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Review

Current treatment strategies for first relapse of high-risk neuroblastoma

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ABSTRACT

More than 50 % of patients with high-risk neuroblastoma (HRNB) will relapse despite intensive multimodal therapy. Most relapses occur within 2 years of diagnosis. Overall survival at relapse is 20 % at 4 years, but long-term survival can be achieved in a patient subset. A biopsy at relapse with in-depth molecular characterization should now become accepted as standard of care to confirm active neuroblastoma and identify potential targets for biomarker-based targeted therapy or immunotherapy. No clear consensus currently exists about optimal therapy because the field lacks umbrella trials covering all phases of relapse treatment (re-induction,

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consolidation, maintenance) in a homogenous strategy. Recruitment into clinical trials (e.g. BEACON2) should be prioritized. Current evidence supports starting re-induction therapy with a camptothecin-based chemotherapy regimen combined with monoclonal antibody therapy targeting GD2 or VEGF (or ALK inhibitors if *ALK*-aberrant) as the first choice. The RIST regimen is a promising first choice for *MYCN*-amplified disease. After an objective response to re-induction therapy, GD2-directed immunotherapy or cellular therapies harnessing the immune system (haploidentical stem cell transplantation, CAR T cells) are of high interest as a consolidation strategy. Long-term maintenance therapy must be feasible as outpatient treatment, have a low toxicity profile and be well-tolerable to suit patients with relapsed HRNB. For optimal care, new options must be tested as maintenance therapy in randomized trials. The most promising salvage options for patients responding insufficiently to treatment are the chemotherapy combinations, topotecan/vincristine/doxorubicin (TVD), topotecan/cyclophosphamide/etoposide (TCE), ifosfamide/carboplatin/etoposide (ICE) or topotecan/cyclophosphamide (TopoCy), or [^{131}I]-mIBG therapy. Early-phase clinical trials are also a possible option in this setting.

1. Introduction

More than 50 % of patients with high-risk neuroblastoma (HRNB) will relapse despite intensive multimodal therapy. Most relapses occur within 2 years of diagnosis [1], yielding a 20 % overall survival (OS) at 4 years [2] and a median progression-free survival (PFS) of 6.4 months [3]. Long-term survival of a first HRNB relapse is, however, achievable for a subgroup of patients. Thus, a first relapse diagnosis should generally not be considered a palliative situation at that time.

Disease heterogeneity, therapy resistance, organ toxicity including poor hematological reserve and quality of life aspects make managing relapsed HRNB challenging. No clear consensus about optimal therapy exists due to the lack of well-designed randomized clinical trials [4] and umbrella trials using a homogenous strategy to cover all relapse treatment phases (re-induction, consolidation, maintenance). Heterogeneity in their inclusion criteria and primary endpoints for efficacy as well as unconsidered bias factors [4] make evaluating and comparing efficacy of different published relapse treatment strategies exceedingly difficult. Historically, clinical response criteria guided inclusion criteria for relapse trials, creating a heterogeneous patient population with (1) progressive disease during first-line induction therapy, (2) refractory disease (up to 20 % of cases) with insufficient metastatic response to first-line induction therapy, (3) first HRNB relapse, (4) first metastatic relapse of initially localized disease and (5) second or subsequent HRNB relapses. The community now agrees that all these subgroups likely represent different biological entities, with which different response rates and survival times are associated.

Bias created by broad inclusion criteria is numerous and comes from varied sources. Relapsed disease responds differently to refractory disease [5,6]. Patients with refractory disease are less likely to show an objective response to chemotherapy, but have a longer time to progression and a better OS [3,4]. The pattern and extent of recurrent disease provides a second source of bias. A higher proportion of unfavorable clinical and biological features (and shorter survival) separates patients whose metastases are distant or both distant and local, from patients with isolated local relapses [7]. Response rates can be objectively higher if bone marrow analysis or [^{123}I]-meta--iodobenzylguanidine (mIBG) scintigraphy can detect disease, as compared to detection with magnetic resonance imaging (MRI) or computed tomography [8–10]. Oligometastatic and widely disseminated disease also have different response rates. Time to relapse forms a third source of bias. The median time to first HRNB relapse is 18–19 months from diagnosis [11,12]. Time to subsequent disease relapse episodes become progressively shorter (8.7 to second, 3.8 months to third), presumably reflecting an ongoing acquisition of chemoresistance within residual neuroblastoma cells. The relationship between time to first relapse and subsequent survival is complex [10], but relapses occurring nearer to diagnosis are generally associated with shorter survival [13]. Early relapse in patients with International Neuroblastoma Staging System (INSS) stage 4 *MYCN*-amplified disease was clearly associated with worse outcome [10]. Patient age and molecular tumor characteristics present a fourth source of bias. Older patients have a worse outcome [14]. Tumors harboring *MYCN* amplifications [15], activating *ALK* alterations [16] or *ATRX* mutations [17] are associated with worse and varied survival times at relapse. Prior and subsequent therapy adds a fifth source of bias, and is difficult to control for in non-randomized phase II efficacy trials and in the absence of a homogenous umbrella treatment protocol for relapsed HRNB. Not all pediatric oncology centers use high-dose chemotherapy and autologous stem cell rescue or immunotherapy in HRNB first-line treatment. HRNB therapy variations may influence responses to relapse therapy. Some published relapse trials continued until disease progressed and others were designed to test for response within a predefined therapy cycle number, after which different consolidation therapies were administered. The subsequent therapy is not standardized, and often not even documented. Survival is the clearest way to directly compare efficacy in different trials, but is thwarted in these cases. The varying treatment strategies administered after the investigative treatment prevent using time to progression as an endpoint. Response is currently the only suitable outcome measure, but may not equate to survival. Altogether, consideration must be given to the lack of comparability of existing clinical trial results and lack of generalizability to the individual patient [4,10]. Despite the effort that has been undertaken to clearly define clinical and biological features predictive of survival in relapsed high-risk neuroblastoma [2,13,18,19], no validated prognostic stratification exists to date. While no definitive long-term survivor subgroup can currently be defined, the proposed strategy remains the default strategy at first relapse. Whether this approach is curative in the long term, can only be ascertained with longer follow-up for each individual patient. Some clinical circumstances of note in which this approach may be reconsidered are very rapidly progressing disease, patients with a severely compromised general condition (e.g., low Lansky performance score) and/or poor response to initial salvage therapy. This paper represents an expert consensus developed through review of the available literature and collaborative discussion among SIOPEN specialists.

2. Diagnosing relapse and defining response

The high diagnostic sensitivity and specificity achievable with [^{123}I]-mIBG scintigraphy [20,21] and previously limited prospects for biopsy-informed curative therapy has historically made it uncommon to collect biopsies from the relapsed tumor and/or metastases. The awareness that tumors evolve between diagnosis and relapse [22–24], the increased spectrum of actionable mutations in relapsed tumors [22,24], the spatiotemporal heterogeneity in mutations observed in individual patients [25–27] and the recently described plasticity in transcriptional circuitries [28,29] all provide strong arguments that molecular profiling at relapse will better inform treatment decisions. A biopsy at relapse with in-depth molecular characterization is strongly recommended to confirm viable active neuroblastoma and identify potential targets for biomarker-based targeted treatment or immunotherapy. Bone marrow infiltration should be routinely assessed at relapse from different sites (cytology and GD2 immunocytology in aspirates, immunohistochemistry in trephine biopies). A new level of

molecular precision in characterization is offered by recent developments in single-cell technologies. Rapidly developing liquid biopsy approaches offer minimally invasive procedures allowing circulating cell-free tumor DNA and RNA profiling [30–35] for more precise and longitudinal monitoring of tumor evolution, resistance mechanisms and therapeutic response biomarkers in the future.

To appropriately assess response, imaging diagnostics should be ideally set at predefined time points (e.g. the first after 2 re-induction cycles) and include [^{123}I]-mIBG scintigraphy or FDG-PET-CT/MRI scans (for non-MIBG-avid lesions) and MRI (primary tumor site ±whole body and brain) or at least CT of all tumor regions. Response to therapy after relapse is evaluated using uniform response metrics for patients with relapsed neuroblastoma [36] that were adapted from the International Neuroblastoma Response Criteria for primary disease [37]. These uniform response metrics will facilitate comparable data collection across international trials and promote more rapid identification of effective treatments for relapsed HRNB [38].

3. Strategies for re-induction therapy

Re-induction therapy aims to achieve a second complete or partial remission. Approaches for relapsed/refractory HRNB have historically added either cytotoxic chemotherapy or the systemic radiopharmaceutical, ^{131}I-mIBG. A questionnaire in SIOPEN member countries revealed considerable heterogeneity in chemotherapy and other regimens currently offered at first HRNB relapse. Patients were often enrolled in the randomized phase II BEACON (NCT02308527) or RIST-rNB-2011 (NCT01467986) trials. Widely used were also ^{131}I-mIBG (combination) therapy and/or 8 different cytostatic drug combinations, partly based on limited, rather historical clinical trial evidence for patients with relapsed HRNB. Patients were sometimes enrolled on different early clinical trials (biomarker- and nonbiomarker-based), or given 3 chemo-immunotherapies or single-agent therapy with an ALK inhibitor (biomarker-based) or temozolomide (nonbiomarker-based). Several previous reviews of HRNB relapse therapy summarize the rationale and data for various chemotherapeutic approaches [4,10,38]. However, as we state in *Section 1*, heterogeneous inclusion criteria and prior/-subsequent treatment prevents a definitive determination of the most effective re-induction strategy [4].

3.1. Established chemotherapy combinations

Camptothecins, topotecan and irinotecan, typically form the cytotoxic chemotherapy backbone [10]. Camptothecins ultimately lead to apoptosis by targeting the topoisomerase I enzyme to stabilize the DNA-bound enzyme form, and cause double-strand DNA breaks during replication [39]. This mechanism of action is distinct from those of chemotherapy agents typically used in first-line therapy, making them particularly attractive for relapse treatment. Both topotecan and irinotecan demonstrated single-agent activity in phase I trials in patients with neuroblastoma [40–42], but results from subsequent phase II trials were rather disappointing. Topotecan as a single agent only achieved 0–10 % overall response rates (ORR; complete, very good partial and partial responses) against neuroblastoma [43–46]. The equivalent ORR for irinotecan as a single agent was 0–14 % [47–49]. A phase I trial administering topotecan and irinotecan showed unacceptable toxicity [50], preventing further development of this combination.

3.1.1. Topotecan combinations

One of the best-characterized combinations is topotecan with the alkylating agent, cyclophosphamide (**TopoCy**) [51]. This pair achieved synergistic cytotoxicity in preclinical studies [52,53], in part through topoisomerase I upregulation by cyclophosphamide. Reported retrospective single-center experiences [51] as well as phase I and II trials confirmed that heavily pretreated patients tolerated the combination with reversible myelosuppression as the only significant toxicity [52–54]. The initial phase II trial used 250 mg/m^2/d cyclophosphamide and 0.75 mg/m^2/d topotecan for 5d every 21d and achieved an ORR of 46 % (6/13 patients with refractory/relapsed HRNB) [53]. This schedule was subsequently tested for refractory/relapsed HRNB in the large, randomized COG 9462 trial [55], and compared against single-agent topotecan (2 mg/m^2/d for 5d every 21d). While TopoCy achieved a 32 % ORR, compared with 19 % for topotecan alone, OS and toxicity did not differ between the treatment arms [55].

Combining high doses of cyclophosphamide (4200 mg/m^2/course) and topotecan (2 mg/m^2/d for 4d) with vincristine (2 mg/m^2; termed **HD-CTV**) achieved a 19 % ORR in patients with refractory HRNB and a 52 % ORR in patients with first HRNB relapse [6]. Response rates were lower for patients with progressive disease (at trial entry) or refractory disease in adults [6]. Combining TopoCy with the topoisomerase II inhibitor, etoposide (termed **TCE**), achieved a 61 % ORR in 31 patients with relapsed HRNB [56], while combining only topotecan and etoposide achieved a 47 % ORR in 36 patients with relapsed HRNB [57].

A phase II European ITCC trial using topotecan in combination with temozolomide (**TOTEM**) against relapsed/refractory neuroblastoma and other pediatric solid malignancies achieved an ORR of 21 % in 38 patients with refractory/relapsed HRNB [58]. **TVD** is the other well-established topotecan-containing regimen. It combines topotecan (1.5 mg/m^2/d for 5 d) with a 48 h infusion of doxorubicin (45 mg/m^2) and vincristine (2 mg/m^2) that are repeated every 21–28d. The initial phase II trial in 25 patients with refractory/relapsed HRNB achieved a 64 % ORR and 4 complete responses [5]. The European HR-NBL1/SIOPEN trial adopted **TVD** as salvage therapy in patients failing to achieve at least partial metastatic responses after induction with rapid COJEC [59].

3.1.2. Irinotecan combinations

Irinotecan is typically administered over an extended schedule to maximize exposure of tumor cells in the cell cycle S-phase [60,61]. The major toxicity of irinotecan is diarrhea, which can be treated by administration of loperamide and a cephalosporin [62]. The best characterized irinotecan combination is with the methylating agent, temozolomide, and termed **IT** or **TEMIRI**. Preclinical studies in neuroblastoma xenograft models confirmed drug synergy based on a model in which temozolomide-induced DNA methylation leads to irinotecan recruitment [61].

A single-institution trial administered 50 mg/m^2/d intravenous irinotecan and 150 mg/m^2/d oral temozolomide over 5d to achieve 2 complete responses among 19 patients with refractory disease and 1 partial response among 17 patients with progressive disease at trial entry [63]. Although the ORR was only 8.3 %, all patients with refractory disease showed some evidence of clinical benefit (7 mixed responses; stable disease in 10) [63]. Toxicities included the expected diarrhea and myelosuppression [63]. The subsequent COG multicenter ANBL0421 trial (NCT00311584) applied lower doses (10 mg/m^2/d irinotecan for 5d/week for 2 weeks; 100 mg/m^2/d temozolomide for 5d/week for 1 week; every 3 weeks) and achieved a 15 % ORR in the 55 enrolled patients [8]. Stable disease was achieved in 53 % of patients [8]. Importantly, TEMIRI therapy achieved stable disease or a partial or complete remission in 14/21 patients who had previously received topotecan, suggesting TEMIRI remains a useful salvage regimen even for patients previously treated with topotecan [8]. TEMIRI has the advantage of being less myelotoxic than **TopoCy** [10]. Although similar frequencies of neutropenia (35–45 %) were reported in relevant phase II trials, Grade 3 or 4 thrombocytopenia was less frequent with TEMIRI (13 %) than TopoCy (60 %) [8,55]. The New Approaches to Neuroblastoma Therapy (NANT) consortium has established 60 mg/m^2/d irinotecan 5d/week for 2 weeks and 75 mg/m^2/d temozolomide 5d/week for 1 week (both oral) every 3 weeks as the recommended phase II doses [60]. The largest randomized trial conducted to date for relapsed/refractory neuroblastoma (n = 160) is the European SIOPEN/ITCC BEACON phase II trial (NCT02308527), which evaluated three

backbone chemotherapy regimens and bevacizumab addition to reduce angiogenesis [64]. The trial included a randomization designed to test whether adding bevacizumab to IT increases the response rate compared with IT alone. BEACON trial results demonstrated that combining bevacizumab and IT improved response rate (23 % ORR) and progression-free survival (1-year PFS 67 %) in patients with refractory/relapsed HRNB [64]. IT (TEMIRI) is currently regarded internationally as an excellent backbone for future studies of new agents.

The randomized phase II RIST-rNB-2011 trial (NCT01467986) evaluated intravenous irinotecan (50 mg/m^2/dose) and oral temozolomide (150 mg/m^2/dose) given 5 days, followed by 2 rest days (IT control arm) compared to a combination of IT with oral dasatinib and rapamycin (**RIST** experimental arm; 4d followed by 3 rest days prior to IT). RIST-rNB-2011 enrolled 129 patients with relapsed (80 %) or refractory (19 %) HRNB [65]. After a median 72-month follow-up, the median PFS was 11 months in the RIST experimental arm and only 5 months in the IT control arm. At one year, RIST achieved 38 % (13 % with IT) EFS in patients with *MYCN*-amplified disease and 65 % EFS (44 % with IT) against cases lacking *MYCN* amplifications. The best ORR with RIST was 67 % (56 % with IT) in cases lacking *MYCN* amplifications and 62 % with RIST (42 % with IT) in the *MYCN*-amplified subgroup. Median OS in the RIST arm was 20 months (IT control arm: 16 months). RIST achieved an OS of 11 months in *MYCN*-amplified cases compared to only 6.5 months in the IT control arm. Rapamycin or dasatinib added no additional toxicity to the IT backbone [65]. RIST-rNB-2011 provided the first evidence that *MYCN*-amplified HRNB can be effectively treated, and demonstrated RIST is an interesting re-induction strategy for this molecularly defined patient subgroup.

The open-label, randomized, phase II selection design in the Children's Oncology Group (COG) ANBL1221 trial (NCT01767194) tested adding temsirolimus to irinotecan–temozolomide treatment in patients with relapsed or refractory neuroblastoma [66]. Patients received oral temozolomide (100 mg/m2 per dose) and intravenous irinotecan (50 mg/m2 per dose) on days 1–5 of 21-day cycles. Patients in the temsirolimus group also received intravenous temsirolimus (35 mg/m2 per dose) on cycle days 1 and 8. Patients received a maximum 17 treatment cycles. The irinotecan–temozolomide–temsirolimus combination did not meet the minimum activity requirement set by the activity design in ANBL1221 [66]. Notably, temsirolimus dosage and application in addition to the overall trial design strongly differed in the ANBL1221 and RIST trials. Thus, data from the two trials cannot be directly compared. The irinotecan-temozolomide-temsirolimus therapy arm only included 5 patients with *MYCN*-amplified disease (29 %) [66]. Independent of the trial design differences, sample size may also have been too small to detect particular benefit for *MYCN*-amplified disease, which the RIST trial reported [65].

3.1.3. ICE

The Memorial Sloan Kettering Cancer Center reported their single-center experience treating 74 patients with relapsed/refractory HRNB with ICE, reviewed together with published reports of relapsed disease treated with ICE [67]. **ICE** combines high-dose ifosfamide (2g/m^2; d1–5), carboplatin (500 mg/m^2; d1–2) and etoposide (100 mg/m^2; d1–5) [10]. Patients predicted to have poor hematological reserves received a preemptive peripheral blood stem cell rescue 72 h after ICE [67]. Overall responses were observed in 9/17 patients with first relapse (ORR 53 %) and 4/26 patients with refractory disease (ORR 15.4 %). ORR was lower (1/34) in patients with progressive disease, although 22/34 achieved stable disease. ICE was associated with predicted grade 4 myelotoxicity, and bacteremia was detected in 26 % of patients [67]. ICE is another potential rescue therapy particularly for patients with stored peripheral blood stem cells.

3.2. Chemo-immunotherapy

3.2.1. Irinotecan and temozolomide combined with dinutuximab plus GM-CSF

The COG phase II ANBL1221 trial (NCT01767194) randomly assigned patients with refractory/relapsed HRNB to receive TEMIRI with temsirolimus (**IT/TEM** arm) or dinutuximab plus granulocyte-macrophage colony-stimulating factor (GM-CSF, **IT/DIN** arm). Patients in both arms received oral temozolomide (100 mg/m^2/dose) and intravenous irinotecan (50 mg/m^2/dose) on d1–5 of 21-day cycles. Temsirolimus (35 mg/m^2/dose) was intravenously administered on days 1 and 8. DIN (17.5 or 25 mg/m^2/day) was intravenously administered on days 2–5 [66]. Myelosuppression, elevated alanine aminotransferase and hypokalemia were the most common toxicities ($\geq$ grade 3) in patients treated in the IT/TEM arm, with IT/DIN arm patients most commonly experiencing pain, hypokalemia, myelotoxicity, fever/-infection and hypoxia [66]. At interim analysis in 36 patients, the IT/DIN arm had a 53 % ORR, compared with 6 % in the IT/TEM arm [66]. This was lower than previously reported for IT alone, suggesting temsirolimus might be antagonistic in this combination. The protocol enrolled an additional 36 patients to the IT/DIN arm, after which the ORR for all patients was 41.5 % [68]. Patients who received prior anti-GD2 therapy also responded to IT/DIN therapy. IT/DIN therapy achieved 67.9 % PFS and 84.9 % OS in patients at one year [68]. Specific immunogenotypes influencing natural killer cell activity were evaluated in ANBL1221 trial participants, and median CD161, CD56 and KIR values did not associate with therapy response in logistic regression models [69]. A retrospective study reported similar results for 146 patients, who had received $\geq$1 IT/DIN/GM-CSF cycle for relapsed or progressive HRNB [70]. The promising results from ANBL1221 changed the paradigm of treatment for patients with a first HRNB relapse.

3.2.2. Irinotecan and temozolomide combined with dinutuximab beta

Dinutuximab beta (**DB**), a GD2 antibody developed in Europe and approved by the EMA in 2017 to treat HRNB, shares the DIN (formerly: ch14.18) protein sequence. However, glycosylation patterns differ greatly between DB and DIN. DB is manufactured in Chinese hamster ovary cells, while DIN is manufactured in murine SP2/0 cells. The altered glycosylation translates into significantly higher antibody-dependent cellular cytotoxicity from DB, as compared to DIN. Furthermore, DB lacks the alpha-gal epitope that is typical for glycoprotein expression in murine SP2/0 cells [71]. The alpha-gal epitope is known to trigger allergic reactions [72] Thus, while the protein sequences remain the same, DB and DIN are structurally and actionably different monoclonal antibodies.

Treating relapsed/refractory HRNB with IT-DB was evaluated retrospectively in 2 patient cohorts [73,74]. IT-DB achieved a 63 % ORR in a 19-patient cohort [74] and a 64 % ORR in a 25-patient cohort, in whom 14 patients had received DB as part of their previous treatment (mostly frontline treatment, post-consolidation phase) [73]. Interestingly, 4 of the 14 patients previously treated with DB achieved a complete remission, while 6 of these patients achieved partial responses (ORR 71 %) [73]. A 32 % complete remission rate was achieved in both cohorts [73,74]. The dosage of dinutuximab beta administered differs in the two studies (50 mg DB/m^2/cycle in 5 days [73] versus 100 mg DB/m^2/cycle in 10 days [74]). IT-DB re-induction therapy was well-tolerated even in patients who were heavily pretreated or had previously received DB. DB dosing into 21-day cycles needs adaptation based on its 8-day half-life to avoid antibody accumulation. The cumulative dose of 50 mg DB/m^2/cycle as a 5-day continuous infusion [73] contrast to the 100 mg DB/m^2/cycle as a 10-day continuous infusion in a 35-day interval used in frontline maintenance [75]. Altogether, significant anti-tumor activity is achieved by combining the IT backbone with DIN plus GM-CSF treatment or DB, with more than half of patients showing objective responses. While the mechanism of action remains to be elucidated and predictors of response to chemo-immunotherapy have

yet to be defined, both regimens have been transferred to first-line therapy and are being evaluated in current COG and SIOPEN clinical trials. This transfer is supported by recent real-world data from the SACHA-France study (NCT04477681), which reported objective responses in 38 % of patients with progressive disease before high-dose chemotherapy [76]. Both regimens are also now widely used to treat patients at first relapse or when refractory HRNB is declared. IT-DB can be considered as one of the best current options for re-induction therapy.

3.2.3. Topotecan and temozolomide combined with dinutuximab beta

The **SIOPEN/ITCC BEACON trial** (NCT02308527) was amended to evaluate whether adding DB to chemotherapy improves efficacy, thus being one of few randomized trials of chemo-immunotherapy to date. Patients aged 1–21 years with refractory or relapsed HRNB were randomized for chemotherapy alone or with DB (1:2 ratio). **BEACON-Immuno** administered 70 mg dinutuximab beta/m^2/cycle over 7d. The factorial design in BEACON allowed some patients to initially be randomized between chemotherapy regimens (temozolomide versus temozolomide-topotecan). This randomization closed soon after DB randomization opened, and all patients subsequently received topotecan-temozolomide. Crossover to DB with topotecan and cyclophosphamide was allowed for patients randomized to chemotherapy alone who experienced disease progression (n = 12). In total, 65 patients were randomized to chemotherapy alone (temozolomide, n = 3; topotecan-temozolomide, n = 19) or with DB (temozolomide-DB, n = 6; topotecan-temozolomide-DB, n = 37). Chemo-immunotherapy achieved 35 % ORR and 57 % 1-year PFS (chemotherapy only: 18 % ORR, 27 % 1-year PFS) in BEACON patients [77]. OS did not differ between the arms [77]. Grade $\geq$ 3 toxicities occurred in 9 patients (chemotherapy alone, 41 %) compared to 13 patients receiving chemo-immunotherapy (30 %), demonstrating that chemo-immunotherapy was well-tolerated [77]. The main randomization in the subsequent BEACON-2 trial (EuCT: 2024–516115–24–00) will focus in tier 1 on comparing the efficacy and safety of IT-DB (arm A) versus bevacizumab-IT (arm B) followed by dose expansion and confirmation in tier 2, which will explore the potential additive effects of combining bevacizumab and DB to the IT chemotherapy backbone.

3.2.4. N5 and N6 GPOH chemotherapy combined with dinutuximab beta

The GPOH induction chemotherapy cycles, **N5** (cisplatin, etoposide, vindesine) and **N6** (vincristine, dacarbazine, ifosfamide, doxorubicin), were combined with long-term DB infusion through compassionate use to treat 25 patients with refractory/relapsed HRNB, who had previously failed one or more second-line therapies (**N5/DB**; **N6/DB**) [78]. Retrospective data analysis revealed no unexpected severe toxicities. Grade 3/4 pain was reported by 4/25 patients in cycle 1, but decreased to 0/9 patients in cycles 3 and 4. Combination with long-term DB infusion achieved a 48 % ORR (12/25 patients, 3 patients with minor responses) with an estimated 27 % EFS and 44 % OS at one year [78]. Based on the encouraging ORR in heavily pretreated patients and acceptable safety profile, this approach is currently being evaluated as salvage therapy (NCT06485947).

3.2.5. Irinotecan, temozolomide and naxitamab plus GM-CSF

Naxitamab (formerly: humanized 3f8) is a humanized GD2–binding monoclonal antibody [79] approved by the FDA for use in combination with GM-CSF to treat patients, who have achieved a partial or minor response to prior therapy or have stable refractory/relapsed HRNB limited to the bone or bone marrow. Accelerated FDA approval was based on interim data from the global clinical phase II **Trial 201** (NCT03363373) and the phase I/II **Trial 12–230** (NCT01757626) [80–82]. NCT03363373 was designed to evaluate the efficacy and safety of naxitamab plus GM-CSF as salvage therapy for patients with primary refractory HRNB or incomplete response in the bone and/or bone marrow to salvage treatment. In total, 74 patients received naxitamab (3 mg/kg/d, d1,3,5) and GM-CSF (d −4–5) every 4 weeks until a complete or partial response was achieved followed by 5 additional cycles every 4 weeks [82]. ORR, as specified by the 2017 International Neuroblastoma Response Criteria [36], was the primary endpoint for the prespecified interim analysis. Patients achieved complete (38 %) and partial (12 %) remissions, with a 50 % ORR, and 93 % OS and 35 % PFS at one year (secondary endpoints). Naxitamab-related grade 3 adverse events included hypotension (58 %) and pain (54 %). NCT03363373 inclusion and exclusion criteria as well as patient population characteristics differed in several aspects from previous clinical trials evaluating DIN or DB. Patients with progressive disease at trial entry were excluded, and disease was limited to the bone/bone marrow. The percentage of *MYCN*-amplified cases (prognostically worse) was lower. Only 27 % of patients had received prior high-dose chemotherapy with stem cell rescue and only 25 % had received prior immunotherapy [82]. These differences are positive selection biases that might lead to overestimating the efficacy of naxitamab plus GM-CSF, if extrapolated to a non-selected population of patients with refractory/relapsed HRNB. A phase II clinical trial of **IT-naxitamab plus GM-CSF** is currently enrolling in Asia and Russia (NCT04560166).

Recently, PFS and ORR were compared in a systematic literature review including patients treated with naxitamab (NCT03363373; n = 52; NCT01757626, n = 38) or DB (NCT02300547; NCT02743429, n = 77) [83]. The DB population was adjusted for sex, *MYCN* amplification and disease site (bone/bone marrow) to balance the aggregated characteristics of the naxitamab population. Compared to naxitamab, DB significantly extended PFS (hazard ratio 0.47; p = 0.015), with an ORR of 60.1 % (ORR, 43.3 % in the naximatab population) in this indirect comparison [83].

3.2.6. Other chemo-immunotherapy combinations

The COG ANBL1821 trial for refractory/relapsed HRNB (NCT03794349) has evaluated the ornithine decarboxylase 1 (ODC1) inhibitor, eflornithine (also known as α-difluoromethylornithine, DFMO), in combination with the IT/DIN/GM-CSF treatment protocol from the COG ANBL1221 trial. Eflornithine is proposed to act by irreversibly inhibiting ODC1, the rate-limiting enzyme in polyamine biosynthesis (see also Table 2). Preclinical data have shown that inhibiting ODC1 enhances tumor cell stress, chemotherapy efficacy and immune modulatory effects [84–86]. Patients with refractory/relapsed HRNB were randomized in ANBL1821 to DIN, irinotecan, temozolomide and GM-CSF without (arm A; n = 44 patients) or with eflornithine (6750 mg/m^2 divided into 3 times/d; arm B; n = 47 patients). Response rates did not differ between the trial arms (arm A: 61.4 %, 27/44; arm B: 57.4 %, 27/47; p = 0.566). Adding eflornithine to chemo-immunotherapy also did not improve 1-year-OS (arm A: 87.0 ± 5.7 %, arm B: 81.4 ± 6.3 %), and was associated with an increased incidence of hearing loss [87].

3.3. Chemotherapy backbone combined with molecular targeted therapy

Lorlatinib was combined with topotecan-cyclophosphamide (NCT03107988) in children (<18 years) with ALK-driven relapsed/refractory HR-NB neuroblastoma [88]. The recommended phase II dose was 115 mg/m2 [88] and hypertriglyceridemia, hypercholesterolemia and weight gain were common adverse events [88]. Adding lorlatinib achieved complete/partial/minor responses in 63 % of patients [88], supporting usage in the relapsed/refractory setting and suggesting lorlatinib should be exploited in phase III clinical trials for newly diagnosed ALK-driven HRNB. Several other interesting combinations are not yet considered for re-induction treatment of first relapse, but have been administered for second or subsequent HRNB relapses (Table 1).

3.4. Conclusions: Strategy for re-induction therapy

The consensus is always to prioritize participation into clinical trials, such as BEACON2. When recruiting trials are unavailable, chemo-

Table 1
Chemotherapy backbone combined with molecular targeted therapy for 2nd or subsequent HRNB relapses.

Combination therapy	Selected examples with ClinicalTrials.gov ID and sponsor	Key considerations
Irinotecan and adavosertib (AZD1775)	NCT02095132COG phase II ADVL1312 clinical trial	Irinotecan with adavosertib (WEE1 G2 checkpoint kinase inhibitor) was well tolerated with no dose-limiting toxicities at the recommended phase II dose administered orally for 5d every 21d [89]. Objective responses were documented for 3 of the 20 patients.
Irinotecan, temozolomide and alisertib (MLN8237)	NCT01601535NANT consortium	Alisertib is a selective and potent oral aurora kinase A inhibitor (indirectly targets MYCN) that induces cell cycle arrest and apoptosis in preclinical NB models [90,91]. The COG ADVL0921 phase I trial treated patients with HRNB or other solid tumors with single-agent alisertib, documenting ORR $< 5\,\%$ [92]. The 2-stage NCT01601535 phase II trial achieved a 21 % partial response rate (19 evaluable patients) using alisertib in combination with irinotecan and temozolomide in 20 patients with r/r HRNB [93]. The estimated PFS at 1 year was 34 %. *MYCN* amplification was associated with inferior PFS. Hematological toxicities were the most common adverse events.
Topotecan, cyclophosphamide and nifurtimox	NCT00601003Giselle Sholler	The nitrofuran, Nifurtimox, has been employed > 50 years to treat Chagas disease, a parasitic infection caused by *Trypanosoma cruzi* [94]. Nifurti- mox demonstrated antitumor activity in preclinical models for pediatric cancers, including HRNB [95,96]. The phase II NCT00601003 trial achieved a 53.9 % ORR against first HRNB relapse (stratum 1) and a 16.3 % ORR against multiple r/r HRNB (stratum 2) using nifurtimox combined with topotecan and cyclophosphamide [97]. BM suppression and reversible neurological complications were the most common side effects.

Abbreviations: BM, bone marrow; NANT, new approaches to neuroblastoma therapy; HRNB, high-risk neuroblastoma; ORR, overall response rate; NB, neuroblastoma; PFS, progression-free survival; r/r, refractory or relapsed.

immunotherapy (IT chemotherapy + GD2-targeting immunotherapy, DB currently the most available in Europe and SIOPEN countries) is the first choice to start re-induction therapy for first HRNB relapse based on current evidence. Further options are **RIST** for relapsed *MYCN*-amplified HRNB or chemotherapy combined with ALK inhibitor for ALK-driven HRNB (Figure 1). Consolidation treatment followed by maintenance treatment (both preferably in clinical trials) should be subsequently planned for patients who achieved ORR to re-induction therapy to ensure improved survival (Figure 1). The **TVD**, **TCE**, **ICE, TopoCy** or **[^{131}I] -mIBG therapy plus vorinostat** (for patients with MIBG-avid disease) combinations are currently potential salvage options for patients with only minor responses to re-induction therapy and/or stable or progressive disease during or at the end of re-induction. Early clinical trials, preferably biomarker-based and with targeted treatment combinations, are also a suitable option in this setting.

4. Consolidation strategy

Consolidation therapy aims to eliminate (minimal) residual disease. Appropriate re-induction therapy can achieve complete responses against refractory/relapsed HRNB, but responses often do not persist. Novel therapy strategies applied as consolidation treatment (or as a main,tenance strategy) may provide more durable responses. Since re-induction treatment also influences EFS and OS, assessing the efficacy of consolidation and maintenance therapies is difficult. A traditional option for consolidation (after a partial or complete response to re-induction therapy) has been [^{131}I]-mIBG therapy. In recent years, immunotherapy approaches have shown potential to precisely target disease with improved toxicity profiles. Cellular therapies that harness the immune system are of high interest as a consolidation strategy for relapsed HRNB.

4.1. Haploidentical stem cell transplantation with dinutuximab beta and low-dose interleukin 2

Haploidentical hematopoietic stem cell transplantation (**haploSCT**) is designed to harness a potential graft-versus-tumor effect, provide the patient with a new healthy immune system and provide direct antitumor effects through intense conditioning chemotherapy. Strong evidence supports T/B cell-depleted haploSCT in combination with DB and subcutaneous interleukin 2 as an efficient consolidation strategy. The safety, feasibility and outcomes of this approach was assessed in a phase I/II trial (NCT02258815) for patients with relapsed HRNB [98]. Treatment success (primary end point) was met by 54.4 % of patients (37/68; median observation=7.8 years; 5-year EFS=43 %, 5-year OS=53 % from trial treatment start), and described patients who received 6 DB cycles, were alive without progressive disease 180d after trial treatment ended and experienced no unacceptable toxicity or higher grade acute or chronic graft-versus-host-disease. Five-year EFS was better among patients achieving complete (52 %) or partial (44 %) responses by re-induction therapy prior to haploSCT compared to patients with no or mixed responses or progressive disease (13 %). For the 43 patients with evidence of disease after haploSCT, 35 % achieved complete remission (15/43 patients) and the ORR was 51 % (22/43 patients) [98]. This concept was deemed feasible for patients with refractory/relapsed HRNB, having only a low risk of graft-versus-host disease or severe viral infections. These promising results with long-term remissions are likely attributable to increased anti-HRNB activity by donor-derived effector cells. In Germany and other European countries, only centers with strong haploSCT expertise use it as part of routine care, otherwise it is not widely used. The haploSCT regimen warrants further investigation in a randomized consolidation trial for patients with relapsed HRNB, who achieved at least partial responses to re-induction therapy.

Functional immune monitoring was employed to gain a better understanding of synergy between haploSCT and DB [99]. Monitoring detected highly functional NK cells capable of antibody-dependent

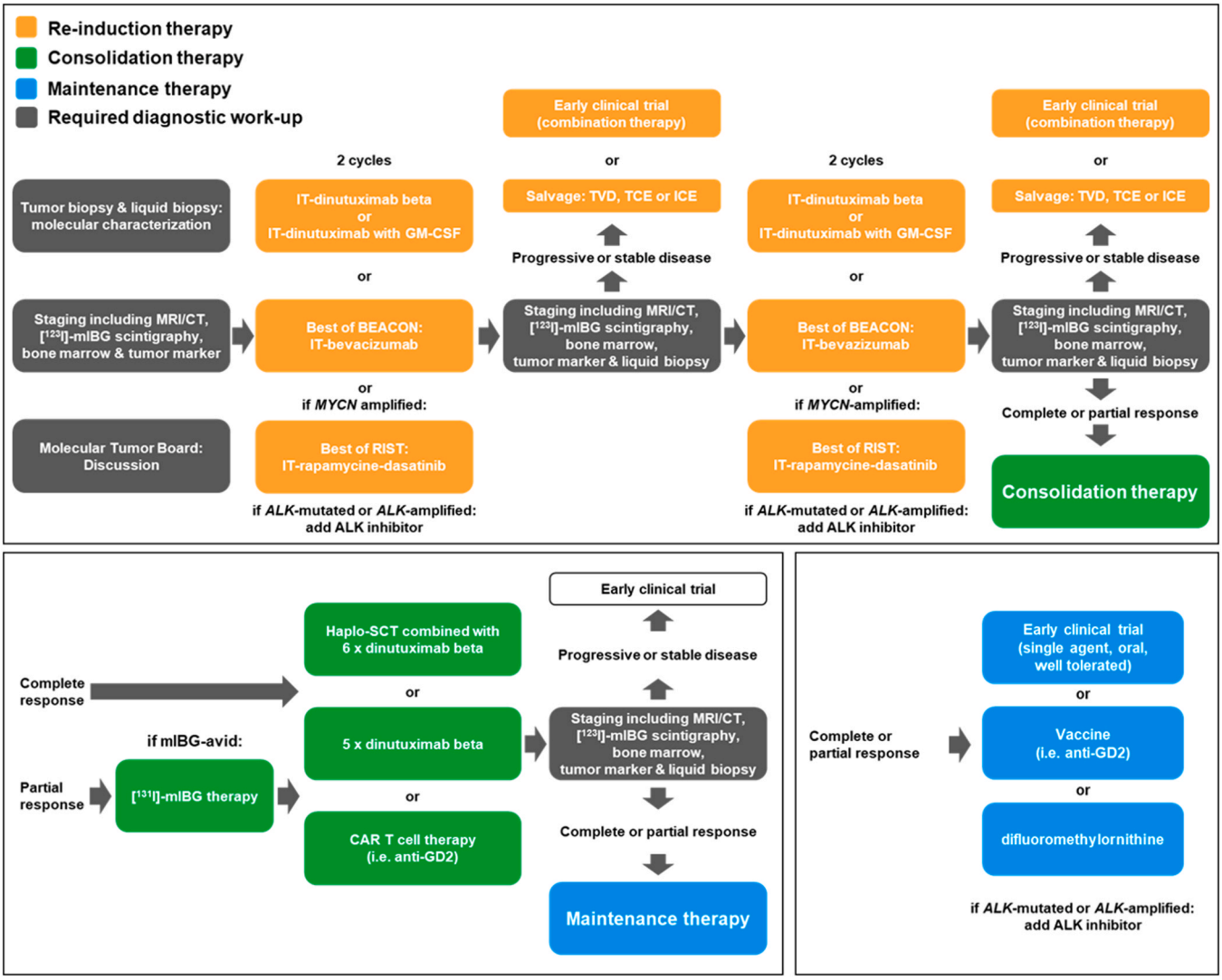

Fig. 1. Current diagnostics and treatment scheme for patients with high-risk neuroblastoma suspected of a first relapse. No clear consensus exists about the optimal therapy due to the lack of umbrella trials covering all phases of relapse treatment (re-induction, consolidation, maintenance) in a homogenous strategy. The required work-up at relapse includes a tumor biopsy with in-depth molecular characterization, bone marrow diagnostics, liquid biopsy and multimodal imaging, and discussion in a molecular tumor board where molecular profiling has been performed and identifies potentially actionable alterations. Patient enrollment into trials in all phases of relapse treatment (re-induction, consolidation, maintenance) should be given preference as first choices when open to recruitment. An ALK inhibitor may be added to a treatment backbone if an ALK mutation or amplification is verified in the relapse sample. No data currently exist that define the optimal backbone or timing to incorporate an ALK inhibitor during relapse treatment. In clinical trials, ALK inhibitors have only been evaluated alone or in combination with standard chemotherapy. Vaccines in the maintenance phase have demonstrated immune responses, but not evidence of efficacy as yet. **ALK**, anaplastic lymphoma kinase; **CAR**, chimeric antigen receptor; **CT**, computed tomography; **GD2**, disialoganglioside 2; **GM-CSF**, granulocyte-macrophage colony-stimulating factor; **ICE**, ifosfamide, carboplatin, etoposide; **IT**, irinotecan/temozolomide chemotherapy backbone; **mIBG**, meta-iodobenzylguanidine; **MRI**, magnetic resonance imaging; **TCE**, topotecan, cyclophosphamide, etoposide; **TVD**, topotecan, vincristine, doxorubicin; **SCT**, stem cell transplantation. **BEACON** refers to the NCT02308527 trial; **RIST**, refers to the NCT01467986 RIST-rNB-2011trial.

cytotoxicity, a relevant DB mechanism of action. Degranulation in NK cell subsets indicated a significant NK cell response induced by DB. Complement-dependent cytotoxicity was shown to be a potent effector cell-independent mechanism to lyse tumor cells. During DB therapy, elevated proinflammatory cytokines and markers indicated a strong anti-GD2 immune response [99]. In-patient functional immune monitoring contributes to our understanding of anti-cancer combinatorial immunotherapy, and should be incorporated into future immunotherapy/cellular therapy trials.

4.2. *Dinutuximab beta long-term infusion*

Previous immunotherapy trials for neuroblastoma associated short-term DB infusions combined with isotretinoin and cytokines with inflammatory side effects and pain. Three trials evaluated long-term continuous DB infusion in patients with relapsed/refractory HRNB. In an open-label, single-arm phase II clinical trial (NCT02743429), patients with refractory/relapsed HRNB, who had not previously received DB therapy and had responded to second-line chemotherapies, received 100 mg/m^2 DB as continuous infusion over 10 days (long-term infusion, **LTI DB**) in 35-day intervals for up to five cycles [100]. Responses were evaluable in 38/40 patients, and the best response rate (secondary endpoint) was 53 % if minor responses were included (37 % ORR counting only partial/complete responses). Responses had a median duration 238d and **LTI DB** achieved 31 % PFS and 66 % OS at 3 years (secondary endpoints) [100]. A second multicenter trial (NCT01701479) achieved 56 % ORR in patients with refractory/relapsed HRNB using long-term DB infusion combined with IL2 and

isotretinoin (2-year EFS=56 %/OS=73 %) [101]. Patients previously treated with a GD2-targeting antibody were also ineligible for this trial. Two-year survival was greater in patients with high-affinity Fc-gamma receptor polymorphisms and high natural killer cell levels. Low-affinity Fc-gamma receptor polymorphisms and > 5 years of age were identified as independent risk factors [101]. In a randomized phase of this trial, 160 patients received up to 5 DB-LTI cycles (100mg/m^2) and oral isotretinoin (160mg/m^2) either with (81 patients) or without (79 patients) interleukin 2 [102]. Comparisons of EFS and OS indicated that IL2 does not add clinical benefit in this setting [102]. A third single-center trial (compassionate use program reviewed by the University of Greifswald medical ethics committee) achieved 47.7 % OS and 33.1 % PFS at 4 years and a best response of 40.5 % (15/37; 5 complete, 10 partial responses) in GD2-targeting immunotherapy naïve patients with refractory/relapsed HRNB using LTI DB (10x10mg/m^2, 24 h) combined with subcutaneous IL2 [103]. Survival of the entire cohort (53/53) and the relapsed patients (29/53) was significantly improved compared to historical controls [103]. The substantially improved treatment tolerance allowed outpatient treatment. Altogether, this dataset formed the core of the submission that led to EMA approval of DB for neuroblastoma.

4.3. Autologous CAR T cells

Genetically engineered autologous T cells present another promising approach for consolidation therapy. Stably transfecting these cells with a chimeric antigen receptor (CAR) redirects the patient's own cytotoxic T cells against tumor-associated antigens (e.g. GD2). CAR T cells combine the specificity of an antibody with T cell cytolytic capacity independent of the major histocompatibility complex (MHC) [104]. CAR T cells have the potential for increased potency and durability, and can cross the blood-brain barrier [105]. Antibodies generally only penetrate the central nervous system (CNS) if the blood brain barrier is disturbed. While still needing confirmation in clinical trials, CAR T cells may be able to combat relapsed HRNB involving the CNS (see also *Section 7*).

CAR T cells directed against different targets have already shown promise in early-phase trials (NCT00085930, NCT02761915, NCT02765243 [106–110]), achieving several objective responses in patients with relapsed/refractory HRNB. The immunosuppressive neuroblastoma microenvironment [111] creates challenges for CAR T cell penetration, persistence and potency [106–108]. These challenges and the paucity of neuroblastoma-specific targets [112] have caused CAR T cell efficacy to be less robust against refractory/relapsed HRNB so far, as compared to their success against hematological malignancies. Next-generation 14G2a-based CAR T cells demonstrated encouraging clinical efficacy against relapsed (n = 14) and refractory (n = 12) HRNB in a recent phase I/II clinical trial (NCT03373097) [113]. No dose-limiting toxicity was recorded in this trial evaluating 3 dosages of autologous, third-generation GD2-CAR T cells expressing the inducible caspase 9 suicide gene (GD2-CART01) in patients with refractory/relapsed HRNB [113]. The optimal recommended dosage was 10×10^6 CAR T cells/kg body weight. The GD2-targeting CAR T cells expanded *in vivo* and were detectable in peripheral blood in 26/27 patients up to 30 months after infusion (median persistence, 3 months; range, 1–30). Cytokine release syndrome occurred in 20/27 patients (74 %, mild in 19 patients). The suicide gene was activated, rapidly eliminating GD2-CART01 cells, in one patient. Among patients receiving the recommended dose, 17 children responded to treatment (ORR=63 %; 9 complete, 8 partial responses) with 60 % EFS and 36 % OS at 3 years. Patients with a low disease burden (SIOPEN score <= 7) showed a particular benefit in subgroup analysis [113]. NCT03373097 demonstrates the feasible and safe use of GD2-CART01 cells to treat refractory/relapsed HRNB, and showed promising efficacy, at least for patients with low disease burden. Next-generation cytokine-engineered CAR T cells to enhance effector function [114] are currently under investigation (EUCT 2022–501725–21–00), and bi-specific CAR T cells that simultaneously target GD2 and B7H3 are in preclinical development [115].

4.4. Novel approaches for consolidation therapy

4.4.1. Allogeneic CAR T cells

Allogeneic CAR T cells targeting GD2 (ALLO_GD2-CART01) could be a therapeutic option for relapsed/refractory HRNB that did not respond to autologous GD2-CART01 or in patients with profound lymphopenia [116]. ALLO_GD2-CART01 was administered to 5 children with HRNB refractory to > 3 different lines of therapy in a hospital exemption setting [116]. Four children had previously received allogeneic hematopoietic stem cell transplantation. All patients experienced grade 2 or 3 cytokine release syndrome, with one case of grade 2 neurotoxicity and moderate acute graft-versus-host disease in 4 patients. Treatment achieved 2 complete responses (1 maintained) [116]. Safety and efficacy of ALLO_GD2-CART01 against relapsed/refractory HRNB deserves further investigation.

4.4.2. GD2-targeting CAR-NKT cells

Vα24-invariant natural killer T cells (NKTs) also have anti-tumor properties that can be enhanced by CARs. Interim results from a first-in-human phase I trial (NCT03294954) of autologous NKTs co-expressing a GD2-targeting CAR with interleukin 15 (GD2-CAR.15) in 12 children with relapsed HRNB recently demonstrated feasibility (ORR: 25 %; 1 complete, 2 partial responses) with no dose-limiting toxicities and a grade 2 cytokine release syndrome in one patient resolved by tocilizumab [117]. The frequency of $CD62L^+$ NKTs in products correlated with CAR-NKT expansion in patients, and was higher in responders (n = 5; ORR or stable disease with reduction in tumor burden) than non-responders (n = 7) [117]. NCT03294954 shows NKTs are safe and can mediate objective responses in patients with relapsed HRNB.

4.4.3. Other Immunotherapy strategies

Given the limitations of passive antibody immunotherapy, particularly for bulky disease, many other immunological approaches are being developed and have yet to enter clinical trials. These include active immunization with anti-idiotype antibody; infusions of dendritic or NK cells, the immunostimulatory antibody, ipilimumab (anti-CTLA4) and checkpoint-inhibitor therapy with pembrolizumab or nivolumab. Whether these strategies form more efficient consolidation or maintenance relapse treatments remains to be elucidated.

4.5. Conclusions: Consolidation phase

DB currently holds a high level of evidence as an efficient consolidation strategy with good tolerability in patients without previous DB exposure. The use of haploSCT combined with DB is conceptually interesting to exploit a fresh immune system with KIR/KIR-L mismatch to drive the DB immune effect against neuroblastoma. CAR T cell therapy targeting GD2 is also a promising consolidation approach in patients with relapsed HRNB who achieved objective responses with previous re-induction therapies. Randomized multicenter trials for both options are needed (Figure 1).

5. Maintenance therapy

Maintenance therapy aims for long-term cure. For maintenance therapy to be well-suited to patients with refractory/relapsed HRNB, they must be feasible as outpatient treatments, have low toxicity and be well-tolerated. Preference should be given to well-tolerated oral treatments, including ALK inhibitors (for patients with ALK altered disease), temozolomide and eflornithine, although early clinical trials with single agents (preferably biomarker-based) or vaccine strategies appear promising (Figure 1; Table 2). Randomized trials of new options in the maintenance setting are needed if we are to achieve optimal care.

Table 2
Single-agent targeted therapies for potential usage in maintenance therapy.

Class of agent/target	*Selected examples* with ClinicalTrials.gov ID and sponsor	Key considerations
ALK inhibitors	*Ceritinib*NCT01742286; Novartis Pharmaceuticals*Alectinib*NCT05770037; Cancer Research UK*Lorlatinib*NCT03107988; NANT consortium	Activating mutations of the ALK tyrosine kinase occur in up to 21.5 % of primary sporadic NBs, with *ALK* amplifications in a further ~4 % of HRNBs [16,24,118] *ALK* mutation frequency increases in relapsed NBs [24]. NBs harbor *ALK* aberrations resistant to crizotinib [119] yet sensitive to 2nd and 3rd generation ALK inhibitors (ceritinib, alectinib, lorlatinib), which have improved inhibition and CNS penetrance compared to crizotinib, and have been evaluated as single agents with/without chemotherapy in biomarker-based phase I/II trials [88,120].
Aurora kinase inhibitors	*Alisertib*NCT01154816; COG	Clinical single-agent results in pediatric patients with r/r solid tumors or ALL were disappointing despite promising preclinical data [92].
ODC1 inhibitors	*Eflornithine (DFMO)*NCT02395666; Giselle Sholler	Results obtained as maintenance therapy for patients with HRNB in first-line therapy and compared to a historical control cohort [121] support additional investigations for eflornithine potential as relapse maintenance therapy.
Vaccines	*GD2/GD3 vaccine* NCT00911560; MSKCC*Anti-idiotype antibody ganglidiomab vaccine* Compassionate-use treatment; University Hospital Greifswald	Vaccines have demonstrated immu- ne responses, but not evidence of efficacy as yet. Findings provide an important basis for prospective clinical trial design. Whether the GD2/GD3 vaccine [122], ganglidio- mab [123] or vaccines against other targets are suitable approaches for maintenance therapy of patients with first HRNB relapses remains to be elucidated.

Abbreviations: ALK, anaplastic lymphoma kinase; ALL, acute lymphoblastic leukemia; COG, Children's Oncology Group; HRNB, high-risk neuroblastoma; MSKCC, Memorial Sloan Kettering Cancer Center; NANT, new approaches to neuroblastoma therapy; NB, neuroblastoma; r/r, refractory or relapsed.

6. Other treatment modalities

6.1. Surgery and radiotherapy

Surgery and radiotherapy (Table 3) should be incorporated to control local disease at first relapse, if feasible, similar to their use in first-line treatment. The role of radiotherapy in oligometastatic disease remains to be elucidated. In first-line therapy, complete resection of the primary tumor has been associated with improved overall survival (OS) [128,129]. In the relapse setting, however, the evidence is largely retrospective and based on small patient cohorts, with no large randomized studies specifically addressing the role of surgical resection after relapse. To date, no significant OS benefit has been demonstrated for isolated surgical intervention when comparing complete versus incomplete resection of abdominal relapses [130]. Nonetheless, retrospective data suggest that a strategy involving gross total resection or multiple surgical interventions as part of a multimodal treatment approach may be associated with improved OS, even in patients with osteomedullary metastases [130,131]. In patients with relapsed neuroblastoma surgical resection may be considered within a multimodal treatment approach [132]. Given the lack of prospective data, we recommend the systematic collection of prospective, standardized data on all surgical interventions after relapse, including extent of tumor resection, timing relative to systemic therapies, postoperative morbidity, local control, and survival outcomes (OS, EFS).

Table 3
Local radiotherapy concepts for first relapse of high-risk neuroblastoma.

Radiation source	Key considerations
External beam radiotherapy	External beam radiotherapy is equally important for local control of primary HRNBs at relapse or in first-line therapy. Symptomatic control in patients with r/r metastatic HRNB is an important additional consideration. External beam radiotherapy can help control soft-tissue lesions [10,124], especially near the spinal cord, and provide relief from painful bone metastases [125]. Feasibility has been demonstrated in the first-line setting [126]. Whether radiotherapy is beneficial to control oligometastatic lesions in patients with relapsed HRNB remains to be determined.
Proton beam radiotherapy	Salvage proton beam irradiation to local or metastatic relapses is documented within the KiProReg and ProReg prospective registry trials. A retrospective analysis (20 patients) demonstrated safety and efficacy [127]. Prospective studies would be desirable to better define the role of proton beam therapy for these patients.

Abbreviations: HRNB, high-risk neuroblastoma; NB, neuroblastoma; r/r, refractory or relapsed.

6.2. Theranostics

Current evidence shows promise for combining ^{131}I-MIBG with vorinostat in patients with mIBG-avid disease (Table 4). The potential of ^{177}Lutetium-DOTATATE remains to be elucidated (Table 4).

7. Special challenge: CNS relapse

Although CNS neuroblastoma metastases are rare at initial HRNB diagnosis, leptomeningeal and/or parenchymal CNS metastases are present in 6–8 % of cases at relapse [143,144]. Rates of CNS relapse have been suggested to be rising, with the CNS representing a sanctuary

Table 4
Theranostic candidates for first relapse of high-risk neuroblastoma.

Theranostics	Key considerations
[^{131}I]-mIBG therapy	The earliest form of molecularly targeted therapy for HNRB was short-range β radiation via [^{131}I]-mIBG therapy [133–135]. Subsequent studies included autologous stem cell rescue to facilitate higher doses (up to 19 mCi/kg body weight) [136,137]. In palliative care, [^{131}I]-mIBG therapy can be useful to provide pain relief [138]. Combining [^{131}I]-mIBG with oral vorinostat (180 mg/m^2/dose) once daily on days 1–12 achieves the greatest overall response (randomized phase II NANT2011–01 trial, NCT02035137, compared to mIBG alone; and mIBG with chemotherapy) [139]). Preliminary efficacy data in the phase I trial NANT2017–01, NCT03332667, showed encouranging antitumor activity and good tolerability of [^{131}I]-mIBG therapy combined with DB in a heavily pretreated population with r/r HRNB [140]. Limited access, logistics and the need of hematopoietic stem cell support are major challenges for [^{131}I]-mIBG therapy. The MINIVAN phase I NCT02914405 study investigated [131I]-mIBG therapy followed by nivolumab and dinutuximab beta antibodies in children with relapsed/refractory neuroblastoma; initial data suggests an overall response rate (PR/CR) of 42.9 % (12/28 patients) [141].
177Lutetium-Dotatate	The LuDO-N multicenter phase II clinical trial (NCT04903899) investigates 177Lu-DOTATATE treatment of r/r HRNB in an an intensified dosing schedule. It builds on experience from the pilot LuDO trial, whose poor results were probably due to administering a subtherapeutic dose [142].

Abbreviations: DB, dinutuximab beta; HRNB, high-risk neuroblastoma; mIBG, meta-iodobenzylguanidine; NIV, nivolumab; r/r, refractory or relapsed.

site protected from systemic chemotherapy or immunotherapy [143, 145]. HR-NBL-1/SIOPEN clinical trial data revealed that the risk of CNS recurrence is linked to patient and disease characteristics, with no impact from high-dose chemotherapy or immunotherapy [146].

CNS relapse remains a major therapeutic challenge, and post-relapse survival is significantly shorter than for patients with CNS-negative disease [146,147]. Patients with CNS metastases are often excluded from traditional phase I and II trials. A recent analysis of relapses after first-line therapy in the European HR-NBL1/SIOPEN trial (NCT01704716) described a median OS after CNS recurrence of only four months [146]. Less than 10 % of the patients survived longer than 3 years [146]. Neurosurgical debulking, craniospanial irradiation and additional treatment options must be considered for patients with CNS disease. These options include intrathecal antibody-based radioimmunotherapy using ^{131}I-8H9 targeting B7H3 (omburtamab, NCT03275402, NCT00089245, [148]), intraventricular chemotherapy (etoposide, topotecan) and/or temozolomide-based systemic chemotherapy regimens. There is a need for randomized trials for patients with HRNB relapse involving the CNS. There are no randomized data comparing craniospinal irradiation (CSI) with focal approaches such as surgery and local radiotherapy in CNS-relapsed neuroblastoma. However, published treatment strategies associated with durable CNS disease control incorporate CSI as part of a multimodal approach [131, 147]. Retrospective series demonstrate that focal irradiation alone is insufficient, with high rates of subsequent CNS failure, whereas CSI-based strategies achieve superior disease control combined to other treatments [149]. CSI has been used consistently both in combination with radioimmunotherapy and in multimodal regimens without radioimmunotherapy, including reports of long-term relapse-free survivors [131,150]. To date CSI is to be considered as a central component of curative-intent therapy for CNS-relapsed neuroblastoma.

8. Conclusions

Patients with relapsed HRNB have typically been heavily pretreated. Since disease has already been exposed to the most effective cytotoxic chemotherapy agents, there is a pressing need to develop less toxic therapies (particularly less myelosuppression) against novel targets. The hope is to overcome tumor resistance and allow personalized treatment for tumor-specific aberrations. Identifying molecular targets for neuroblastoma has been difficult, as unlike in many adult cancers, whole-genome sequencing has demonstrated that recurrent mutations of specific oncogenes are rare with the exception of *ALK*. The current level of evidence justifies offering treatment to patients with first HRNB relapse, which is definitely not yet a palliative situation.

How best to identify, prioritize and combine novel agents to improve treatment of first HRNB relapse remain the challenges to moving forward. The field benefits from a long history of international collaboration. Coordinated efforts have been enabled through the **ACCELERATE** pediatric strategy fora and through dedicated international workshops on new drug development for HRNB [151–153]. These coordinated efforts will allow us to develop the most promising combinations in a timely way to best benefit our patients.

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors did not use AI tools.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

G.B. has received Travel Grants and has had educational roles in educational events organised by EUSA pharma and Recordati Rare Diseases. P.B. had a consulting role (institutional) for Recordati and Merck; participated in educational activities (institutional) organized by EUSA Pharma.

P.B. is member of the Executive Committee of SIOPEN, which receives royalties for the sales of dinutuximab beta.

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H.N.L. has worked as consultant of Recordati Rare Diseases and is co-founder of AnYxis Immuno-Oncology.

G.S. is a recipient of research grants from BMS, MSDAvenir, Roche and Pfizer, and member of a board of experts for Recordati Rare Diseases.

L.M. is a past member of data monitoring committees for clinical trials sponsored by Novartis, Shionogi and Incyte; had a consulting role for Novartis, Norgine, Boehringer, Ymabs, Merck, Roche, Bayer and Shionogi; participated in educational activities organized by Bayer and EUSA Pharma and received travel expenses from EUSA Pharma. L.M. is president of SIOPEN (Neuroblastoma Research Network), an

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