Therapeutic potential of targeting MYCN
A case series report of neuroblastoma with MYCN amplification
Can Huang, PhD, Shayi Jiang, MD, PhD*, Jingwei Yang, MD, Xuelian Liao, MD, Yanhua Li, MD, Shanshan Li, MD

Abstract
Introduction: Neuroblastoma (NB) with MYCN amplification has a poor prognosis and high mortality. The potential molecular biological relationship between clinical features and MYCN amplification should be explored.

Methods: NB patients were examined by fluorescence in situ hybridization (FISH) for MYCN amplification in the tumor mass or bone marrow samples to determine whether MYCN was amplified. A series of eleven MYCN-amplified NB patients were included. The age, primary site, tumor size, specific biomarkers, and invaded organs were analyzed. All patients accepted standardized treatment of surgery, chemotherapy, and radiotherapy. Progression-free survival (PFS) and overall survival (OS) were evaluated.

Results: The median age at diagnosis was 24 months. Nine patients (81.8%) were in stage IV, with high serum neuron-specific enolase (NSE) expression, normal urine vanillylmandelic acid (VMA) level and extensive metastases. All patients accepted a chemotherapy protocol with 8 to 10 cycles, and 9 patients (81.8%) were sensitive to the initial chemotherapy protocol. At the end of follow-up, four patients (36.3%) died with a median OS of 15 months. Five patients (45%) survived with a median PFS of 13 months. Two patients were still receiving chemotherpay.

Conclusion: Given the effect of MYCN amplification on poor outcome in NB, novel treatments targeting MYCN should be developed for patients with NB.

Abbreviations: EFS = event-free survival, FISH = fluorescence in situ hybridization, HDCT/auto-SCT = tandem high-dose chemotherapy and autologous stem cell transplantation, INSS = International Neuroblastoma Staging System, MDT = multi-disciplinary team, MRP1 = multiple resistance protein 1, NB = neuroblastoma, NSE = neuron-specific enolase, OS = overall survival, PD = progressive disease, VMA = urine vanillylmandelic acid.

Keywords: abdomen mass, MYCN, neuroblastoma, pediatric, targeting

1. Introduction
Neuroblastoma (NB), which is an embryonal malignancy, can develop anywhere in the sympathetic nervous system, especially in the abdomen. Its incidence is increasing worldwide. Patients with stage I to III and IVS disease according to the International Neuroblastoma Staging System (INSS) present an excellent outcome. However, the prognosis of children with stage IV disease is very poor, with a 5-year event-free survival (EFS) of <50%, even if myeloablative chemotherapy, radiotherapy, immunotherapy, and aggressive surgery are given. MYCN amplification has been considered to be highly correlated with rapid disease progression and poor outcomes; however, the potential molecular biological relationship between clinical features and MYCN amplification should be further explored.

This case series study was approved by the Ethics Committee of Children’s Hospital of Shanghai and based on the clinical data of children with MYCN-amplified NB from a single centre. According to known molecular biology results of MYCN amplification, we analyzed the relationship between amplification, clinical characteristics, and adverse prognosis to inquire about the possibility of MYCN as a therapeutic target.

2. Methods
Consecutive child patients (6 males and 5 females) were seen at Shanghai Children’s Hospital between August 2017 and September 2019. The diagnosis and staging of NB met the criterion. The patients were examined by fluorescence in situ hybridization (FISH) using a two-color molecular probe for MYCN amplifications (ThermoFisher) in tumor mass or bone marrow tissue to determine whether MYCN was amplified. The presence of more than four copies of MYCN was regarded as amplification. Patients with MYCN amplification were included in the study.
The tumor volume of the primary site and the longest dimension were tested at diagnosis and after four cycles of induction chemotherapy based on contrast-enhanced CT. Serum neuron-specific enolase (NSE) levels at diagnosis and after four cycles of induction chemotherapy were calculated. Vanillylmandelic acid (VMA) was tested in 24-h urine collection. All patients were treated according to protocols for NB depending on the children’s age and stage and biological features of the disease.[9,10] High-risk patients received four cycles of neoadjuvant chemotherapy (two cycles of cyclophosphamide + topotecan, followed by cisplatin + etoposide and cyclophosphamide + doxorubicin + vindesine + mesna within a 4-week interval). Radical tumor resection was usually performed, followed by tandem high-dose chemotherapy and autologous stem cell transplantation (HDCT/auto-SCT), local radiotherapy and differentiation therapy with 13-cis-retinoic acid. All the treatment procedures were carried out by the multi-disciplinary team (MDT) members, which included experienced oncologists, surgeons, and interventional radiologists. Regular evaluation was performed every 2 months in our department from the end of local radiotherapy. Progressive disease (PD) was defined as any new lesion or an increase in any measurable lesion by >25%.

3. Results

Table 1 lists the clinical characteristics of the patients. The age at diagnosis ranged from 6 to 52 months, with a median of 24 months. Before the initial treatment, four cases were diagnosed by histopathology of the tumor, and 7 cases were diagnosed by bone marrow examinations, which included morphology, flow cytometry, and histopathology. Of the 11 patients with MYCN amplification, nine were in stage IV (81.8%), one was in stage III (9.1%), and one was in stage I (9.1%). Primary tumors were all located in the abdomen, including the adrenal glands in 10 patients (90.9%) and the paravertebral ganglia in one patient (9.1%). The size of the primary tumors was more than 500 cm³, with the longest dimension being over 9 cm in 8 of the 11 patients (72.7%). The most common site of metastasis was bone marrow (72.7%), followed by bone (63.6%) and liver (54.5%). Almost all patients had a higher serum level of NSE than the normal range. NSE was <370 ng/mL in only one patient who had stage I disease, whose primary tumor had a diameter of <5 cm. Eight patients underwent testing for VMA in a 24-h urine sample, of whom 7 had normal levels and 1 had an elevated level. All primary tumors were examined by pathology after 4 to 6 courses of chemotherapy (10/11, 90.9%) or before chemotherapy (1/11). Pathological findings of the primary tumors included poorly differentiated and undifferentiated NB cells in nine cases (81.8%). Because a small number of tumor cells remained, the degree of differentiation could not be judged in the other 2 patients.

The treatment details are shown in Table 2. Eight of 10 (80%) patients who received chemotherapy before resection of the primary tumor showed a good response, with primary tumors reduced by 60% to 99% and serum NSE levels reduced by more than 80% after 4 cycles of chemotherapy. One patient (#7) who did not respond to chemotherapy underwent a second tumor biopsy, and chemotherapy with drugs to which the patient’s tumor was sensitive was chosen according to a chemo-drug experiment on biopsy tissues in mice; the patient had a partial response to the second chemotherapy. Except for two patients (#3 and #7), the other patients all underwent radical resection of the primary tumor. During treatment, nonhematologic and hematologic toxicities occurred in almost all patients, but no toxicity-related deaths occurred.

At the end of follow-up, four patients (#1, #3, #6, and #8; 36.3%) experienced PD and died 1 or 2 years after diagnosis with a median overall survival of 15 months; three of these patients died of lethal brain metastasis. Five patients (45%) survived with a median progression-free survival time of 13 months. Two patients were still under chemotherapy.

4. Discussion

Stage IV NB has a poor outcome. It has been a long-held assumption that treatments for MYCN-amplified NB need to be more intensive and effective. This concept has provided details on the clinical characteristics and therapeutic responses to current therapeutic strategies of children with MYCN-amplified NB in China. Our data from these patients showed that all the children were <5 years old, and the primary tumors were located in the retroperitoneum, which are similar to the results in the literature.[10] We further found that tumors were far more common in the adrenal glands than in the ganglia in the abdomen. NSE and VMA, which are specific biomarkers for the NB response arising from the secretory functions of tumor cells,

Table 1

| Patient# | Age (months) | Primary site | Volume | Longest dimension | Involved sites | Stage | Serum NSE (ng/mL) | 24h urine VMA (mg/day) |
|----------|--------------|--------------|--------|-------------------|---------------|-------|-----------------|-----------------------|
| 1        | 6            | Right adrenal| 67     | 5.6               | Marrow, bone (>one site), liver | N      | >370            | 1.1                   |
| 2        | 15           | Right adrenal| 1027   | 11.3              | Marrow, bone (>one site), liver, mediastinum | N      | >370            | Unknown               |
| 3        | 48           | Right adrenal| 37     | 4.2               | Marrow, bone (>one site), liver, orbit | N      | >370            | Unknown               |
| 4        | 9            | Paravertebral ganglia | 875 | 12.9 | None but tumor mass rupture | III | >370 | 1.1 |
| 5        | 23           | Right adrenal| 761    | 11.2              | Marrow, bone (>one site), liver, orbit, pelvic cavity | N | >370 | 3.6 |
| 6        | 52           | Left adrenal | 574    | 9.2               | Marrow, bone (>one site), liver, orbit, brain | N | 365.7 | 136 |
| 7        | 24           | Left adrenal | 1485   | 12.5              | Marrow | N | >370 | 4 |
| 8        | 22           | Right adrenal| 1135   | 11.9              | Bone (>one site), liver, lung, chest wall, greater omentum | N | >370 | 2.8 |
| 9        | 25           | Left adrenal | 928    | 11.9              | Marrow, bone (>one site) | N | >370 | Unknown |
| 10       | 30           | Left adrenal | 899    | 12.4              | Marrow | N | >370 | 7.4 |
| 11       | 13           | Right adrenal| 39     | 3.4               | None | I | 64.84 | 1.2 |
were present at different levels, with most patients having higherNSE and lower VAM levels than the respective normal ranges, in accordance with the findings of Lee JW, who showed that NB with MYCN amplification was related to a higher NSE, lower VMA, and poorer differentiation.[11] VMA is probably secreted from more mature tumor cells. No ganglioma cells were found, and most cells were poorly differentiated or undifferentiated in pathological sections of all the children’s tumors. These results can explain the low levels of VMA and indicate that NB cells with MYCN amplification are extremely immature. At the time of diagnosis, extensive metastasis had already appeared, especially metastasis to bone marrow.

All patients, except for one with stage I disease, accepted neoadjuvant chemotherapy protocols based on age, MYCN amplification status, image-defined risk factors (IDRFs) and INSS stage, and the chemotherapy drugs included cyclophosphamide, ifosfamide, cisplatin, carboplatin, and Adriamycin. One child who was not sensitive to the indicated chemotherapy regimen adopted adjusted schemes after a drug sensitivity test. He was eligible for removal of the primary tumor after four cycles of modified chemotherapy. Therefore, for children who do not respond to initial chemotherapy, a drug sensitivity test of tumor tissue is a feasible choice for identifying strategies to which the tumor will be sensitive. In our study, most patients responded well to therapy, with an obvious decrease in NSE and tumor volume. The tumor cells in the bone marrow were usually cleared out after two cycles of chemotherapy, with negative results on morphology and flow cytometry analyses. However, disease progression rapidly occurred in the form of metastasis at metastatic sites after all courses of treatment in four patients, with a median OS of 15 months after diagnosis. Although five patients are still alive, only the survival period of the patient with stage I disease exceeded 15 months. This result shows that the currently used therapeutic strategy, comprising chemotherapy, HDCT/ auto-SCT, surgery, and radiotherapy, can effectively inhibit the primary tumor but does not control metastatic cells well. Perhaps more intensive or novel treatments should be explored. In a prospective study, high-dose combined chemotherapy did not improve the prognosis of stage IV patients with MYCN amplification.[12] The efficacy of anti-GD2 antibody in relapsed or refractory patients remains under investigation.[13] Although immunotherapy with maintenance therapy was found to increase 2-year survival in patients with recurrent or refractory NB,[14] it was not analyzed in patients with MYCN amplification, and the 5-year and longer survival values are still unknown. Patients with MYCN amplification often have recurrent or refractory disease. Thus, a more effective treatment modality should target MYCN.

Approaches combating MYCN amplification may need to be considered. MYCN is a member of the MYC oncogene family. It is a transcription factor that can regulate the expression of many target genes and participates in cell growth, apoptosis, tumor invasion, and metastasis. In normal cells, the role of MYCN, which is located on the terminal end of the short arm of chromosome 2, is to shorten the cycle of cell growth, promote proliferation, and inhibit cell differentiation. Since MYCN promotes the proliferation of cells and inhibits differentiation, the MYCN-amplified cells are often undifferentiated or poorly differentiated,[15,16] which is consistent with the poor differentiation of the tumor cells in our clinical patients. In differentiated neurons, the expression of MYCN is downregulated. MYCN-driven transgenic mouse models and primary neural crest cells successfully recapitulate human disease,[17,18] demonstrating the key role of MYCN in NB tumorigenesis. Furthermore, MYCN is related to tumor drug resistance. A large prospective study showed that MYCN amplification was associated with higher expression of multiple resistance protein 1 (MRP1).[19] In an in vitro study, high expression of MYCN increased the expression of MRP1, resulting in failure of chemotherapy due to low intracellular drug concentration.[20] Therefore, inhibiting the expression of MYCN at the transcriptional or translational level may improve antitumor efficacy. A series of proteasomes have been identified,[21] which can be synthetic lethal targets in this type of NB. In our observational study, the size of the cohort was relatively small, and the follow-up duration was short, which might limit the value of the analysis in the study. However, based on these findings, MYCN-amplified NB should be considered carefully, and more novel treatment modalities should be considered by pediatric oncologists.

5. Conclusions
MYCN amplification is associated with poor prognosis although patients have an encouraging initial response to currently used
chemotherapies. New treatment modalities need to be explored to improve outcomes, especially those targeting MYCN.

**Author contributions**

All the Authors made substantial contributions to conception and design of the study. Can Huang and Shiyi Jiang conceived the study and wrote the paper; Jingwei Yang, Xuelian Liao, Yanhua Li, Shanshan Li performed literature search and reviewed the paper.

**References**

[1] Hubbard AK, Spector LG, Fortuna G, et al. Trends in international incidence of pediatric cancers in children under 5 year of age: 1988-2012. JNCI Cancer Spectr 2019;3:kz007.

[2] Brodeur GM, Seeger RC, Barrett A, et al. International criteria for diagnosis, staging, and response to treatment in children with neuroblastoma. J Clin Oncol 1988;6:1874–81.

[3] Iehara T, Hiyama E, Tajiri T, et al. Is the prognosis of stage 4S neuroblastoma in patients 12 months of age and older really excellent? Eur J Cancer 2012;48:1707–12.

[4] Newman EA, Abdessalam S, Aldrink JH, et al. Update on neuroblastoma. J Pediatr Surg 2019;54:383–9.

[5] Matthay KK, Reynolds CP, Seeger RC, et al. Long-term results for children with high risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: a children’s oncology group study. J Clin Oncol 2009;27:1007–13.

[6] Kreissman SG, Seeger RC, Matthay KK, et al. Purged versus non-purged peripheral bloodstem-cell transplantation for high-risk neuroblastoma (COGA3973): a randomised phase 3 trial. Lancet Oncol 2013;14:999–1008.

[7] Westermark UK, Wilhelm M, Frenzel A, et al. The MYCN oncogene and differentiation in neuroblastoma. Semin Cancer Biol 2011;21:256–66.

[8] Sung KW, Son MH, Lee SH, et al. Tandem high-dose chemotherapy and autologous stem cell transplantation in patients with high-risk neuroblastoma: results of SMC NB-2004 study. Bone Marrow Transplant 2013;48:68–73.

[9] Lee JW, Lee S, Cho HW, et al. Incorporation of high-dose 131Imetaiodobenzylguanidine treatment into tandem high-dose chemotherapy and autologous stem cell transplantation for high risk neuroblastoma: results of the SMC NB-2009 study. J Hematol Oncol 2017;10:108–18.

[10] Lee JW, Son MH, Cho HW, et al. Clinical significance of MYCN amplification in patients with high-risk neuroblastoma. Pediatr Blood Cancer 2018;65:e27257.

[11] Verly IR, Van Kuilenburg AB, Abeling NG, et al. Catecholamines profiles at diagnosis: increased diagnostic sensitivity and correlation with biological and clinical features in neuroblastoma patients. Eur J Cancer 2017;72:235–43.

[12] Suits S, Tajiri T, Sera Y, et al. Improved survival for patients with advanced neuroblastoma after high-dose combined chemotherapy based in part on N-myc amplification. J Pediatr Surg 2000;35:1737–41.

[13] Jabbari P, Hanai S, Rezaei N. State of the art in immunotherapy of neuroblastoma. Immunotherapy 2019;11:831–50.

[14] Ploessl C, Pan A, Maples KT, et al. Dinutuximab: an anti-GD2 monoclonal antibody for high-risk neuroblastoma. Ann Pharmacother 2016;50:416–22.

[15] Dang CV. MYC on the path to cancer. Cell 2012;149:22–35.

[16] Adhikary S, Eilers M. Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol 2005;6:635–45.

[17] Weiss WA, Aldape K, Mohapatra G, et al. Targeted expression of MYCN causes neuroblastoma in transgenic mice. EMBO J 1997;16:2985–95.

[18] Olsen RR, Otero JH, Garcia-Lopez J, et al. MYCN induces neuroblastoma in primary neural crest cells. Oncogene 2017;36:5075–82.

[19] Haber M, Smith J, Bordow SB, et al. Association of high-level MRP1 expression with poor clinical outcome in a large prospective study of primary neuroblastoma. J Clin Oncol 2006;24:1546–53.

[20] Haber M, Bordow SB, Gilbert J, et al. Altered expression of the MYCN oncogene modulates MRP gene expression and response to cytotoxic drugs in neuroblastoma cells. Oncogene 1999;18:2777–82.

[21] Wang JC, Jiang J, Shen H, et al. FDA-approved drug screen identifies proteasome as a synthetic lethal target in MYC-driven neuroblastoma. Oncogene 2019;38:6737–51.