8%)
High risk	59 (94%)	56 (92%)
Disease status at enrolment		
Refractory	14 (22%)	10 (16%)
Relapse	49 (78%)	50 (82%)
First relapse	43 (88%)	41 (82%)
Second relapse	4 (8%)	8 (16%)
Third relapse	2 (4%)	1 (2%)
Unknown	0	1 (2%)

(Table 1 continues in next column)

imputing missing assessments before a disease progression was identified and by conservatively imputing all missing assessments as disease progression. Post-hoc analyses in patients who completed phase 2 in remission were conducted to assess the impact of consecutive therapies on overall survival. All safety data are presented descriptively for each treatment group and were analysed as treated. Response rates are presented descriptively. Response rates in the MYCN-non-amplified and in the MYCN-amplified subgroup and best overall response (complete response or partial response) were assessed post hoc. Missing values regarding the response were counted as non-responders. The Karnofsky scores

	Control group (n=63)	RIST group (n=61)
(Continued from previous column)		
CNS involvement at enrolment	8 (13%)	6 (9%)
Dinutuximab before enrolment	0	1 (1%)
Surgery before enrolment	7 (11%)	9 (15%)
Histology		
Neuroblastoma	61 (97%)	57 (93%)
Ganglioneuroblastoma	2 (3%)	3 (5%)
Unknown	0	1 (2%)
Primary tumour site at diagnosis		
Adrenal	33 (52%)	32 (52%)
Abdomen, not adrenal	4 (6%)	6 (10%)
Abdomen, adrenal unknown	13 (21%)	10 (16%)
Pelvis	3 (5%)	1 (2%)
Thorax	7 (11%)	4 (7%)
Neck	0	0
Other	3 (5%)	7 (11%)
Unknown	0	1 (2%)
Metastatic sites at diagnosis		
Metastasis found	59 (94%)	57 (93%)
Soft tissue (including lymph nodes, liver, skin, and other)	39 (66%)	42 (74%)
Bone marrow	56 (95%)	48 (84%)
Bone	39 (66%)	40 (70%)
1 metastatic compartment	6 (10%)	10 (16%)
>1 metastatic compartment	53 (84%)	47 (77%)

Data are n (%) unless otherwise specified. RIST=irinotecan–temozolomide and dasatinib–rapamycin. Data on race or ethnicity were not collected.

Table 1: Baseline characteristics of the intention-to-treat population

of each visit were summarised descriptively, and scores were compared between both treatment groups using a Wilcoxon Mann–Whitney test. The threshold of significance for all secondary endpoints was $p < 0.05$.

Data preparation and statistical analyses were performed with SAS software (version 9.4), and R software (version 4.2.2). An independent data monitoring committee reviewed safety and recruitment. This trial is registered at ClinicalTrials.gov, NCT01467986, and is closed to accrual.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Aug 26, 2013, and Sept 21, 2020, 129 patients were recruited and were randomly assigned to the RIST group (63 patients) or the control group (66 patients). At data cutoff (Sept 9, 2022), 61 patients from the RIST group and 63 patients from the control group were available for the primary endpoint analysis (intention-to-treat population; figure 1). 78 (63%) of 124 patients in the

intention-to-treat population were male and 46 (37%) were female, and 116 (94%) had International Neuroblastoma Staging System stage 4 disease (table 1; appendix pp 24–27 for the *MYCN*-amplified subpopulations). Data on race or ethnicity were not collected. The median patient age at enrolment was 5·4 years (IQR 3·7–8·1; 53 (43%) were aged 1·5–5·0 years). Formally, there was no limitation for previous treatment beyond the inclusion and exclusion criteria. However, 128 (99%) patients in first relapse were treated as study participants of the NB2004-HR protocol (NCT00410631), which was the standard of treatment in the participating countries at that time.¹⁸ The remaining patients were treated according to equivalent protocols of other societies before enrolment. None of the relapsed patients received irinotecan or temozolomide before enrolment in this trial. Of the 15 (12%) patients with second or third relapses, 12 patients received some form of chemotherapy only. In three patients (two in the control group and one in the RIST group) anti-disialoganglioside monoclonal antibody, metaiodobenzylguanidine therapy, or radiotherapy was added. *MYCN* was amplified in 48 (39%) patients. 29 (29%) of 99 patients who had relapse at enrolment had a first relapse within 18 months, with a balanced distribution of *MYCN* amplification between the RIST group and the control group. 115 (93%) patients were classified as high risk (International Neuroblastoma Risk Group¹⁹) at diagnosis. 15 (62%) of the 24 patients with refractory disease had *MYCN* amplification, all older than 2 years, and 21 (88%) patients were enrolled with metastatic disease. The median overall follow-up for both groups was 72 months (IQR 31–88), the median follow-up was 76 (35–89) months for the RIST group and 34 (30–88) months for the control group.

In the intention-to-treat population, there were 49 progression-free survival events in the RIST group and 54 in the control group. Median progression-free survival was 11 months (95% CI 7–17) in the RIST group and 5 months (2–8) in the control group (HR 0·62, one-sided 90% CI 0·81; $p=0·019$; post-hoc two-sided 95% CI 0·42–0·92; figure 2A). This result was confirmed in the per-protocol population (HR 0·53, one-sided 90% CI 0·75; post-hoc two-sided 95% CI 0·31–0·90; $p=0·018$). In a multivariable Cox regression model adjusting for known risk factors for progression or death, median progression-free survival with RIST was significantly longer compared with control (table 2). Furthermore, the post-hoc analysis with the interaction term of treatment**MYCN* status was statistically significant. In order not to violate the proportional hazards assumption, age and time to relapse were added as continuous covariates; all variables hold the assumptions of proportional hazards (data not shown).

Median progression-free survival in patients with amplified *MYCN* was 6 months (95% CI 4–24; 18 events in the RIST group) in the RIST group versus 2 months (2–5; 23 events in the control group) in the control group

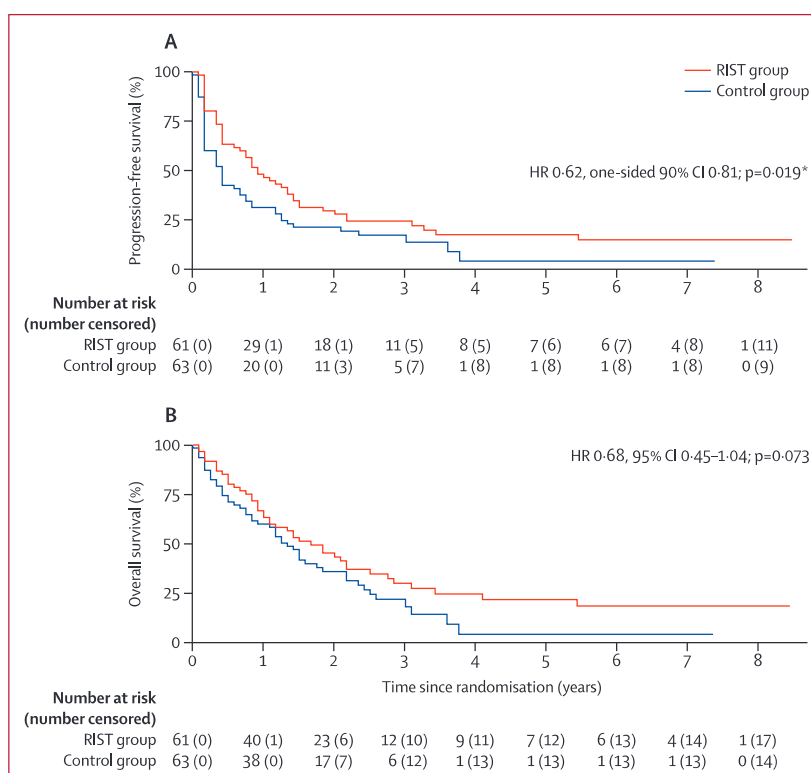


Figure 2: Progression-free survival (A) and overall survival (B) in intention-to-treat population
RIST=irinotecan–temozolomide and dasatinib–rapamycin. *Post-hoc two-sided 95% CI 0·42–0·92.

(HR 0·45 [95% CI 0·24–0·84], $p=0·012$; appendix p 14). Median progression-free survival in patients without amplified *MYCN* was 14 months (95% CI 9–17; 31 events) in the RIST group versus 8 months (4–15; 31 events) in the control group (HR 0·84 [95% CI 0·51–1·38], $p=0·49$; appendix p 16). Additional progression-free survival data by *MYCN* status are shown in the appendix (p 30). The post-hoc multivariable model in patients with *MYCN* amplification is shown in the appendix (pp 28–29). Progression-free survival in the per-protocol analysis stratified by treatment groups and according to *MYCN* is summarised in the appendix (pp 18–19).

Response rate in the RIST group was 75% (46 of 61 patients) after phase 1 and 61% (37 of 61 patients) after phase 2. Post-hoc best overall response rates and response rates in the *MYCN*-amplified subgroup are summarised in table 3. Among patients with intracranial recurrence (14 [11%]), the response rate was similar between treatment groups for the *MYCN*-amplified subgroup and the *MYCN*-non-amplified subgroup. Among patients with *MYCN* amplification ($n=7$), responses were observed in one (25%) of four patients in both phases in the RIST group and one (33%) of three patients in both phases in the control group. Among patients without *MYCN* amplification ($n=7$), responses were observed in two (100%) of two patients in both phases in the RIST group and five (100%) of five patients

	Hazard ratio (95% CI)	p value	Event/patients
Treatment group	..	0.002	..
MYCN status	..	0.017	..
Treatment*MYCN status	..	0.018	..
MYCN-A: RIST vs control	0.35 (0.18–0.68)	..	41/48
MYCN-NA: RIST vs control	1.00 (0.58–1.70)	..	60/74
Control: MYCN-NA vs MYCN-A	0.50 (0.28–0.88)	..	54/63
RIST: MYCN-NA vs MYCN-A	1.43 (0.70–2.91)	..	47/59
Dinutuximab before enrolment			
No	1 (ref)	..	100/121
Yes	1.40 (0.17–11.18)	0.754	1/1
Disease status			
Relapse	1 (ref)	..	79/98
Refractory	2.23 (1.28–3.90)	0.005	22/24
Time from diagnosis to first relapse	0.98 (0.97–0.999)	0.038	..
Sex			
Female	1 (ref)	..	37/46
Male	1.32 (0.86–2.03)	0.201	64/76
Age at diagnosis (years)	1.00 (0.93–1.08)	0.911	
Surgery			
Yes	1 (ref)	..	10/16
No	1.75 (0.89–3.51)	0.113	91/106
Histology			
Neuroblastoma	1 (ref)	..	96/117
Ganglioneuroblastoma	1.36 (0.53–3.51)	0.525	5/5
CNS recurrence			
No	1 (ref)	..	90/108
Yes	0.89 (0.44–1.79)	0.734	11/14

In the post-hoc multivariable Cox regression analysis, we adjusted for all relevant covariables. MYCN-A=MYCN amplified. MYCN-NA=MYCN non-amplified. RIST=irinotecan–temozolomide and dasatinib–rapamycin.

Table 2: Multivariable Cox regression of progression-free survival of high-risk parameters (n=122)

	Intention-to-treat population		MYCN-amplified subgroup	
	Control group	RIST group	Control group	RIST group
Complete response	11 (17%)	15 (25%)	5 (21%)	9 (38%)
Partial response	24 (38%)	26 (43%)	5 (21%)	6 (25%)
Minor response*	0	2 (3%)	0	2 (8%)
Stable disease	6 (10%)	7 (11%)	1 (4%)	3 (13%)
Progressive disease	20 (32%)	10 (16%)	12 (50%)	4 (17%)
Overall response rate	35 (56%)	41 (67%)	10 (42%)	15 (62%)

RIST=irinotecan–temozolomide and dasatinib–rapamycin. *Minor response was defined as no new lesions; >50% reduction of any measurable lesion (primary or metastasis) with <50% reduction in any other; <25% increase in any existing lesion by radiological means (ie, MRI) or via metaiodobenzylguanidine scintigraphy in all sites. Reduction of catecholamines and nervous system-specific enolase less than 50%.

Table 3: Overall best response by treatment regimens (post-hoc analysis)

in phase 1 and four (80%) of five patients in phase 2 in the control group. Median duration of response in the RIST group was 17 (95% CI 11–26) months.

Median overall survival for RIST was 20 (95% CI 13–30) months versus 16 (10–22) months in the control group (HR 0.68, 95% CI 0.45–1.04; $p=0.073$; figure 2B; RIST

43 deaths in the RIST group and 49 deaths in the control group). Post-hoc analysis of median overall survival in patients with MYCN amplification and those without MYCN amplification is shown in the appendix (pp 15, 17) and overall survival in the per-protocol population stratified by treatment groups and MYCN status are in the appendix (pp 20–21).

51 (41%) of 124 patients (29 [48%] of 61 patients in the RIST group, 22 [35%] of 63 patients in control group) finished phase 2 therapy in remission and were eligible for consolidation therapy. 49 (40%) of 124 patients received some form of consolidation therapy. RIST was continued until recurrence in eight (16%) patients (four in each treatment group) and 16 (33%) patients (nine patients in the RIST group and seven in the control group) received a conventional form of consolidation therapy and 25 (51%) patients (15 in the RIST group and ten in the control group) additionally received a haplo-identical haematopoietic stem-cell transplantation with or without anti-disialoganglioside monoclonal antibody (appendix pp 13, 31). Overall survival in patients who had consecutive therapies who completed phase 2 in remission, including in the MYCN-amplified subgroup, is shown in the appendix (pp 22, 23, 31). The consecutive physician-based consolidation with a haploidentical haematopoietic stem-cell transplantation was well balanced between treatment groups, with 15 (54%) of 28 patients in the RIST group and ten (48%) of 21 patients in the control group. However, only four (44%) of nine patients without MYCN amplification in the RIST group (44%) and five (62%) of eight patients without MYCN amplification in the control group survived post-transplantation. Conversely, five (83%) of six patients with MYCN amplification in the RIST group and zero of two patients with MYCN amplification in the control group survived post-transplantation (appendix pp 23, 31). The median duration of exposure to therapy was 156 days (IQR 149–165) in the RIST group and 150 days (141–165) in the control group. Patient samples were not sufficiently available to conduct analyses of laboratory parameters.

65 (97%) of 67 patients who were given RIST and 52 (86%) of 60 patients who were given control had an adverse event, and 14 (21%) patients given RIST and eight (13%) patients given control had a grade 3 or worse adverse event (table 4). Haematological adverse events were the most common grade 3 or worse adverse events—namely, neutropenia (54 [81%] of 67 patients in the RIST group vs 49 [82%] of 60 patients in the control group), thrombocytopenia (45 [67%] vs 41 [68%]), and anaemia (39 [58%] vs 38 [63%]). Infections of grade 3 or worse occurred in 12 (18%) patients in the RIST group and 13 (22%) patients in the control group. Diarrhoea of grade 3 or worse was observed in 14 (21%) patients in the RIST group and 11 (18%) patients in the control group. Nine serious treatment-related adverse events were reported; five in the control group (one glomerulopathy,

	RIST group (n=67)*				Control group (n=60)†			
	Grade 1–2	Grade 3	Grade 4	Grade 5	Grade 1–2	Grade 3	Grade 4	Grade 5
General condition	51 (76%)	10 (15%)	4 (6%)	0	44 (73%)	5 (8%)	2 (3%)	1 (2%)
Haematological event								
Anaemia	28 (42%)	29 (43%)	10 (15%)	0	20 (33%)	28 (47%)	10 (17%)	0
Leukocytopenia	5 (7%)	26 (39%)	33 (49%)	0	10 (17%)	24 (40%)	24 (40%)	0
Neutropenia	9 (13%)	12 (18%)	42 (63%)	0	8 (13%)	15 (25%)	34 (57%)	0
Thrombocytopenia	16 (24%)	32 (48%)	13 (19%)	0	11 (18%)	31 (52%)	10 (17%)	0
Gastrointestinal event								
Stomatitis	17 (25%)	2 (3%)	0	0	12 (20%)	0	0	0
Vomiting	49 (73%)	5 (7%)	2 (3%)	0	38 (63%)	6 (10%)	0	0
Diarrhoea	38 (57%)	9 (13%)	5 (7%)	0	35 (58%)	7 (12%)	4 (7%)	0
Infection	41 (61%)	12 (18%)	0	0	28 (47%)	13 (22%)	0	0
Fever	42 (63%)	0	0	0	33 (55%)	0	0	0
Laboratory abnormality								
Creatinine increased	28 (42%)	0	0	0	17 (28%)	0	0	0
Proteinuria	16 (24%)	0	0	0	14 (23%)	0	0	0
Haematuria or haemoglobinuria	10 (15%)	0	0	0	11 (18%)	0	0	0
Hyperbilirubinemia	6 (9%)	3 (4%)	0	0	7 (12%)	0 (0%)	1 (2%)	0
Transaminase increased	46 (69%)	14 (21%)	1 (1%)	0	35 (58%)	14 (23%)	0	0
Other								
Peripheral neurotoxicity	6 (9%)	0	0	0	4 (7%)	1 (2%)	0	0
Skin changes	27 (40%)	1 (1%)	0	0	13 (22%)	1 (2%)	0	0

Most common treatment-related adverse events in patients who received at least one dose of study drug, as treated. Grade 1–2 events reported in at least 10% patients and all grade 3–5 events are shown. RIST=irinotecan–temozolomide and dasatinib–rapamycin. *One patient refused result of randomisation and received irinotecan–temozolomide therapy. †Six patients refused result of randomisation and received RIST therapy.

Table 4: Adverse events

two urinary tract infections, one diarrhoea, and one prolonged atrio-ventricular transmission) and four in the RIST group (one complex fracture of the distal tibia after an accident, one sinusoidal obstruction syndrome after a mistletoe treatment, and two urinary tract infections). No treatment-related severe hepatic toxicity leading to diseases, such as veno-occlusive disease or sinusoidal obstruction syndrome, was reported. Some adverse reactions, such as elevated creatinine concentration, skin changes, and cardiac changes, were observed more frequently in the RIST group but were transient (data not shown). Overall, no dose adjustments were made in the dasatinib–rapamycin course. The irinotecan–temozolomide course was delayed in 23 (19%) patients (11 in the RIST group and 12 in the control group), and the chemotherapy dose was reduced to allow for haematological recovery in 11 (9%) patients (five in the RIST group and six in the control group). Premature termination of study therapy due to toxicity was necessary in two patients in the control group and three patients in the RIST group. Causes of death were disease related in 47 (96%) patients in the control group and 39 (91%) patients in the RIST group, and treatment related in none of the patients in the control group and one (2%) patient in the RIST group due to multiorgan failure.

The QoL assessments at the respective timepoints showed no significant difference between RIST and control (appendix p 32).

Discussion

With a median follow-up of 72 (IQR 31–88) months, results from the RIST-rNB-2011 trial showed that RIST significantly improved progression-free survival and resulted in a substantially (but not statistically significant) longer overall survival in relapsed or refractory neuroblastoma. These trial results are consistent with the pilot series.¹⁵ Several in-vitro studies showed synergy between mTOR inhibitors and multikinase inhibitors, as well as in combination with chemotherapy. In neuroblastoma, a synergistic effect was most prominent in *MYCN*-amplified tumours and cell lines.^{8,10,13,20–23} The pathway-directed drug combination in RIST-rNB-2011 had a clinically significant and sustained impact on progression-free survival and overall survival, limited to the *MYCN*-amplified subgroup in post-hoc analyses. Accordingly, only 17% of patients with *MYCN* amplification treated with RIST compared with 50% of patients with *MYCN* amplification treated in the control group had progressive disease. In a multivariable analysis of the unselected patient population, including the most relevant high-risk criteria, the progression-free survival

with RIST was improved compared with the control group and stable after the first 2 years throughout follow-up. The additional post-hoc analysis with the interaction term of treatment*MYCN status confirms the significant impact of RIST, exclusively in patients with MYCN amplification.

37 patients with relapsed or refractory neuroblastoma were enrolled in a randomised trial comparing temsirolimus versus dinutuximab with an irinotecan–temozolomide backbone.⁷ The overall response rate was 53% with dinutuximab versus 5.6% with temsirolimus. In the consecutive non-random extension of the cohort treated with irinotecan–temozolomide–dinutuximab, the reported overall response rate was 42%.²⁴ The worse outcome of irinotecan–temozolomide plus temsirolimus compared with the RIST trial can be multifactorial. First, the COG ANBL1221 trial had a sample size that was four times smaller than that of the RIST-rNB-2011 trial. Also, RIST follows a metronomic concept with no interruptions of molecular-targeted medication, compared with 13 days of medication-free intervals in irinotecan–temozolomide–temsirolimus study.²⁴ Moreover, the addition of the synergistically acting dasatinib in RIST and the timing of the targeted drugs might also have contributed to the better outcomes.

The metronomic approach of RIST requires a hierarchical repetition of cycles consisting of pathway-directed drugs preceding chemotherapy. Furthermore, timing of administration in a combination therapy is poorly understood and difficult to translate in vivo. In-vitro data showed a synergistic effect, also via induction of cell-cycle arrest in G1, repetitively synchronising transition of cells into the chemo-sensitive S phase.^{10,13,20,21} A previous study²⁵ showed that treatment response of neuroblastoma cells is dictated by their expression of MYCN and the cell-cycle phase before treatment. Although all MYCN-non-amplified cells enter therapy-induced senescence, MYCN-amplified cells disable their cell-cycle checkpoints, forcing renewed proliferation despite treatment-induced DNA damage. Furthermore, in agreement with reduced metastatic sites found in RIST-treated patients, an in-vitro analysis²⁶ revealed less expression of cancer stem cell-like markers in targeted neuroblastoma spheroids. Intracranial metastasis of neuroblastoma is rare, but indicates a dismal prognosis.²⁷ Although only a few patients with MYCN-amplified intracranial metastasis were enrolled in our study, the outcome remains poor.

The safety profile of the RIST group was similar to the control group. Treatment delays occurred often due to haematopoietic exhaustion not reaching thresholds for trial continuation, mostly in patients with early relapse. However, the post-hoc sensitivity analyses supported that these delays had no negative effect on the endpoints of the trial.

A possible limitation of the study with regard to the results is the free choice of subsequent consolidation

therapy. Haploidentical haematopoietic stem-cell transplantation as consecutive physician-based consolidation was well balanced since half of the patients in remission of both groups received a haematopoietic stem-cell transplantation for consolidation. However, five (83%) of six patients with MYCN amplification and haematopoietic stem-cell transplantation in the RIST group survived versus none of two patients in the control group. The better survival of patients with MYCN amplification treated with RIST and a haploidentical haematopoietic stem-cell transplantation might be explained by those patients achieving minimal residual disease, permitting a durable cellular consolidation therapy. Therefore, treatment with RIST could induce remission and a sustained progression-free survival in MYCN-amplified relapsed or refractory neuroblastoma, permitting consolidation therapy with either chemotherapy, haploidentical haematopoietic stem-cell transplantation²⁸ with or without a chimeric antigen receptor T-cell therapy to reach a cure.²⁹ Whichever concept for consolidation is the most suitable needs to be defined in future trials. In the light of substantial adverse effects of current first-line therapies, the results of the RIST trial initiate the discussion on a first-line concept, including a RIST-like approach in MYCN-amplified neuroblastoma.

Another potential limitation of the study is the prolonged recruitment period of the RIST trial. To our knowledge, the RIST trial is one of the trials for neuroblastoma with the largest enrolment for a rare disease like relapsed or refractory neuroblastoma that has evaluated progression-free survival in a classical two-arm randomised design. In retrospect, more modern statistical methods could have reduced the sample size to shorten the recruitment time. However, only this long follow-up could have led to the observed progression-free survival outcomes.

Although the revised International Neuroblastoma Response Criteria were only published after the start of the study in 2017, nuclear medicine imaging, among others, was already implemented in the protocol for the assessment of the primary endpoint. Therefore, the necessary measurements were available to apply the revised criteria to all included patients, improving the assessment of bone and bone marrow disease, which are often the only sites of tumour involvement in relapsed or refractory disease.

Furthermore, at least a third of the patients in both groups were excluded from the per-protocol population due to protocol violations, and some patients in remission were withdrawn from the trial prematurely to explore alternative therapies. Regarding the latter, we hypothesise that this temporary remission was seen as an opportunity for an alternative modality of consolidation. The better outcome of the per-protocol population might be the consequence of a more robust minimal residual disease, which was reached with completion of all courses that allowed for a durable progression-free survival. The

observed toxicity in both groups leading to protocol violations was almost exclusively related to irinotecan–temozolomide, so equally effective alternatives could be considered in the future.

Although patients received different first-line drugs, most (99%) patients received first-line treatment as study participants of the NB2004-HR (NCT00410631) protocol. Furthermore, a recent analysis on two different induction regimens concluded that no significant difference in outcomes was observed.³⁰ We believe that this represents an adequate homogeneity of pre-treatment to support the results of the RIST trial.

In conclusion, to our knowledge, the trial shows for the first time that the addition of rapamycin–dasatinib to irinotecan–temozolomide can improve long-term progression-free survival and overall survival, exclusively in patients with *MYCN*-amplified relapsed or refractory neuroblastoma, demonstrating that *MYCN* amplification is a clinically relevant molecular target. The ubiquitous availability of the drugs used in the study, most in oral liquid formulation, the outpatient applicability, and the acceptable adverse event profile suggests that RIST could be a potential treatment option for even of the most fragile patients with neuroblastoma.

Contributors

SC conceptualised the RIST trial and wrote the study protocol and the first draft of the manuscript. SC, SE, FZ, MJ, AT, and JF secured funding for the trial. FZ, MK, and SC were the methodological leads, contributed to the study design, and performed the statistical analysis. HL, MS, GE, KB, P-G S, BG, PL, MR, K-M D, DS, AB, NG, SK, MJ, MP, D-T S, MM, PH, IK, DD, SS, GS, IT-v-L, MF, NJ, MP, AL, MN, TI, HC, IS, RC, NN, GC, JF, RL, JF, MD, FB, BF, SB, TB, JG, AE, and TS were involved in the recruitment and treatment of participants. PH, DH, KM, SZ, and MR performed the blinded histopathology, neuropathology, and imaging analysis. SC, AT, JF, GS, TH, TS, HL, and AE were members of the clinical steering committee that contributed to the study design, were responsible for oversight throughout the trial, and contributed to data interpretation. SC, FZ, HL, TS, AT, and JF wrote the final manuscript. All authors had access to data reported in this study. SC and FZ accessed and verified the data. SC had final responsibility for the decision to submit for publication. All authors assume responsibility for the accuracy and completeness of the data and analyses, as well as for the fidelity of the trial and this report to the protocol.

Declaration of interests

We declare no competing interests.

Data sharing

Deidentified data (patient characteristics and outcome) will be made available to other researchers on request, subject to the approval of a formal written data access request starting from publication and ending 2 years after publication. Trial documentation, including the protocol, are available on request by contacting the corresponding author. Data recipients are required to enter a formal data sharing agreement that describes the conditions for release and requirements for data transfer, storage, archiving, publication, and intellectual property. Requests are reviewed by the trial steering committee in terms of scientific merit and ethical considerations including patient consent. Data sharing is allowed if proposed projects have a sound scientific or patient benefit rationale, as approved by the trial steering committee. The ethics committee that initially approved the trial should also approve any specimen or data use not covered by already collected informed consent forms and might request new written consent. Restrictions relating to patient confidentiality and consent will be enforced by aggregating and anonymising identifiable patient data. Additionally, all indirect identifiers that might lead to deductive disclosures will be removed. Data sharing requests should be directed to the corresponding author.

Acknowledgments

The RIST-rNB-2011 trial was funded by an unrestricted grant from Deutsche Krebshilfe. We thank the patients and their families, the nurses, the site staff, the clinical research coordinators, and project managers. We thank the members of the independent data monitoring committee: Stefan Suci, Lawrence Faulkner, and Jaume Mora (appendix p 8).

References

- 1 Basta NO, Halliday GC, Makin G, et al. Factors associated with recurrence and survival length following relapse in patients with neuroblastoma. *Br J Cancer* 2016; **115**: 1048–57.
- 2 London WB, Bagatell R, Weigel BJ, et al. Historical time to disease progression and progression-free survival in patients with recurrent/refractory neuroblastoma treated in the modern era on Children's Oncology Group early-phase trials. *Cancer* 2017; **123**: 4914–23.
- 3 Moreno L, Rubie H, Varo A, et al. Outcome of children with relapsed or refractory neuroblastoma: a meta-analysis of ITCC/SIOPEN European phase II clinical trials. *Pediatr Blood Cancer* 2017; **64**: 25–31.
- 4 Kushner BH, Kramer K, Modak S, Cheung NK. Irinotecan plus temozolomide for relapsed or refractory neuroblastoma. *J Clin Oncol* 2006; **24**: 5271–76.
- 5 Wagner LM, Villablanca JG, Stewart CF, et al. Phase I trial of oral irinotecan and temozolomide for children with relapsed high-risk neuroblastoma: a new approach to neuroblastoma therapy consortium study. *J Clin Oncol* 2009; **27**: 1290–96.
- 6 Bagatell R, Norris R, Ingle AM, et al. Phase 1 trial of temsirolimus in combination with irinotecan and temozolomide in children, adolescents and young adults with relapsed or refractory solid tumors: a children's oncology group study. *Pediatr Blood Cancer* 2013; **61**: 833–39.
- 7 Mody R, Naranjo A, Van Ryn C, et al. Irinotecan-temozolomide with temsirolimus or dinutuximab in children with refractory or relapsed neuroblastoma (COG ANBL1221): an open-label, randomised, phase 2 trial. *Lancet Oncol* 2017; **18**: 946–57.
- 8 Johnsen JI, Segerström L, Orrego A, et al. Inhibitors of mammalian target of rapamycin downregulate MYCN protein expression and inhibit neuroblastoma growth in vitro and in vivo. *Oncogene* 2008; **27**: 2910–22.
- 9 Kling MJ, Griggs CN, McIntyre EM, et al. Synergistic efficacy of inhibiting MYCN and mTOR signaling against neuroblastoma. *BMC Cancer* 2021; **21**: 1061.
- 10 Corbacioglu S, Kilic M, Westhoff MA, Reinhardt D, Fulda S, Debatin KM. Newly identified c-KIT receptor tyrosine kinase ITD in childhood AML induces ligand-independent growth and is responsive to a synergistic effect of imatinib and rapamycin. *Blood* 2006; **108**: 3504–13.
- 11 Westhoff MA, Faham N, Marx D, et al. Sequential dosing in chemosensitization: targeting the PI3K/Akt/mTOR pathway in neuroblastoma. *PLoS One* 2013; **8**: e83128.
- 12 Schramm A, Köster J, Marschall T, et al. Next-generation RNA sequencing reveals differential expression of MYCN target genes and suggests the mTOR pathway as a promising therapy target in MYCN-amplified neuroblastoma. *Int J Cancer* 2013; **132**: E106–15.
- 13 Segerström L, Baryawno N, Sveinbjörnsson B, et al. Effects of small molecule inhibitors of PI3K/Akt/mTOR signaling on neuroblastoma growth in vitro and in vivo. *Int J Cancer* 2011; **129**: 2958–65.
- 14 Herrmann MD, Lennerz JK, Bullinger L, et al. Transitory dasatinib-resistant states in KIT(mut) t(8;21) acute myeloid leukemia cells correlate with altered KIT expression. *Exp Hematol* 2014; **42**: 90–100.
- 15 Corbacioglu S, Steinbach D, Lode H, et al. The RIST design: a molecularly targeted multimodal approach for the treatment of patients with relapsed and refractory neuroblastoma. *Proc Am Soc Clin Oncol* 2013; **31**(15_suppl): 10017 (abstr).
- 16 Park JR, Bagatell R, Cohn SL, et al. Revisions to the International Neuroblastoma Response Criteria: a consensus statement from the National Cancer Institute Clinical Trials Planning Meeting. *J Clin Oncol* 2017; **35**: 2580–87.
- 17 Panageas KS, Ben-Porat L, Dickler MN, Chapman PB, Schrag D. When you look matters: the effect of assessment schedule on progression-free survival. *J Natl Cancer Inst* 2007; **99**: 428–32.

- 18 Berthold F, Faldum A, Ernst A, et al. Extended induction chemotherapy does not improve the outcome for high-risk neuroblastoma patients: results of the randomized open-label GPOH trial NB2004-HR. *Ann Oncol* 2020; **31**: 422–29.
- 19 Monclair T, Brodeur GM, Ambros PF, et al. The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. *J Clin Oncol* 2009; **27**: 298–303.
- 20 Waetzig R, Matthes M, Leister J, et al. Comparing mTOR inhibitor rapamycin with torin-2 within the RIST molecular-targeted regimen in neuroblastoma cells. *Int J Med Sci* 2021; **18**: 137–49.
- 21 Bender A, Opel D, Naumann I, et al. PI3K inhibitors prime neuroblastoma cells for chemotherapy by shifting the balance towards pro-apoptotic Bcl-2 proteins and enhanced mitochondrial apoptosis. *Oncogene* 2011; **30**: 494–503.
- 22 He Y, Sun MM, Zhang GG, et al. Targeting PI3K/Akt signal transduction for cancer therapy. *Signal Transduct Target Ther* 2021; **6**: 425.
- 23 Zafar A, Wang W, Liu G, et al. Molecular targeting therapies for neuroblastoma: Progress and challenges. *Med Res Rev* 2021; **41**: 961–1021.
- 24 Mody R, Yu AL, Naranjo A, et al. Irinotecan, temozolomide, and Dinutuximab With GM-CSF in children with refractory or relapsed neuroblastoma: a report from the Children's Oncology Group. *J Clin Oncol* 2020; **38**: 2160–69.
- 25 Ryl T, Kuchen EE, Bell E, et al. Cell-Cycle Position of single MYC-driven cancer cells dictates their susceptibility to a chemotherapeutic drug. *Cell Syst* 2017; **5**: 237–50.e8.
- 26 Kaess C, Matthes M, Gross J, et al. Evaluating the RIST molecular-targeted regimen in a three-dimensional neuroblastoma spheroid cell culture model. *Cancers (Basel)* 2023; **15**: 1749.
- 27 Matthay KK, Brisse H, Couanet D, et al. Central nervous system metastases in neuroblastoma: radiologic, clinical, and biologic features in 23 patients. *Cancer* 2003; **98**: 155–65.
- 28 Flaadt T, Ladenstein RL, Ebinger M, et al. Anti-GD2 antibody dinutuximab beta and low-dose interleukin 2 after haploidentical stem-cell transplantation in patients with relapsed neuroblastoma: a multicenter, phase I/II trial. *J Clin Oncol* 2023; **41**: 3135–48.
- 29 Del Bufalo F, De Angelis B, Caruana I, et al. GD2-CART01 for relapsed or refractory high-risk neuroblastoma. *N Engl J Med* 2023; **388**: 1284–95.
- 30 Garaventa A, Poetschger U, Valteau-Couanet D, et al. Randomized trial of two induction therapy regimens for high-risk neuroblastoma: HR-NBL1.5 International Society of Pediatric Oncology European Neuroblastoma Group Study. *J Clin Oncol* 2021; **39**: 2552–63.