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REVIEW

High-risk neuroblastoma: ATRX and TERT as prognostic markers and therapeutic targets. Review and update on the topic

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Abstract High-risk neuroblastoma continues to show a very high mortality, with a 5-year survival rate of 50%. While MYCN amplification is the main genetic alteration associated with high-risk tumours, other molecular mechanisms, such as alterations in ATRX and TERT, remain poorly understood.

ATRX and TERT biomarkers, which are associated with a more aggressive neuroblastoma pattern, should be considered for accurate prognostic stratification.

We highlight the promising results of the clinical trial involving the combination of adavosertib and irinotecan, which encourages further clinical trials with adavosertib targeting NB with ATRX mutations. Preclinical results with BET inhibitors (OTX015 and AZD5153) and with 6-thio-2′-deoxyguanosine, targeting NB with TERT mutations, are promising. Both represent future therapeutic targets, emphasizing the need to prioritize research using these models.

Abbreviations: ALK, anaplastic lymphoma kinase; ALT, alternative lengthening of telomeres; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; ATRX, alpha-thalassemia/mental retardation, X-linked; ATRX LoF, ATRX loss of function; BET proteins, bromo- and extra-terminal domain proteins; CDK, cyclin-dependent kinases; DAXX, death-associated protein 6; EZH2, enhancer of zeste homolog 2 (EZH2) is a histone-lysine N-methyltransferase enzyme; FH, favourable histology; GN, ganglioneuroma; GNBi, ganglioneuroblastoma, intermixed; GNBn, ganglioneuroblastoma, nodular; IFF proteins, in-frame fusion proteins; INPC, International Neuroblastoma Pathology Classification; INRG, International Neuroblastoma Risk Group Staging System; NB, neuroblastoma; NDDS, Neuroblastoma Drug Development Strategy; PARP, Poly (ADP-ribose) polymerase; REST, RE-1 Silencing Transcription Factor; SWI/SNF, switch/sucrose non-fermentable; TERT, telomerase reverse transcriptase; TMM, telomere maintenance mechanisms; UH, unfavourable histology; WEE1, nuclear kinase belonging to the Ser/Thr family of protein kinases.

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PALABRAS CLAVE
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Neuroblastoma de alto riesgo: ATRX y TERT como marcadores pronósticos y dianas terapéuticas. Revisión y actualización del tema

Resumen El neuroblastoma de alto riesgo sigue mostrando una tasa de mortalidad muy alta, con una tasa de supervivencia a 5 años del 50%. Aunque la amplificación de MYCN es la principal alteración genética asociada con tumores de alto riesgo, otros mecanismos moleculares, como las alteraciones en ATRX y TERT, aún no se entienden completamente.

Los biomarcadores ATRX y TERT, vinculados a un patrón de neuroblastoma más agresivo, deberían tenerse en cuenta para una estratificación pronóstica exacta.

Destacamos los resultados prometedores del ensayo clínico con la combinación de adavosertib e irinotecán, lo que motiva la realización de nuevos ensayos clínicos con adavosertib dirigido al neuroblastoma con mutaciones en ATRX. Los resultados preclínicos con inhibidores BET (OTX015 y AZD5153) y con 6-tio-2'-deoxiguanosina, dirigidos al neuroblastoma con mutaciones en TERT, son prometedores. Ambos representan dianas terapéuticas futuras y se subraya la necesidad de priorizar la investigación utilizando estos modelos.

Introduction

Neuroblastoma: overview, prognosis, and pretreatment risk classification

Neuroblastoma is the most common extracranial solid paediatric tumour. One of its most significant characteristics is the heterogeneity of the clinical presentation, as it can regress or mature spontaneously or following chemotherapy. This accounts for approximately half of the cases; however, in the rest of the patients, it behaves aggressively with rapid disease progression, metastatic dissemination, and resistance to multimodal treatments, including chemotherapy or combinations of immunotherapy and stem cell transplantation.[1,2]

Many factors influence prognosis, but the most important are the stage of the tumour and the age of the patient.[2,4] The International Neuroblastoma Risk Group (INRG) pretreatment risk stratification guideline evaluates 16 combinations of known prognostic factors, including age, stage, histology, and molecular characteristics such as MYCN amplification status, chromosome 11q status, and tumour cell ploidy (see Table 1).[7]

These factors allowed the classification of patients into four prognostic groups, as indicated in Table 2, facilitating the selection of the most appropriate treatment and enabling the prediction of 5-year survival.[7]

In neuroblastoma, high-risk tumours continue to exhibit a very high mortality, with a 5-year survival rate of less than 50%. Genomic amplification of the MYCN proto-oncogene has been established as an independent marker for high-risk disease and is detected in 40% of these tumours.[3] However, the molecular mechanisms of more than half of these high-risk tumours are still poorly understood, including the molecular determinants of ATRX and TERT. It is essential to deepen the understanding of the genetic basis of high-risk neuroblastoma to improve prognostic stratification and identify new therapeutic targets that may contribute to improving survival.[3,12] Therefore, the objective of this review is to evaluate the impact of ATRX and TERT biomarkers on prognostic prediction in high-risk NB and their potential as therapeutic targets, as well as to review and update the literature on the status of preclinical and clinical research on ATRX and TERT.

Pathological aspects

For histopathological study, the International Neuroblastoma Pathology Classification (INPC) is currently used, which is based on a modification of the Shimada system. The presence of Schwannian stroma and gangliocytic differentiation is indicative of a favourable prognosis. Peripheral neuroblastic tumours have two main cellular components: neuroblastic/ganglion cells and Schwann cells. However, in tumours with unfavourable histology, the Schwannian component is reduced or absent, and tumour differentiation is limited or absent.[5,6,10]

The INPC describes four categories of peripheral neuroblastic tumours: **Neuroblastoma (NB)**, Schwannian stroma-poor; **Ganglioneuroblastoma, intermixed (GNBi)**, Schwannian stroma-rich; **Ganglioneuroma (GN)**, Schwannian stroma-dominant; and **Ganglioneuroblastoma, nodular (GNBn)**, composite Schwannian stroma-rich/stroma-dominant and stroma-poor.

The category of Neuroblastoma, Schwannian stroma-poor, further presents three subtypes:

Undifferentiated neuroblastoma: It is constituted by neuroblastic cells without apparent neuropil. From a histological point of view, it is part of the family of small round cell tumours and requires a differential diagnosis with Ewing family of tumours, solid alveolar rhabdomyosarcoma, Wilms tumour with blastemal predominance, and lymphoblastic lymphoma. This differential diagnosis requires support from

Table 1 International neuroblastoma treatment risk groups.

INRGSS	Age (months)	Histologic classification	Grade of tumour differentiation	MYCN	11Q deletion	Ploidy	Pretreatment risk group
L1/L2		GN maturing, GNBi					Very low
L1		Any except GN maturing or GNBi		NoAmp			Very low
				Amplification			High
L2	<18	Any except GN maturing or GNBi	Differentiating	NoAmp	No		Low
				NoAmp	Yes		Intermediate
	≥18	Nodular GNB, neuroblastoma	Poorly differentiating or undifferentiating	NoAmp	No		Low
				NoAmp	Yes		Intermediate
				NoAmp			Intermediate
				Amplification			High
M	<18			NoAmp		Hyperdiploid	Low
	<12			NoAmp		Diploid	Intermediate
	12–18			NoAmp		Diploid	Intermediate
	<18			Amplification			High
	≥18						High
MS	<18			NoAmp	No		Very low
				NoAmp	Yes		High
				Amplification			High

GNBi: ganglioneuroblastoma intermixed, GN: ganglioneuroma, NoAmp: MYCN non-amplified.

Table 2 International neuroblastoma treatment risk groups.

Pretreatment risk group	Five-year event-free survival (%)	Percentage of patients (%)
Very low	>85	28.2
Low	>75 to ≤85	26.8
Intermediate	≥50 to ≤75	9
High	<50	36.1

ancillary techniques such as immunohistochemistry [IHC] and molecular techniques.[2,5,9]

Poorly differentiated neuroblastoma: This tumour is poor in Schwannian stroma, with evident neuroblasts and neuropil. The presence of differentiating cells should be less than 5% of the tumour population. In this subtype, Homer–Wright pseudorosettes are characteristic.

Differentiating neuroblastoma: This tumour presents more than 5% tumour cellularity with differentiation features.[2,5]

Examples of the different histological subtypes are shown in Fig. 1.[8]

This classification also differentiates between favourable histology (FH) and unfavourable histology (UH), making it one of the most powerful prognostic factors in patients with neuroblastoma. Furthermore, it correlates with the genetic basis of neuroblastoma.[5,6]

The category of favourable histology, characterized by spontaneous regression or appropriate tumour differentiation/maturation for age, is common in younger children, generally under 1.5 years old. In contrast, unfavourable histology is frequent in older children and is molecularly heterogeneous. MYCN amplification is the most common genomic anomaly in tumours with UH. Abnormal telomere maintenance or elongation, overexpression of telomerase reverse transcriptase (TERT), and the alternative lengthening of telomeres (ALT) phenotype are other molecular alterations observed in UH.[5] Ikegaki and Shimada proposed four subgroups of unfavourable histology (UH) neuroblastomas based on the expression of distinctive proteins detectable by molecular assays and immunohistochemistry. These four subgroups of UH are: the MYC subgroup, the TERT subgroup, the ALT subgroup, and the Null subgroup, of which the MYC, TERT, and ALT subgroups are defined as extremely unfavourable histology (EUH) tumours.[5,6]

TERT: structure and function

Telomerase reverse transcriptase (TERT) is the catalytic subunit of the telomerase enzyme, which maintains telomeric ends by adding specific short repetitive DNA sequences. The enzyme comprises a protein component with reverse transcriptase activity, encoded by the TERT gene, and a

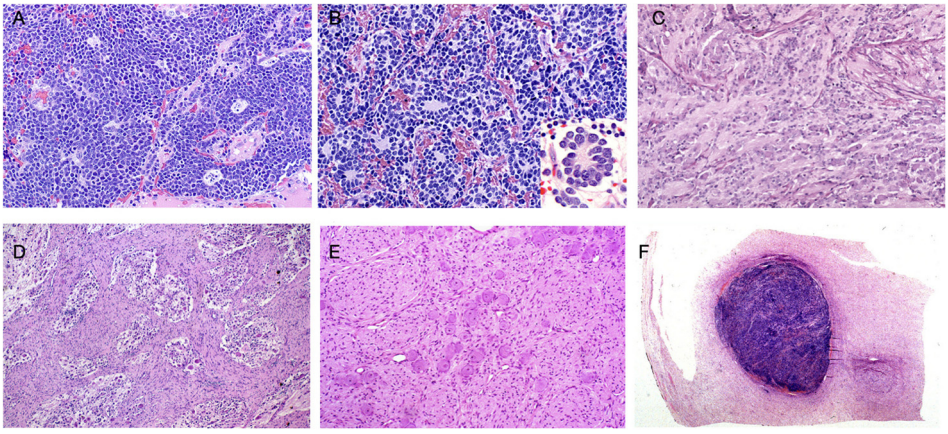

Figure 1 Categories of peripheral neuroblastic tumours. (A) Undifferentiated neuroblastoma. (B) Poorly differentiated neuroblastoma. (C) Differentiating neuroblastoma. (D) Ganglioneuroblastoma, intermixed. (E) Ganglioneuroma. (F) Ganglioneuroblastoma, nodular. (Courtesy of H. Shimada, E. d'Amore, S. Navarro et al. of the INPC Committee).

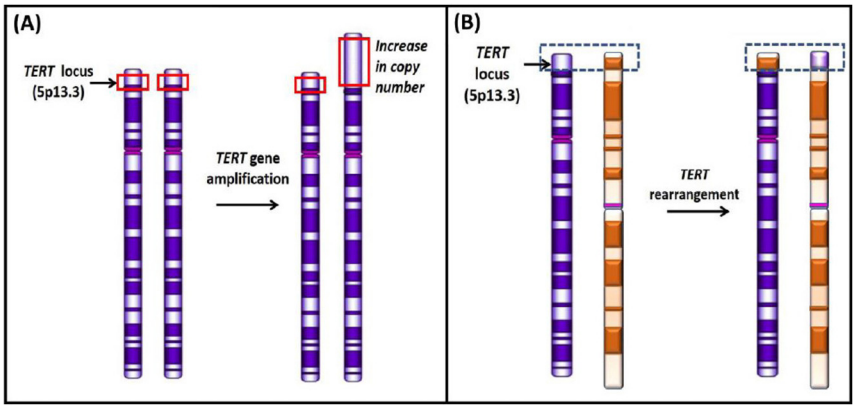

Figure 2 Schematic representation of genetic mechanisms of telomerase activation. (A) TERT gene amplification resulting in an increase in TERT copy number at the 5p15.33 locus. (B) Genomic rearrangement in TERT resulting in inter-chromosomal translocation.[13]

telomerase RNA component (TER or TERC). The TERT gene is located on chromosome 5p15.33 in humans.[11]

The telomerase complex is essential for telomere homeostasis, which is a crucial factor in regulating ageing and cancer development. It is estimated that more than 80% of tumours adopt regulatory strategies such as telomere maintenance mechanisms (TMM). These mechanisms include changes in TERT, such as TERT gene rearrangements, gene amplifications of TERT and TERC, structural variants of TERT, somatic and germline mutations in the TERT promoter, epigenetic changes of TERT, or transcription factor binding.[11,12] Several studies have indicated that chromosomal rearrangements at the TERT locus are associated with the development of tumours such as neuroblastoma[12,13] (Fig. 2).

Furthermore, a smaller percentage of tumour cells achieve immortality through a telomerase-independent mechanism known as alternative lengthening of telomeres (ALT), which is largely associated with ATRX mutations.[11]

ATRX: structure and function

ATRX (alpha-thalassemia, mental retardation, X-linked syndrome) was identified as the gene responsible for a rare developmental disorder characterized by thalassemia and intellectual disability. The ATRX gene is located on the q21.1 band of the long arm of the X chromosome. It encodes an ATP-dependent helicase of the SWI/SNF (switch/sucrose non-fermentable) chromatin remodeller family. ATRX is part of a protein complex that regulates gene expression by ATP-dependent chromatin remodelling. It is also involved in various biological activities, including DNA repair, transcriptional regulation, and nucleosome reorganization[17–19] (Fig. 3).

Several studies have highlighted that ATRX mutations, particularly loss-of-function mutations, are associated with a specific subgroup of tumours characterized by alternative lengthening of telomeres (ALT), an aberrant form of telomere maintenance independent of telomerase activity, based on homologous recombination.[12,19] However, ATRX mutations are not present in all ALT cells, indicating that these mutations alone are not sufficient to induce ALT; additional unidentified mechanisms are needed. It has also been suggested that genetic alterations in DAXX can result in ALT.[12]

In neuroblastoma, ATRX mutations include point mutations in coding regions and deletion/insertion-induced frameshift mutations that result in functional loss. Additionally, it has been noted that large deletions in the N-terminal

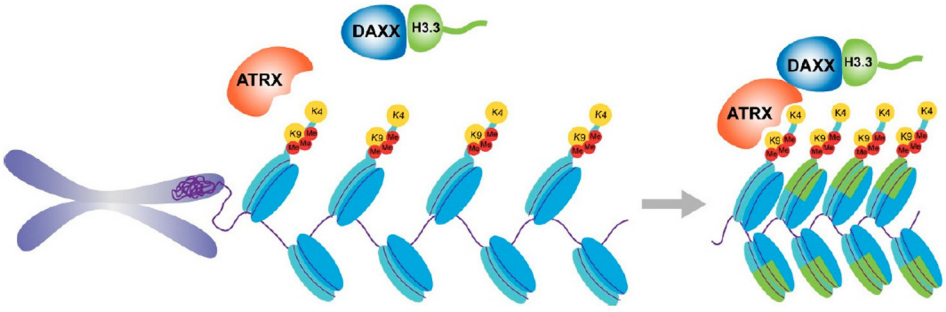

Figure 3 The main role of ATRX is to interact and bind with the chaperone protein DAXX, forming a chromatin remodelling complex that deposits H3.3 at telomeres and preserve heterochromatin.[18]

region of ATRX generate ''in-frame fusion'' (IFF) proteins, which also contribute to aggressive neuroblastoma.[18,31]

Most high-risk neuroblastomas are affected by genetic alterations in MYCN, TERT, or ATRX, all of which converge on telomere lengthening mechanisms, as shown in Fig. 4. In contrast, low-risk tumours seem to lack these mechanisms, which may explain their inability to proliferate indefinitely and their potential for spontaneous regression.[3]

ATRX and TERT as prognostic factors and therapeutic targets

ATRX and TERT as prognostic factors

Koneru et al.[23] classified high-risk neuroblastoma patients into three subgroups with significant differences in survival based on TERT expression and ALT status. They concluded that neuroblastomas with a telomere maintenance mechanism (TMM) could be classified as true high-risk tumours, regardless of other prognostic markers currently used. Their findings indicated that patients with both an ALT phenotype and TERT-positive had very poor overall survival, with no differences between them. However, Roderwieser et al.[22] did observe substantially better overall survival rates for neuroblastoma with an ALT phenotype compared to patients with TERT alteration. This discrepancy may be attributed to differences in methods used to detect these molecular alterations. Koneru et al. proposed that the use of the ''c-circle assay,'' a specific ALT marker, could be valuable for the detection and stratification of these patients.[23]

Roderwieser et al.[22] highlighted that telomerase activation and ALT mechanism characterize distinct subgroups of NB with adverse outcomes. Consistent with these findings, other authors[25,28] also concluded that cases associated with telomere stability – whether resulting from telomerase activation through TERT rearrangements or the ALT pathway linked to ATRX mutations – are related to higher risk and poor outcomes. Together, these studies underscore the correlation between TMM and high-risk NB, highlighting their significance as adverse prognostic factor.

Roderwieser et al. and Peifer et al.[22,25] found that TERT rearrangements were more common in patients aged 18 months or older and with stage 4 disease at diagnosis, and they associated these rearrangements with an unfavourable prognostic marker. The ALT mechanism was mainly observed in tumours of patients aged 18 months or older at diagnosis and was found in approximately half of patients diagnosed after the age of 5; genomic inactivation of ATRX contributed to the ALT phenotype in approximately half of the cases. Roderwieser et al.[22] concluded that both telomerase activation and ALT activation are strong, independent prognostic markers and may be considered for patient risk assessment in clinical practice. However, Valentijn et al.,[27] although they did relate genetic defects in TERT and ATRX to clinical parameters for aggressiveness, concluded that they were not independent prognostic markers.

It is worth noting that the ALT mechanism has been validated as a robust diagnostic and prognostic biomarker for certain types of cancer, including primary pancreatic neuroendocrine tumours (PanNET). The presence of ALT or the loss of ATRX protein detected by immunohistochemistry is highly specific for certain neuroendocrine tumours and is used in diagnosing other CNS tumours, such as mutant IDH astrocytomas.[38] These findings support the inclusion of ALT in prognostic stratification.

Additionally, it has been suggested that ALT activation may be associated with a more indolent but eventually fatal course of the disease.[22] Consistent with this, Cheung et al.[24] discovered a very significant association between ATRX mutations and age at diagnosis, noting that children with ATRX-mutated tumours tended to be over 5 years old at diagnosis and presented a chronic course of the disease.

From the analysis of the studies, we can conclude that ATRX and TERT markers are associated with a more aggressive pattern of neuroblastoma, defining different patient subgroups with adverse outcomes and very poor survival. Cases related to telomere maintenance mechanisms, either by telomerase activation through TERT rearrangements or by the ALT mechanism, are strongly correlated with ATRX mutations and ALT phenotype. These findings highlight a higher risk and provide a molecular definition of this subtype of neuroblastoma. Additionally, ATRX mutations are associated with older age at diagnosis and a prolonged, refractory clinical subtype, so it could serve as a molecular marker to help identify patients with a chronic but progressive clinical course.

ATRX as a therapeutic target

Currently, there is limited evidence supporting ATRX as a therapeutic target. In a phase 2 clinical trial evaluating the efficacy of adavosertib (AZD1775), a WEE1 inhibitor,

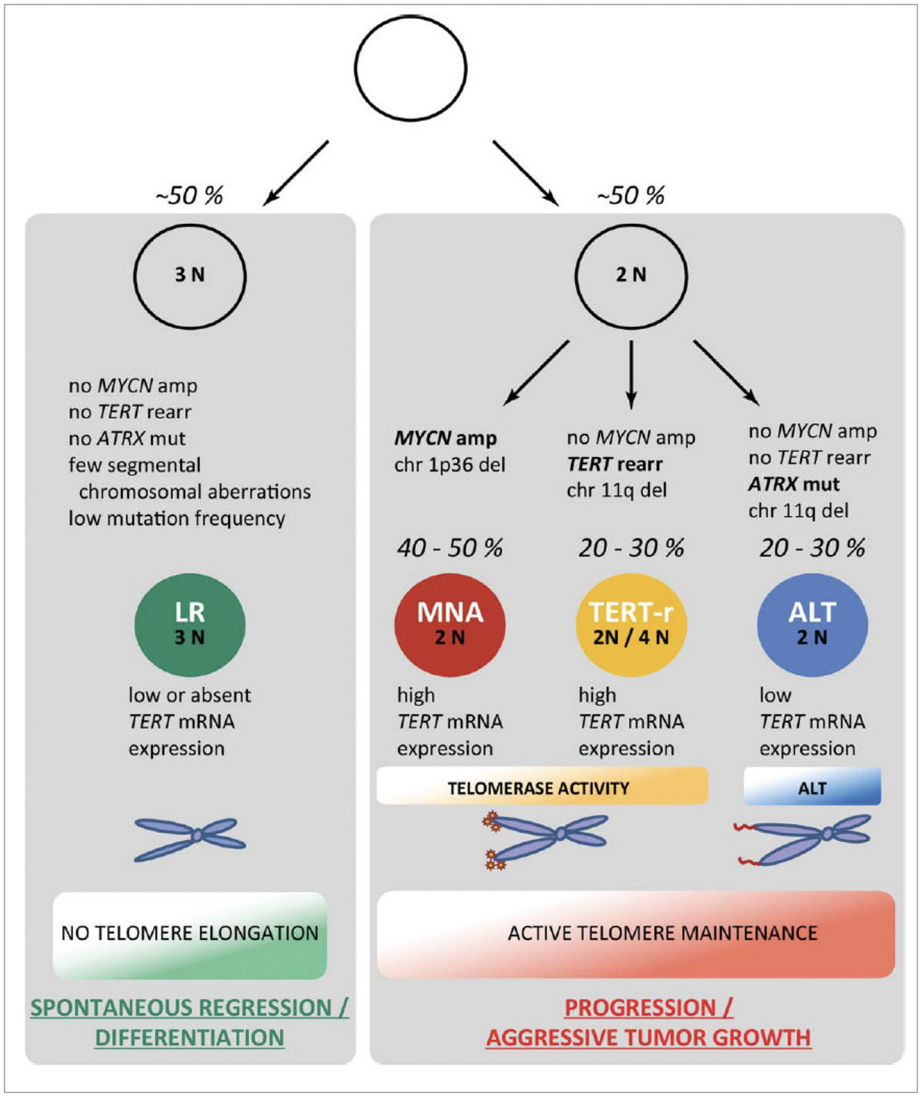

Figure 4 Pathogenesis of neuroblastoma based on recurrent genomic alterations. High-risk (HR) neuroblastoma differs from low-risk (LR) neuroblastoma in mechanisms of telomere maintenance and elongation: either by (1) telomerase activation resulting from TERT rearrangements (TERT-r) or MYCN amplification (MNA) or (2) by alternative lengthening of telomeres (ALT). 2N, near-diploid karyotype; 3N, near-triploid karyotype; 4N, near-tetraploid karyotype; amp, amplification; rearr, rearrangement; mut, mutation; del, deletion.[3]

and irinotecan in paediatric solid tumours: neuroblastoma, medulloblastoma, and rhabdomyosarcoma[29] – the neuroblastoma cohort met the protocol-defined efficacy endpoint, with an estimated objective response rate of 16.7%. The combination was well tolerated. Of the three patients who achieved an objective response, two of them had ALT-positive tumours. As mentioned earlier, ALT is associated with an aggressive, refractory subtype of NB with a poor prognosis, and ATRX mutations can be identified in half of this patient group. Although an association between objective response to the combination of adavosertib and irinotecan and ALT status could not be demonstrated due to lack of data on mutation status, it can be said that ALT is a therapeutic target for refractory neuroblastoma. Further clinical studies are needed to evaluate the efficacy of adavosertib and other WEE1 inhibitors in neuroblastoma and other tumours with ATRX mutations. It is worth noting that this study is the first phase 2 clinical trial with adavosertib in paediatric patients and the first with irinotecan. The results support the need for further research with adavosertib in neuroblastoma.

In preclinical studies, S.L. George et al.[30] investigated the combination of PARP inhibitors with various chemotherapeutic agents used for treating relapsed neuroblastoma. They identified a preferential sensitivity to the combination of olaparib and irinotecan and an increased *in vivo* survival in NB models with ATRX mutations. Currently, phase 1 paediatric clinical trials are exploring olaparib for solid tumours,[26] so olaparib can be considered a potential therapeutic target for this patient group. Additionally, preclinical studies have shown that other tumours with ATRX mutations or deletions, such as high-grade gliomas or hepatocellular carcinomas,

show sensitivity to irinotecan and to WEE1 inhibitors like adavosertib.[14,33]

It is worth highlighting the study by Qadeer et al.,[31] which highlighted the potential of REST inhibitors to restore neuronal differentiation programmes and stop proliferation in NB with IFF (in frame fusion) proteins, resulting from ATRX mutations, alone or in combination with EZH2 inhibitors. It was concluded that EZH2 inhibitors should be considered a therapeutic strategy for treating patients with neuroblastoma with these mutations, and further preclinical studies with these inhibitors in neuroblastoma models are of interest. Additionally, somatic mutations have been reported in other chromatin remodellers and alterations in ATRX in a series of paediatric cancers that are also sensitive to EZH2 inhibitors, such as tazemetostat, an agent used in clinical trials in patients with follicular lymphoma, with results showing its therapeutic potential and limited toxicity.[20]

It is noteworthy that the second Neuroblastoma Drug Development Strategy (NDDS) forum highlighted ALT, ATRX, and TERT as high-priority targets, for which there are currently no specific drugs available.[21] Regarding ALT as a potential therapeutic target and possible ALT inhibitors, Moreno et al., Shimada et al., Akter et al.[6,21,37] considered several drugs as candidates. It was pointed out that ATR (Ataxia Telangiectasia and Rad3-related protein) could play a key role in maintaining ALT, and ATR inhibitors could be clinically relevant. However, it was emphasized that not all ALT cancer cell lines show hypersensitivity to ATR inhibitors. Additionally, ATM (Ataxia telangiectasia mutated) inhibitors have been found to reverse chemoresistance to temozolomide and irinotecan-chemotherapeutic agents used in NB treatment. This implies that the combination of ATM and ATR inhibitors could be more effective than either of these inhibitors alone.[6] George et al.[30] also identified a preferential sensitivity to the ATM inhibitor KU60019 in NB models with ATRX mutations. Currently, phase 1 clinical trials are evaluating the ATM inhibitor AZD0156 in combination with olaparib or irinotecan for adults with advanced solid tumours (NCT02588105).[15]

Similarly, these compounds, all of which are still in preclinical investigation, were identified as options targeting ALT and ATRX. Tetra-Pt (bpy), a novel cisplatin derivative, has been shown to inhibit tumour growth in ALT cells *in vivo*, but has not been evaluated in NB models. CX5461, an RNA polymerase I inhibitor, selectively acts against cells with ATRX mutations. Pifithrin-α, a p53 inhibitor, decreases tumour growth in ALT xenografts. Additionally, trabectedin is another compound to consider as it has been found to be effective against ALT cancer cell lines.[6,21,37]

TERT as a therapeutic target

At the NDDS forum, TERT and telomerase were also identified as critical therapeutic targets, and potential drugs targeting telomerase were evaluated. Imetelstat, a telomerase inhibitor, was evaluated in paediatric clinical trials, but its clinical development was halted due to high toxicity.[21] However, preclinical studies by Fischer-Mertens et al. reported that imetelstat acts synergistically with etoposide in inhibiting tumour growth in neuroblastoma with high telomerase activity.[36] Similarly, sorafenib, a multikinase inhibitor, has been considered for its potential to act synergistically with imetelstat in inhibiting tumour growth in preclinical models, with the hypothesis that it might be effective in NB with elevated telomerase activity.[6] BIBR1532 is a potent non-nucleoside telomerase inhibitor; however, it has not yet been evaluated in clinical trials, and concerns regarding its toxicity similar to those associated with imetelstat persist.[12,21] Telomestatin is a compound that also inhibits telomerase activity by stabilizing G-quadruplexes, inducing apoptosis *in vitro* in neuroblastoma cell lines expressing high telomerase activity, but it is not currently in clinical development either.[12]

It is interesting to note the role of BET (bromodomain extra-terminal) protein inhibitors, which are important transcriptional regulators. Preclinical research in neuroblastoma with TERT rearrangements by Chen et al. indicated that the BET inhibitor OTX015 and the proteasome inhibitor carfilzomib act synergistically by blocking TERT expression, reducing tumour progression, and improving survival in patient-derived neuroblastoma xenografts. Therefore, combined treatment with OTX015 and carfilzomib may lead to a first clinical trial of therapy specifically targeted at patients with TERT rearranged neuroblastoma.[32] While OTX015 has not yet been tested in clinical trials with paediatric patients, it is currently undergoing phase 2 clinical trials in adults. Another BET inhibitor, BMS-986158, is in a phase I clinical trial for paediatric cancer patients (NCT03936465).[34] Regarding carfilzomib, an approved oncology drug, is also being investigated in phase 1 clinical trials for paediatric cancer patients (NCT02303821).[16]

Regarding the function of BET inhibitors in neuroblastoma with TERT rearrangements, while *in vitro* studies have generally shown positive responses, tumour regression *in vivo* is uncommon with monotherapy.[21,37] Therefore, it is relevant to consider combination therapies, such as the study by Chen et al. mentioned earlier, or other studies such as that by Huang et al.,[35] which investigated the combination of BET inhibitors (JQ1 and AZD5153) and the CDK inhibitor dinaciclib. Huang et al. proposed that the epigenetic modulators Brd4 and cyclin-dependent kinases (CDKs) were involved in the coactivation of various transcriptional regulators, resulting in increased TERT expression. They found that the synergistic action of these inhibitors suppressed TERT expression and multiple TERT-associated genes in NB cell lines overexpressing TERT and MYCN amplification. Additionally, the BET inhibitor AZD5153 and dinaciclib reduced tumour growth and substantially increased survival *in vivo* in TERT rearranged xenografts, leading to the conclusion that the combined use of AZD5153 and dinaciclib is a novel epigenetic therapeutic strategy targeting neuroblastoma with TERT overexpression.[35]

Another novel compound to analyze is the nucleoside analogue 6-thio-2′-deoxyguanosine (6-thio-dG), targeting telomerase. It is recognized by telomerase and is incorporated into *de novo* synthesized telomeres, causing telomere dysfunction specifically in telomerase-expressing cells. Although it has not yet been evaluated in clinical trials, it is believed to be less toxic than traditional telomerase inhibitors and has been shown to have preclinical utility in neuroblastoma.[12,21,37] Roderwieser et al.[22] demonstrated that 6-thio-dG is effective *in vitro* and *in vivo* in neuroblas-

toma with TERT activation, as it decreased the viability of cell lines with telomerase activation and inhibited tumour growth of TERT rearranged cells in xenografts. Additionally, Fischer-Mertens et al.[36] observed that, while 6-thio-dG alone was already highly effective *in vivo*, its combination with etoposide acted synergistically and almost completely abolished tumour growth in TERT rearranged NB models. Therefore, 6-thio-dG is a priority compound in the investigation of future treatments targeting neuroblastoma with high telomerase activity, and specifically with TERT rearrangements.

Conclusions

Genetic alterations in ATRX and TERT are associated with a more aggressive pattern of neuroblastoma and define different patient subgroups with adverse outcomes and very low survival rates. Together, we highlight the clinical and biological relevance of TERT and ATRX as biomarkers and they should be considered for proper prognostic stratification in high-risk neuroblastoma.

Despite the relevance of ATRX and TERT as therapeutic targets, targeted clinical trials remain scarce and preclinical research is limited. The promising results of the clinical trial combining adavosertib and irinotecan in paediatric NB patients highlight the need for further clinical trials with adavosertib targeted at NB with ATRX mutations. Preclinical studies have shown promising results with several compounds, urging for more preclinical research on NB with the ALT phenotype and ATRX mutations. Unfortunately, there are still no targeted clinical trials for NB with TERT alterations, but preclinical results are encouraging, with special mention of BET inhibitors (OTX015 and AZD5153) in combination with other compounds, and 6-thio-2′-deoxyguanosine, targeting telomerase.

In conclusion, both ATRX and TERT are promising therapeutic targets, and preclinical research with these models should be prioritized, with the aim of translating these findings into future clinical trials. This approach aims to offer high-risk neuroblastoma patients more personalized and effective treatment options.

Informed consent

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Ethical considerations

Not applicable.

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Conflict of interest

Not applicable.

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