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Applications and prospects of targeted therapy for neuroblastoma

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ABSTRACT

Background Neuroblastoma is an extremely malignant tumor in children. For advanced or recurrent cases, existing treatment modalities are limited and efficacy remains disappointing. With the improvement in understanding of molecular biology of neuroblastoma and the development of clinical trials of targeted drug therapy, a variety of targeted therapies for neuroblastoma have appeared.

Data sources All the recent literatures on targeted therapies of neuroblastoma on PubMed were searched and reviewed.

Results This article reviewed targeted therapies of neuroblastoma going through clinical trials and obtained preliminary results. The features, advantages and disadvantages of targeted radiation therapy, immunotherapy, gene and pathway molecular inhibitor and angiogenesis inhibitor were discussed.

Conclusion This study provides references for better understanding the current progress of targeted therapies for neuroblastoma.

INTRODUCTION

Neuroblastoma is an extremely malignant and aggressive childhood tumor, which is prone to distant metastasis. Despite the improvement of multimodal treatment regimens including induction chemotherapy and surgery, intensive consolidation chemotherapy, irradiation and autologous hematopoietic stem-cell rescue, the outcome for children with advanced or recurrent diseases has been improved only modestly. With the promotion of molecular biological research of neuroblastoma, a variety of targeted therapies have been developed for clinical trials, providing promising intervention therapies for high-risk neuroblastoma, especially for relapsed/refractory disease. In this review, we presented the current status and prospects of targeted therapies for neuroblastoma going through clinical trials and obtained preliminary results.

The significance of targeted therapy for neuroblastoma

About half of children with neuroblastoma have distant metastasis at the time of diagnosis. Multimodality approaches including inducing chemotherapy and surgery, consolidation chemotherapy with autologous hematopoietic stem-cell rescue, radiation therapy and immunotherapy provide the standard-of-care treatment strategy for high-risk neuroblastoma to date.[1] More than 50% of patients diagnosed with high-risk neuroblastoma were either resistant to conventional chemotherapies or relapsed after treatment. Patients with recurrent or refractory neuroblastoma had particularly low survival rates according to an analysis of large registry-based results.[2 3] The 5-year overall survival (OS) postrelapse was 20%, and the 5-year OS was only 8% for patients in stage 4 with postrelapse in a report from the International Neuroblastoma Risk Group project.[3] In 35 phase I/II clinical trials (from August 2002 to January 2014) for recurrent/refractory neuroblastoma conducted by Children's Oncology Group (COG), the 4-year OS was reported to be 20%±2%.[4] The analysis of these trials showed that most patients with high-risk neuroblastoma did not continue to receive other treatments that beyond inducing chemotherapy due to insufficient response to chemotherapy. For example, in a randomized phase III trial conducted by HR-NBL1/SIOPEN, 51.5% of the high-risk patients failed to continue beyond induction therapy.[5 6] Therefore, the development of novel agents is imperative.

Recurrent neuroblastomas usually harbor heavy mutational burden but reduced subclonal heterogeneity compared with primary tumors at diagnosis. In a retrospective study of 138 patients whose tumors had been sequenced at diagnosis, second-look surgery and relapse revealed a substantial mutational evolution during treatment and progression. A variety of mutated genes are targetable and promising.[7] Remarkably, anaplastic lymphoma kinase (ALK) was the most commonly mutated gene at diagnosis and gained a higher frequency of aberrations in recurrent tumors. An enrichment of activating mutations in the RAS-MAPK pathway was also observed in tumors after

chemotherapy or at relapse.[7 8] Therefore, targeted therapies have become a promising approach to treating patients with neuroblastoma (especially relapsed and refractory cases) and to improving the prognosis.

With the intensive study of the etiology of neuroblastoma, more and more targeted drugs have been introduced. Several new drugs have been tested in clinical trials or are currently being tested, which include targeted radiation therapy, targeted immunotherapy, gene and pathway molecular inhibitor and angiogenesis inhibitor. The following sections discuss these drugs in detail.

Approaches to targeted therapies for neuroblastoma

Targeted radiation therapy

Metaiodobenzylguanidine (MIBG) is a compound that can be combined with radioactive iodine (^{131}I) to deliver targeted radiation therapy. It is an analog of norepinephrine and was first used as a radioactive tracer for imaging of the adrenal medulla. Tumors cells derived from sympathetic nervous system tissues, such as neuroblastoma, express the norepinephrine transporter (encoded by SLC6A2 gene), which is thought to have high specificity and sensitivity to MIBG.[9] MIBG labeled with ^{131}I could make it radioactive, which could achieve the purpose of treatment by killing tumor cells.[10] Several clinical trials have shown that the response rate to treatment can reach 37% by gradually increasing the frequency and cumulative dose of ^{131}I-MIBG in neuroblastoma, and the MIBG therapy also showed high effectiveness and good tolerance.[11–13] Now, ^{131}I-MIBG is mainly applied to clinical treatment of high-risk, recurrent and refractory patients.

Targeted immunotherapy

Anti-GD2 antibodies

Disialoganglioside (GD2) is a b-series ganglioside and plays an essential role in embryonic development.[14] GD2 is highly expressed on the surface of neuroblastoma tumor cells, whereas its expression in normal tissues is limited. What is more, interfering with GD2 expression has a significant antitumor effect making this surface glycolipid antigen an ideal target for immunotherapy of neuroblastoma.[15] There are currently three types of clinically used GD2 antibodies: mouse monoclonal antibodies (mAb), human-mouse chimeric antibodies, and humanized antibody.

Active immunotherapy

Neuroblastoma cells can avoid the attack of T cells and natural killer (NK) cells by downregulating human leukocyte antigen and adhesion molecules. At the same time, its cell surface carries abundant gangliosides and sialic acid-containing sugars and proteins, making it even immunosuppressive.[16] Therefore, it is of necessity to develop active immunotherapy of neuroblastoma, the most representative of which are anti-idiotype vaccine and bivalent ganglioside vaccine.

Adoptive T- cell therapy

Adoptive cell therapy refers to the therapy isolating a large number of tumor-specific lymphocytes (eg, T-lymphocytes) and re-injecting them into the patient after genetic modification and in vitro culture.[16] The high-risk group neuroblastoma mainly focuses on chimeric antigen receptor (CAR)-T-cell therapy. Adoptive cell therapies aiming at high-risk neuroblastoma mainly concentrate on CAR-T-cell therapy. CAR consists of a single-chain variable fragment (anti-GD2), a transmembrane domain and an extracellular domain of an inner domain. CAR connects tumor cell surface antigens and provides co-stimulatory signals to T cells, enabling T cells to directly recognize and kill tumor cells beyond the major histocompatibility complex presentation mechanism.[17]

Gene and pathway molecular inhibitor

ALK inhibitors

ALK belongs to the insulin receptor protein-tyrosine kinase superfamily and is considered as an oncogene in human cancers. ALK aberrations in neuroblastoma include copy number variation (CNV), amplification and mutation. ALK copy number gain is approximately 15%–25% of neuroblastoma, amplification is seen in 4% of high-risk cases and mutation is found in 6%–10% of cases. The most common point mutations of ALK are R1275Q (43%), F1174L (30%) and F1245C (12%), which could induce autophosphorylation of the tyrosine kinase domain and abnormal activation of the ALK receptor.[16 18 19] The results of preclinical studies confirmed that ALK was a promising therapeutic target, which had the following advantages and characteristics. First, ALK abnormalities can be inhibited by small-molecule blockers, which are easy to prepare and are convenient to apply. Second, abrogation of ALK was effective both in the wild-type and in the mutated neuroblastoma cells.[20] Third, ALK alterations are usually associated with MYCN amplification. ALK is a transcriptional target of MYCN, whereas ALK activates transcription of MYCN in neuroblastoma cell lines.[21] Therefore, ALK inhibitors also have a therapeutic effect on neuroblastoma in the high-risk cases with MYCN amplification.

Targeting MYCN-dependent transcription and N-Myc protein stability

The transcription factor MYCN belongs to the MYC family, which is a crucial for regulating cell proliferation, cell growth, cell differentiation and survival in embryonic central nervous system cells. The amplification of the MYCN gene is the most common focal gene mutation in sporadic neuroblastoma, and it is also a strong indicator of the poor prognosis. However, the MYCN gene is still difficult to serve as a direct therapeutic target. MYCN can be repressed by targeting the transcription process of MYCN as well as by reducing the stability of the N-Myc protein.[16] For example, bromodomain and extra terminal (BET) inhibitor can inhibit BET family proteins, which are the transcription factors binding at the promoter

of MYCN gene, and can suppress the transcription of MYCN.[22] Small molecule inhibitor of Aurora-A kinase destroys Aurora-A kinase/N-Myc protein complex, maintaining neuroblastoma cell proliferation, destabilizing N-Myc protein to mediate tumor regression.[23]

Pathway inhibitors

Aberrantly activated gene pathways are important drivers in the malignant progression of neuroblastoma. Aberrations of ALK also induce activation of multiple downstream signaling like phosphatidylinositol 3-kinase/protein kinase B/mammalian target (PI3K/AKT/mTOR) and Ras/mitogen-activated protein kinase (RAS-MAPK) signal transduction pathways.[24] PI3K/AKT/mTOR pathway is a key intracellular signaling pathway for tumorigenesis, promoting cell growth, proliferation, metastasis, angiogenesis and glucose metabolism.[25] Abnormal activation of this pathway is common in high-risk neuroblastoma.[26] In recent years, several novel agents have been developed to inhibit the occurrence and progress of neuroblastoma by blocking different targets in the PI3K/AKT/mTOR pathway.

Angiogenesis inhibitor

Angiogenesis is a key process for the continuous growth and metastasis of neuroblastoma. To date, a large number of pro-angiogenic factors have been identified, whose complicated interaction induces angiogenesis in neuroblastoma, including vascular endothelial growth factor (VEGF), interleukin 8 (IL-8), fibroblast growth factor 2 (FGF-2), transforming growth factor-α, platelet-derived growth factor A (PDGF-A), erythropoietin and angiopoietins.[27] Expression of VEGF and VEGF receptor (VEGFR) was associated with high-risk and high-stage neuroblastoma, and these factors were sensitive targets to antiangiogenic therapy.[28 29] Antiangiogenic agents include single-pathway inhibitors and multipathway inhibitors. For example, bevacizumab is a single-pathway antiangiogenic antibody against VEGF that inhibits the binding of VEGF to the receptors Flt-1 (VEGFR-1) and KDR (VEGFR-2).[30] Ponatinib and imatinib are novel inhibitors for multiple tyrosine kinases involved in angiogenesis including FGFR1–4, RET, PDGFR, c-KIT, FLT3, MEKK2 and the VEGFR 1 and 2.[31]

Advantages and disadvantages of currently applied targeted agents

Advantages of targeted therapies

High specificity

Due to focusing on a certain target of neuroblastoma tumor cells, drugs usually have higher specificity. 3F8 was the earliest mouse mAb used to treat neuroblastoma, belonged to IgG3 and has a high affinity for GD2.[32] Hu3F8 is a humanized mAb of 3F8, which has longer retention on the GD2-positive cell surface. Besides, GD2 anti-idiotype vaccine induced GD2-specific humoral immune response against gangliosides on the surface of neuroblastoma cells in mice and specifically killed neuroblastoma cells.[33] Eleven of 13 children exhibited an IgM and/or IgG antibody response against NeuGcGM3 in the phase I trial of the anti-idiotype vaccine racotumomab. JQ1 is a BET inhibitor targeted BRD4 from the MYCN promoter, results in inhibition of MYCN transcription[34] and leads to cell cycle arrest and apoptosis, which may be beneficial for patients with MYCN-amplified neuroblastoma.

Great antitumor activity

Various targeted drugs showed great antitumor activity. A retrospective analysis of 39 patients with recurrent or refractory neuroblastoma who were treated with ^{131}I-MIGB monotherapy demonstrated an objective response rate (ORR) of 46%.[13] The human-murine chimeric anti-GD2 antibody ch14.18 (dinutuximab) had the same complement-mediated cytotoxicity (CMC) potency and 50-fold to 100-fold higher antibody-dependent cell-mediated cytotoxicity (ADCC) compared with mouse mAb 14G2a.[35] The humanized anti-GD2 mAb hu14.18 has enhanced ADCC and has reduced CMC compared with mouse mAb, with a great ORR of 61.5% when combining with chemotherapy, IL-2, granulocyte-macrophage colony-stimulating factor (GM-CSF) and infusion of NK cells.[36] Phase I trial of third-generation CAR-T GD2-CAR3 showed an increment of circulating IL-15 levels as an expansion of GD2-CAR3T cells was observed. The significant expansion of CD45/CD33/CD11b/CD163+ bone marrow cells in all children indicated that GD2-CAR3 was effective in early antitumor responses.[37]

Improved prognosis

A variety of targeted drugs improved the prognosis of patients with high-risk neuroblastoma, especially in targeted immunotherapies. A comprehensive therapy including ^{131}I-MIGB could improve the prognosis of refractory neuroblastoma, with a 3-year OS of 62%±8% in the new approaches to neuroblastoma therapy phase II study.[38] Compared with retinoic acid (RA) alone, anti-GD2 mAb ch14.18 combined with GM-CSF plus IL-2 and RA significantly improved 2-year progression-free survival (PFS) (66%±5% vs 46±5%, p=0.01) and OS (86%±4% vs 75±5%, p=0.02).[39] In the phase I trial of a bivalent gangliosides vaccine combined with immunological adjuvant OPT-821 and β-glucan, the result showed astonishing antitumor activity with a 2-year event-free survival of 80%±10% and OS of 93%±6%.[40] The combination of Aurora A kinase inhibitor MLN8237, irinotecan and temozolomide in phase I trial has shown the ORR of 31.8% and the 2-year PFS of 52.4%.[41] The phase II trial has demonstrated antitumor activity (1-year PFS 34%) of combination, particularly in children with MYCN non-amplified neuroblastoma.[42] The AKT inhibitor perifosine showed a 3-year PFS rate of 36% in a phase I trial in patients with relapsed/refractory neuroblastoma, and 9 of 27 children without MYCN amplification had a median PFS of 54 months. Multikinase angiogenesis inhibitor imatinib revealed the complete remission (CR) rate of 21% at the time of the first report and 10-year OS

of 12.5% in prolonged phase II study, suggesting imatinib is efficient in some subjects with relapsed/refractory neuroblastoma.[43]

Less side effects

Targeted drugs usually have fewer side effects, which leads to better compliance and better clinical use. Hu14.18K322A is a humanized anti-GD2 mAb to 14G2a, which has a single point mutation (K322A) designed to prevent activation of the complement cascade, thus reducing complement-mediated pain and the possibility of allergic reaction.[44] Dose-limiting grade 3 or 4 toxicities have included sensory neuropathy, serum sickness and posterior reversible encephalopathy syndrome. Grade 3 or 4 pain has been observed in most patients.[44] Compared with anti-GD2 mAbs, the regimen including the bivalent gangliosides vaccine had advantages of no neuropathic pain.[40] The common side effects of MIBG were myelosuppression and diarrhea, which showed a good application prospect.[45] The ALK inhibitor crizotinib was well tolerated without evidence of cumulative toxic effects in its phase I consortium study of pediatric refractory solid tumors, with common side effects of mild nausea, mild vomiting and mild visual disturbances.[46]

Disadvantages of targeted therapies

Limited antitumor activity

Although anti-GD2 immunotherapies showed good cytotoxicity against neuroblastoma, several targeted drugs such as gene and pathway inhibitors showed limited antitumor activity in early phase clinical trials. COG developed a phase I trial of crizotinib in children with refractory neuroblastoma. In this trial, only 1 of 11 children harboring ALK translocation had CR, 2 children remained SD and the remaining cases had progressive disease.[47] The phase II trials of mTOR inhibitor temsirolimus demonstrated that the 1-year PFS rate of patients with relapsed/refractory neuroblastoma was only 24.7% of temsirolimus plus irinotecan/temozolomide, which was significantly lower than the efficacy of ch14.18 plus irinotecan/temozolomide with 1-year PFS rate of 76.5%. Moreover, the angiogenesis inhibitor bevacizumab combined with irinotecan and temozolomide did not improve the response rate of refractory neuroblastoma compared with irinotecan/temozolomide treatment in phase II study.[30]

Drug resistance

The utility of some targeted drugs has been limited due to drug resistance and disease progression. ALK inhibitor is an example, and the early phase clinical trials of ALK inhibitor crizotinib showed high disease progression rate.[47 48] Vitro studies revealed that cell lines of neuroblastoma harboring F1174 and F1245 mutated ALK increased ATP-binding affinity and reduced ability to competitively inhibit ATP, resulting in resistance.[49] Another study found that no additional mutations or CNV occurred in ALK, whereas the level of tyrosine kinase receptor activation altered, with significantly increased EGFR phosphorylation in crizotinib-resistant neuroblastoma cell line.[50] Besides, the study showed that activation of receptor tyrosine kinases and PI3K signaling promoted drug resistance of BET inhibitor in neuroblastoma, informing efficacious synergistic therapies.[51]

Immunogenicity

Although several targeted immunotherapies have obtained good curative effects, they still have common shortcomings, one of which is immunogenicity. Previous studies have found the incidence of antidrug antibody was 70%–80% for human antimouse antibody (m3F8), 19%–21% for human antichimeric antibody (ch14.18) and 40% for human antihuman antibody (hu14.18K322A), which resulted in a faster elimination of antibodies from the body due to neutralizing antibodies and affected the half-life of antibodies in the body.[52] Also, Surek and Ektomab are anti-GD2 trifunctional bispecific full-length antibodies, containing the Fab fragments to the GD2/GD3-specific antibody ME361 and the T cell-specific CD3 antigen, which recruit cytotoxic lymphocytes and target them towards GD2-positive tumors. Since they consist of mouse and rat original antibody fragments, the immunogenicity and specificity of them also limit the prospects of their utilization. In addition, ME361 antibodies display cross-reactivity to GD3, which may increase side effects by influencing healthy body tissues.[53]

Difficult to penetrate tumor tissue

Adoptive cell therapy for neuroblastoma faces multiple hurdles, and the first is the difficulty in penetrating the tumor to discharge their cytotoxic function. Unlike the high level of migrating efficacy of CAR-T cells against hematological malignancies, solid tumors including neuroblastoma secrete chemokines, such as CXCL12 and CXCL5 which inhibit T-cell migration into the tumor regions.[54] Besides, the abnormal vasculature hinders effective infiltration, and in the surrounding matrix and the physical barrier of tumors, immunosuppressive bone marrow cells can be attracted to the tumor microenvironment, thereby preventing T-cell infiltration.[54] Solving these problems is a major challenge for CAR-T therapy.

CONCLUSION

In the past decade, a variety of clinical trials of novel targeted agents have been conducted. Targeted immunotherapy has played a prominent role in it. Anti-GD2 chimeric mAb ch14.18 became the first drug approved by the US Food and Drug Administration (FDA) for first-line treatment of high-risk neuroblastoma in nearly 30 years, which was a breakthrough in the treatment of neuroblastoma. The widespread administration of anti-GD2 mAb has shown extraordinary antitumor efficacy, has greatly improved the survival rate of children with neuroblastoma and is presently the most promising drug in the treatment of neuroblastoma. The combination

of cytokines and anti-GD2 mAbs enhanced the synergy effect, leading to stronger cytotoxicity efficacy. The development of CAR-T therapy in neuroblastoma is still in an early stage. The current barriers of CAR-T are mainly target selection, antibody site screening, optimization of CAR structure, and enhancement of the migrating efficacy.

In addition, therapies targeting actionable mutations and abnormally activated signaling pathways are also a hot topic of current researches, due to the relatively high mutant frequency of recurrent neuroblastoma. Several novel agents and combinations of small molecular inhibitors have been tested preclinically and in early phase clinical trials. However, most results of clinical trials were disappointing, with limited antitumor activity and low rates of objective response. Moreover, it was usually unable to obtain tumor tissues at the time of relapse for acquiring information about mutations and abnormal pathways when children were enrolled in clinical trials. The ignorance of molecular pathways may restrict the efficacy of the drugs.

Despite a myriad of targets, the number of high-risk neuroblastomas for randomized clinical trials is limited. Early identification of patients with neuroblastoma at a very high risk of treatment failure has become a trend for early intervention by novel agents to avoid patients becoming refractory or relapsed cases.[6] This requires a deeper understanding of the molecular biology of the relapsing process and resistant mechanism of neuroblastoma, and an early predictive model including molecular biomarkers.

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