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Promising therapeutic targets in neuroblastoma

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Abstract

Neuroblastoma, the most common extra- cranial solid tumor in children, is derived from neural crest cells. Nearly half of patients present with metastatic disease, and have 5-year EFS of less than 50%. New approaches with targeted therapy may improve efficacy without increased toxicity. The current review will evaluate three promising targeted therapies, including ^{131}I-metaiodobenzylguanidine (MIBG), a radiopharmaceutical taken up by the human norepinephrine transporter expressed in 90% of neuroblastomas, immunotherapy with monoclonal antibodies targeting the GD2 ganglioside, expressed on 98% of neuroblastoma cells, and inhibitors of ALK, a tyrosine kinase which is mutated or amplified in approximately 10% of neuroblastoma and expressed on the surface of most neuroblastoma cells. Early phase trials have confirmed the activity of ^{131}I-MIBG in relapsed neuroblastoma, with response rates of about 30%, but the technical aspects of administration of large amounts of radioactivity in young children and the limited access have hindered incorporation into treatment of newly diagnosed patients. Anti-GD2 antibodies have also demonstrated activity in relapsed disease, and a recent phase III randomized trial showed a significant improvement in event-free survival for patients receiving chimeric anti-GD2 (ch14.18) combined with cytokines and isotretinoin after myeloablative consolidation therapy. A recently approved small molecule inhibitor of ALK has promising pre-clinical activity for neuroblastoma, and is currently in phase I and II trials. This is the first agent directed to a specific mutation in neuroblastoma, and marks a new step toward personalized therapy for neuroblastoma. Further clinical development of targeted treatments offers new hope for children with neuroblastoma.

Introduction

Neuroblastoma, the most common extra- cranial solid tumor in children, is derived from primordial neural crest cells that ultimately inhabit the sympathetic ganglia and adrenal medulla. The clinical behavior, which ranges from spontaneous maturation to inexorable progression despite multimodal intensive therapy, is attributable to molecular differences in the tumor. The high-risk clinical prognostic factors of age greater than 18 months and advanced stage, are closely associated with unfavorable biologic risk factors, including unfavorable histopathology, tumor amplification of the *MYCN* oncogene, and loss of heterozygosity of 1p and 11q, or other partial chromosome deletions(1). The 5-year event-free survival (EFS) for high-risk neuroblastoma is less than 50%, including patients with

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metastatic neuroblastoma greater than 18 months of age, and patients with locoregional or metastatic neuroblastoma with tumor *MYCN* gene amplification(2). The best outcome reported until recently for high-risk neuroblastoma was achieved with intensive combination induction chemotherapy and surgery, followed by myeloablative therapy with hematopoietic stem cell rescue, and then differentiation therapy with isotretinoin(3), the first tumor-targeted therapy with demonstrated activity in neuroblastoma.

Although there are extensive laboratory studies and some early clinical trials investigating small molecule inhibitors and antibodies targeting relevant genetic pathways implicated in neuroblastoma proliferation, such as PI3kinase, mTOR, IGF1R and many others, the current review will put into perspective two of the most successful extremely tumor-specific agents in current use which may further improve outcome, and evaluate a third more recent addition to the armamentarium. The first is ^{131}I-metaiodobenzylguandine (MIBG), which targets the norepinephrine transporter (expressed in 90% of neuroblastoma tumors) for cell-specific uptake, then destroys the cell with the targeted radiation (4). The second is anti-GD2 antibody, targeting GD2 ganglioside, expressed on >98% of neuroblastoma and mediates immune destruction of the cells. This antibody combined with cytokines improves survival for patients with high-risk disease (5). The third target is the *ALK* gene, which encodes a receptor tyrosine kinase and has now been implicated as an oncogenic driver in neuroblastoma. *ALK* mutations occur in 8–12% of neuroblastoma at diagnosis (6, 7), and germline, *ALK* mutations are responsible for the majority of familial cases (8, 9). Small molecule inhibitors with proven utility in ALK-rearranged cancers (10) have shown promise in pre-clinical studies in neuroblastoma, with early phase clinical trials underway. Other targeted approaches to neuroblastoma and other pediatric malignancies are addressed elsewhere in this issue (11–15).

Targeting the human norepinephrine transporter (hNET) with MIBG

The observation that 90% of tumors are MIBG-avid provides the rationale for utilizing ^{131}I-MIBG as a targeted radiopharmaceutical for high-risk neuroblastoma. MIBG is an aralkylguanidine norepinephrine analogue originally developed to visualize tissue of sympathetic neuronal origin, which has now become an essential tool for neuroblastoma staging and response (Figure 1) (16). Early clinical trials (Table 1A) in relapsed neuroblastoma showed that ^{131}I-MIBG was effective in producing significant response rates and that the only severe acute toxicity was myelosuppression, which could be abrogated by hematopoietic stem cell transplant (HCT) (17–21). The activity administered has varied, with set total amounts of 50 to 100 mCi given in earlier European studies regardless of patient size, while one UK study based dose on the amount needed to limit whole body radiation to 1–2 Gy. The US studies used weight-based dosing, which correlated significantly with whole body radiation received (22). The maximal practical weight-based dose in a phase I trial was established as 18 mCi/kg. This dose was then tested in a large phase II study of 164 patients, of whom 30% required hematopoietic stem cell support to prevent prolonged myelosuppression. Minor acute toxicities were grade 1 or 2 nausea and vomiting, parotid pain and rarely changes in blood pressure. Late toxicities included a 12% incidence of grade 2 hypothyroidism, despite the routine use of SSKI to block thyroid uptake of free radioactive iodide (23), and the rare occurrence of ovarian failure or myelodysplastic syndrome with acute myeloid leukemia in less than 5% of patients (24). Importantly, almost all the studies have reported impressive response rates in relapsed disease, with the largest Phase II showing 37% of patients with a partial or complete response (20).

Increasing tumor radiation dose

Attempts to further improve response rate included increasing the dose, using a different iodine isotope, or a higher specific activity form of the molecule (Table 1A). Due to radiation safety limitations, the increase in the dose was accomplished by administering a rapid sequence double infusion given two weeks apart and supported by HCT. In this fashion, a single patient could receive as much as 42 mCi/kg over a two-week period in a recent phase I study; however, the response rate in that limited number of patients did not appear to be different than the standard 18 mCi/kg dose (25). Other investigators have reported benefit from repeated MIBG infusions given six to 12 weeks apart, with continued improved response in some of the patients with each successive infusion (26, 27). One small phase I trial used ^{125}I-MIBG, rather than the usual ^{131}I-MIBG, based on the hypothesis that the different isotope would be more effective for microscopic tumors or single cells, since the Auger electrons travel only a few nanometers, whereas the beta particles of ^{131}I are more effective in tumors > 1 millimeter. Myelosuppression was significant, despite a lower whole body radiation dose (28). Recently, a the New approaches to Neuroblastoma Therapy (NANT) phase I trial of “no-carrier added” ^{131}I-MIBG was completed, based on pre-clinical data that non-radioactive “carrier” MIBG molecules in the standard preparation of MIBG (specific activity 1.2 MBq/μg) inhibit uptake of ^{131}I-MIBG, resulting in less tumor radiation and increased risk of cardiovascular toxicity (29). In the no-carrier added preparation of MIBG, virtually every molecule is radioactive (specific activity of 165 MBq/μg). The NANT trial showed a similar toxicity and response profile to the standard preparation, but had the advantage of being able to be infused over 30 min, instead of 90 to 120 minutes (30).

Combining MIBG with radiosensitizers or chemotherapy

Another approach to improving efficacy has been using combination therapy, by adding chemotherapy or other radiosensitizers to the MIBG (Table 1A). Exploratory studies showed the feasibility of giving 7–15 mCi/kg of ^{131}I-MIBG followed10–14 days later by myeloablative doses of carboplatin, etoposide and melphalan (31–34). A Phase I study of 24 patients with refractory neuroblastoma established the MTD at 12 mCi/kg of ^{131}I-MIBG, with carboplatin 1,500 mg/m^2, etoposide 1,200 mg/m^2, and melphalan 210 mg/m^2. The dose-limiting toxicities were mucositis, vascular leak, veno-occlusive disease, and sepsis, but only one toxic death (35). A subsequent NANT phase II study of 50 patients reported the main toxicity as veno-occlusive disease, and 7/50 responses in this highly refractory group of patients (36).

Other studies focused on using the ^{131}I-MIBG combined with chemotherapy or radiosensitizers in a non-myeloablative regimen in refractory patients. Italian investigators treated 16 patients with relapsed or refractory neuroblastoma with 200 mCi ^{131}I-MIBG combined with cisplatin and cyclophosphamide with or without etoposide and vincristine, and obtained 12 partial responses(37). Two studies have combined MIBG with a camptothecin, with tolerable toxicity and measurable responses, including the European study of topotecan with double infusion of MIBG(38), and the NANT Phase I study of irinotecan and vincristine with MIBG (39). Recently, preclinical data showed additive growth inhibition of the combination of a histone deacetylase inhibitor, vorinostat, with radiation in a metastatic murine neuroblastoma model (40). In addition, vorinostat increased uptake of MIBG in the neuroblastoma tumors, via increased expression of hNET(41). These findings led to a NANT phase I study of vorinostat combined with ^{131}I-MIBG, which is currently continuing enrolling at Dose Level 5 with no dose-limiting toxicity to date (MIBG, 18 mCi/kg; vorinostat, 230 mg/m^2). Other radiosensitizers are under study in pre-clinical testing for neuroblastoma, which may prove useful in the future (42).

A few recent studies have tested the incorporation of MIBG therapy into induction for newly diagnosed patients (Table 1). Forty-one patients at an Amsterdam center treated with two cycles of ^{131}I-MIBG prior to addition of chemotherapy had a response rate to the MIBG of 66% (43). German studies evaluated the addition of MIBG therapy at the end of induction and prior to myeloablative therapy for patients with residual MIBG positive disease, with a response rate of 46%, but no improvement in overall survival (44). A Children's Oncology Group pilot trial (ANBL09P1) will test the addition of MIBG with vincristine and irinotecan prior to myeloablative therapy for all high-risk patients, regardless of residual disease, and if feasibility and tolerability is demonstrated, a randomized trial will be undertaken.

Future perspectives for MIBG

^{131}I-MIBG therapy is a promising strategy which now requires randomized testing in newly diagnosed high risk patients. The practicality of this targeted radiopharmaceutical is rapidly increasing, with nine North American and at least seven European pediatric centers regularly administering MIBG therapy, and more centers in development. Due to the whole body radiation accompanying the tumor dose, further testing of compounds that will increase tumor uptake and sensitivity without increasing normal organ toxicity is essential, as is the investigation of different radioisotopes, such as the alpha emitter, astatine-211 (45). Better pre-therapy tumor dosimetry using SPECT-CT with tracer doses of ^{131}I-MIBG (46), or PET-CT with ^{124}I-MIBG (47) may also help to personalize the dose.

GD2-targeted immunotherapy of high-risk neuroblastoma

Promising results have been observed with immunotherapy targeting a surface glycolipid molecule, disialoganglioside (GD2), which is uniformly expressed by neuroblastoma and glioma, some melanoma, and sarcomas (48, 49). In normal human tissues, GD2 expression is weak and restricted to neurons, melanocytes and peripheral pain fibers (50). Thus, GD2 is an ideal antigen target for immunotherapy of neuroblastoma. Three first generation (mAbs 14G2a, ch14.18 and 3F8) and three second generation GD2-directed antibodies (Hu14.18-IL2, hu14.18K332A, and mAb1A7) have been investigated for immunotherapy of neuroblastoma (Table 1B, Figure 2).

First generation anti-GD2 mAbs

14G2a is an IgG 2a murine anti-GD2 monoclonal antibody. In two phase I trials, the dose of mAb14G2a was escalated from 25 to 500mg/m^2/course (Table 1B). Toxicities mainly consisted of reversible pain, tachycardia, fever, changes in blood pressure, hyponatremia and urticaria, and were more severe in adult patients. Pain is thought to occur due to binding of antibody to peripheral nerve fibers expressing GD2 (50). These two phase I trials included 19 neuroblastoma, 3 osteosarcoma and 11 melanoma patients. Therapeutic activity was observed, with 1 complete response and 2 partial responses in neuroblastoma patients, and 6 minor responses including 3 in neuroblastoma patients (51, 52). Pharmacokinetic studies revealed a beta t ½ of 18.3 ± 11.8 h (53). As antibody-dependent cellular cytotoxicity (ADCC) is the key antitumor mechanism of therapeutic antibodies, and IL-2 was shown to augment lymphocyte-mediated ADCC *in vitro* (54) and anti-tumor activity of 14G2a *in vivo* (55), a phase I trial of14G2a in combination with IL2 was conducted in 31neuroblastoma patients and 2 osteosarcoma, with1 complete response in osteosarcoma and 1 partial response in neuroblastoma patients (56).

3F8 is a murine IgG3 anti-GD2 antibody against GD2 developed in the 1980's, with similar side effects and indications of anti-neuroblastoma activity as 14G2A (57). A phase II study of 3F8 showed that 13/34 patients with Stage 4 neuroblastoma in first or subsequent response remained progression free for 40–130 months (57). A follow-up phase II report of

3F8 + GM-CSF in 136 patients showed an overall 5-year EFS of 38% for patients without prior relapse, and a better outcome for patients with the *FCGR2A* (R/R) genotype polymorphism, which favors the binding of the IgG3 antibody. (58)

MAb Ch14.18 consists of the variable regions of murine IgG3 anti-GD2 mAb 14.18 and the constant regions of human IgG1-κ (59). Phase I clinical trials confirmed activity in relapsed neuroblastoma and a similar toxicity profile as mAb14G2a (60, 61). As expected, the half-life of ch14.18 was longer than 14G2a, with a beta t 1/2 of 66.6_+27.4h. (60, 62). Since GM-CSF not only raises the number of leukocytes but also enhances their anti-GD2 mediated ADCC (63), a pilot study of ch14.18 + GM-CSF showed responses in patients with recurrent/refractory neuroblastoma (64). This led to a phase II national study which confirmed the efficacy of ch14.18 + GM-CSF, with 2CR, 2 PR, 1 MR and 2 SD in 32 patients with recurrent/refractory neuroblastoma (65). Since most responses were in bone marrow or bone, it was hypothesized that this approach would be most effective in the setting of minimal residual disease (MRD). Subsequently, the feasibility of giving ch14.18 in combination with GM-CSF, IL2 and isotretinoin during the early post-transplant period was demonstrated in 2 pilot-phase I studies (66, 67).

These clinical trials led to the pivotal randomized COG phase III study, to determine if immunotherapy with ch14.18 combined with GM-CSF and IL2 on the backbone of isotretinoin would improve survival compared to isotretinoin alone for children with high-risk neuroblastoma in first response after myeloablative therapy and stem cell rescue. Eligible patients were randomized after stem cell transplantation to 6 cycles of isotretinoin (standard) or isotretinoin with 5 intercalated cycles of ch14.18 combined with GM-CSF or IL2 in alternating cycles (immunotherapy). Analysis of 226 eligible patients showed that EFS was significantly higher for 113 patients randomized to immunotherapy, with 2-yr estimated EFS from randomization of 66%±5% vs. 46%±5% (p=0.0115) for the 113 randomized to istotretinoin alone. Overall survival (OS) was also significantly higher for immunotherapy group (86%±4% vs 75%±5% at 2 yrs, p=0.0223) (5). This major advance is the first effective immunotherapy for high-risk neuroblastoma and also the first successful immunotherapy to target a non-protein antigen.

Although ch14.18 in combination with IL2 and GM-CSF has been shown to improve the outcome of high-risk neuroblastoma, the treatment is associated with significant toxicities, especially in cycles containing IL2. Furthermore, the exact contribution of cytokines *in vivo* remains unclear. To address these issues, SIOPEN is conducting a study that randomizes patients with high-risk neuroblastoma to ch14.18 alone or in combination with subcutaneous infusion IL2 to ameliorate toxicities associated with intravenous administration of IL2.

Second generation GD2-targeted immunotherapy and future perspectives

With the successful demonstration that ch14.18 + cytokines significantly improved outcome of patients with high-risk neuroblastoma, a COG phase III trial is ongoing to collect comprehensive toxicity data for regulatory approval of ch14.18. In addition, immunophenotypes such as FCGR3A and FCGR2A polymorphism (58, 68) or Killer-Immunoglobulin-like Receptors (KIR) -ligand mismatch (69) that may affect ADCC activity and thereby clinical response to ch14.18 is under investigation. Meanwhile, second-generation anti-GD2 antibodies and an anti-idiotype antibody vaccine have been developed and are currently in early phase clinical trials.

Hu14.18-IL2 is a fusion protein of humanized anti-GD2 antibody (hu14.18) and IL-2. The maximum tolerated dose in a phase I trial was 12 mg/m^2/d, approximately 50% that of ch14.18. Clinical toxicities were similar to those reported with IL-2 and anti-GD2 mAbs, and antitumor activity was noted in three of 27 neuroblastoma patients, although there were

no measurable complete or partial responses (70). A phase II study of this immunocytokine showed 5 CR in 23 patients with neuroblastoma evaluable only by MIBG and/or bone marrow histology, but no responses for patients with measurable disease (71). In this study, patients with KIR -ligand mismatch seemed to be associated with better clinical response to immunotherapy with anti-GD2. (69) A larger Phase II study adding GM-CSF is ongoing in the COG.

Hu14.18K332A is a humanized ch14.18 with a mutation to alanine at lysine 322 that limits its ability to fix complement and thereby reduces the pain associated with ch14.18, while retaining ADCC capabilities. Preclinical studies in rats confirmed that hu14.18K322 elicited significantly less allodynia than ch14.18 (72). Preliminary findings of a phase I clinical trial of hu14.18K322 showed the MTD to be 70 $mg/m^2/d \times 4$ with reduced neuropathic pain (73).

mAb1A7 is an anti-idiotype antibody directed against a murine anti-GD2, 14G2a, and in effect, mimics the GD2 antigen. Yu et al conducted a clinical trial of mAb 1A7 as a surrogate GD2 vaccine in 31 patients with high risk neuroblastoma who achieved first or subsequent responses (74). There were no systemic toxicities seen with subcutaneous injections given periodically over two years, but only local reactions, transient fever in four patients and serum sickness in one. All patients generated anti-mAb1A7 and their immune sera displayed CDC and ADCC activities. Sixteen of 21 patients who enrolled during first remission had no evidence of disease progression at a median of 6 years, while only 1 of 10 patients in second remission remains progression free. These findings indicated that mAb1A7 vaccine has little toxicity, is effective in inducing biologically active anti-GD2, and may be useful in controlling MRD (75) (unpublished data from Yu et al).

In light of the observed low toxicity profile of mAb1A7, and hu14.18K332A, it will be desirable to replace ch14.18 with one of these products, or possibly to substitute the Hu14.18-IL2 for the treatment with the ch14.18 with exogenous IL2. Thus, it will be important to ascertain whether mAb1A7 vaccine or the mutant anti-GD2 mAb will have similar therapeutic efficacy as ch14.18 in future clinical trials. Given the documented synergism between chemotherapy and anti-cancer mAbs such as rituximab (76) and trastuzumab (77), it will be timely to assess the efficacy of anti-GD2 mAbs with chemotherapy for treatment of neuroblastoma. Recently, genetic engineering of human T lymphocytes to express GD2-directed chimeric antigen receptors (CAR) has been produced by grafting the specificity of 14G2a onto a T-cell receptor (78). These CAR-T cells have been used to mediate tumor regression in patients with neuroblastoma and offer another testable strategy for GD2-directed immunotherapy (79).

ALK as a Therapeutic Target in Neuroblastoma

The least developed of the targeted therapies for neuroblastoma is that directed to the ALK (anaplastic lymphoma kinase) tyrosine kinase receptor. This lag can be attributed to the relatively recent identification of aberrations in the *ALK* gene (6–9). Nonetheless, these findings have led to clinical testing of ALK targeting in a remarkably short time, largely because of the availability of crizotinib, a dual ALK/MET inhibitor that has shown efficacy in adults with *ALK*-rearranged cancers, such as non-small cell lung cancer, inflammatory myofibroblastic tumor and anaplastic large cell lymphoma (10) (80, 81).

ALK meets most criteria to be classed as a valid molecular target (82). Targeting mutated ALK in neuroblastoma is likely to produce clinical benefit, for the following reasons: (i) point mutations and amplifications, which occur in approximately 8–10% and 2% of primary neuroblastoma tumors respectively, are oncogenic both *in vitro* and *in vivo*, leading to constitutive phosphorylation of ALK and of downstream signaling molecules critical for cell proliferation and survival. (6–9) Of approximately 12 mutations reported in

neuroblastoma, the most common affect the R1275 and F1174 residues (83). (ii) Cells expressing mutated *ALK* exhibit "oncogene addiction" with ALK inhibition leading to cell death (6). (iii) Most importantly, these aberrations are amenable to inhibition by small molecules - unlike the most unequivocal genetic marker of aggressive neuroblastoma, amplification of the MYCN oncogene (6).

Crizotinib and other ALK inhibitors

The first drug to be FDA approved for the treatment of ALK-rearranged cancers was crizotinib, an orally bioavailable ATP-competitive 2,4-pyrimidinediamine derivative (PF2341066; Pfizer Inc.)(84). Crizotinib binds to the inactive conformation of ALK and has shown striking efficacy against ALK-rearranged tumors such as non-small-cell lung cancer (NSCLC) and inflammatory myofibroblastic tumor. In early phase clinical testing, the overall response rate was 57% in 82 patients with *EML4-ALK* positive NSCLC (10) (80, 81). Currently, crizotinib is being tested in an ongoing phase I/II trial for children with neuroblastoma and other solid tumors bearing *ALK* mutations and rearrangements (NCT00939770, ClinicalTrials.gov). Preclinical testing has demonstrated the sensitivity of neuroblastoma cell lines with *ALK* amplification and the R1275Q mutation to crizotinib, marked by complete and sustained regression of xenografts (85), By contrast, the F1174L mutation, which possesses greater transforming capacity than other *ALK* mutations (83), and is associated with a poor response to standard therapy (83), has shown only minimal response in cell lines and none at all in neuroblastoma xenografts bearing this mutation (85). ALK^{F1174L} also mediates acquired resistance to crizotinib in adults with *ALK* translocation-positive cancers (86). The structural basis of this resistance is not yet understood, but could reflect the observation that this mutation is not in direct contact with the crizotinib-binding site within the ATP-binding pocket (86) or that it has a greater affinity for ATP than crizotinib (85). In view of the lack of sensitivity of the ALK^{F1174L} to crizotinib, the pediatric phase I study has been amended to increase the recommended phase II dose based on preclinical data that suggests that the resistance can be overcome in a dose-dependent manner (85).

Aggressive efforts to develop new ALK inhibitors are under way. An example is CH5424802 (Chugai Pharmaceuticals Ltd) (87), an orally available, benzo[b]carbazole derivative that potently inhibited the growth of neuroblastoma cells expressing amplified *ALK*. This agent also displayed *in vitro* inhibitory activity against neuroblastoma cells expressing ALK^{F1174L}, which was shown to be comparable to that achieved in cells with wild-type ALK. Whether CH5424802 will demonstrate efficacy for *in vivo* models of neuroblastoma with mutated *ALK* must be determined. Additionally, LDK378 (Novartis Pharmaceuticals), an orally active inhibitor of the ALK kinase, is currently being tested in Phase 1 trials in adult patients (NCT01283516, ClinicalTrials.gov), and may hold promise for the treatment of neuroblastoma patients with mutated ALK.

As seen with other kinase inhibitors, eventual resistance to crizotinib and other ALK inhibitors will likely develop, either through mutations within the ALK kinase domain or by upregulation of an alternative signaling pathway (86, 88). This phenomenon has already been described in adult patients, and will undoubtedly emerge in children who initially respond to crizotinib. Preclinical models of resistance are needed that would enable prediction of resistance mechanisms and design drug combinations to delay or circumvent resistance. Effective targeting of resistance mutations would be aided by resolution of the crystal structure of ALK in its active confirmation, and of the F1174L mutant in particular.

Future Prospects for clinical application of ALK targeting

Although the number of patients whose tumors have *ALK* aberrations is approximately half that associated with *MYCN* oncogene amplification, there are several reasons why inhibition of ALK would be a feasible therapeutic option in neuroblastoma. Firstly, ALK is expressed on the surface of most neuroblastoma tumor cells (89) and is restricted to the brain following development. Thus ALK, like, GD2, would be an ideal tumor-associated antigen for targeting with immune-based therapies (Figure 3). Anti-ALK antibodies may be beneficial not only to patients with aberrant ALK in their tumors, alone or in combination with small molecule inhibitors (90), but also to the larger number of patients whose tumors express wild-type ALK.

Secondly, a proportion of neuroblastoma cells overexpressing phosphorylated ALK that is neither mutated nor amplified respond to ALK depletion by undergoing apoptosis. Such tumor cells are also responsive to ALK inhibition by small molecules (91) and may be even be amenable to RNA interference (RNAi)-based therapeutic strategies (92). Thus, ALK is apparently activated in such cells by alternative mechanisms, suggesting that ALK inhibition may afford a useful therapeutic strategy for a larger subset of patients than previously projected.

Thirdly, ALK inhibition may provide an effective targeting strategy against *MYCN*-amplified tumors, which comprise nearly half of all high-risk neuroblastomas. *ALK* F1174L co-segregates with *MYCN* amplification in patients, and this combination is associated with a particularly poor prognosis, as demonstrated by the fatal outcome of 9 of 10 children with *ALK* F1174L/*MYCN* amplified tumors(83). ALK F1174L also has been shown to potentiate the oncogenic activity of *MYCN in vivo,* although by itself, ALK F1174L appears to be insufficient to cause the malignant transformation of neural crest derived m cells (93, 94). This latter observation may in part explain the occurrence of ALK mutations in low stage neuroblastomas. Both wild-type and F1174L ALK upregulate MYCN expression *in vitro* (95), and *in vivo* (R. George, unpublished). Moreover these two genes signal through a common signaling pathway, PI3K/AKT/mTOR, and targeting ALK with short hairpin RNAs (shRNA) has been shown to inhibit cell growth in tumor lines with concomitant *MYCN* amplification (6). Together, these observations suggest that inhibition of ALK, whether wild-type or mutant, could be a means to induce the death of *MYCN*-amplified tumor cells, either alone or when combined with emerging strategies that target MYC, such as BET bromodomain inhibition (96).

Neuroblastoma has been at the forefront of pediatric cancers in terms of stratification of patients based on molecular aberrations, the prototypical example of which is *MYCN* amplification. More recently, the discovery of *ALK* mutations has bolstered the prospects of effective molecularly targeted therapy for high-risk tumors, although considerable work needs to be done before ALK inhibition can occupy the same high-priority ground as hNET-MIBG and anti-GD2 antibody therapy.

Conclusion

These targeted approaches to high-risk neuroblastoma provide new ammunition to fight both the measurable and microscopic disease in these patients. The remaining challenges will be to determine how to combine these agents with standard cytotoxic agents and when to introduce them during therapy, whether in the neo-adjuvant setting, consolidation, or to treat minimal residual disease. Perhaps the greatest challenge for this orphan disease is how to obtain easy worldwide access and commercialization of these therapies. MIBG and anti-GD2 antibodies have both been under investigational testing with proven activity in neuroblastoma since the 1980's, yet neither has been developed commercially, due to the

very small market. Hopefully, the impressive activity of MIBG in phase II studies and the recent success in a randomized trial of the ch14.18 moAb will provide impetus to acceptance of new drug applications by the FDA. Paradoxically, there has already been approval of an ALK small molecule inhibitor due to activity in the more common adult cancers, although it is still in early clinical trials for neuroblastoma. Anti-GD2 antibodies and MIBG both provide targeting for most neuroblastomas, but the acute toxicity of the former and the radiation isolation and then possible late effects of the latter limit their utility to pediatric centers with expertise. The oral ALK inhibitors currently are currently limited to a subset of tumors, but offer a truly personalized approach to treatment.

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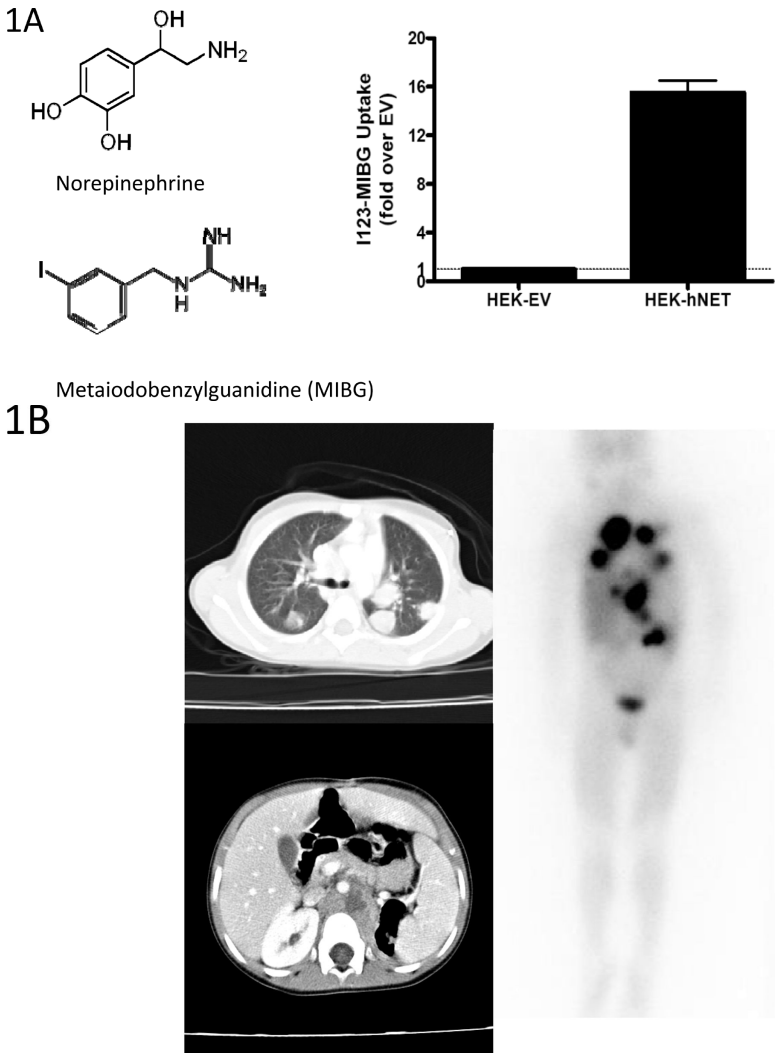

Figure 1.

MIBG uptake in neuroblastoma

A. The left panel shows the similar structures of norepinephrine and metaiodobenzylguanidine (MIBG), which both are taken up via the human norepinephrine transporter (hNET). On the right, HEK293 cells transfected with hNET showed ~15 fold higher uptake of ^{123}I-MIBG over the HEK-empty vector (EV) transfected cells.

B. The CT scan on the left shows the lung metastases and retroperitoneal mass in a child with recurrent neuroblastoma at the time of ^{131}I-MIBG therapy. The right panel shows the uptake of ^{131}I-MIBG in the tumors at 4 days after infusion of 18mCi/kg (total activity infused = 340 mCi).

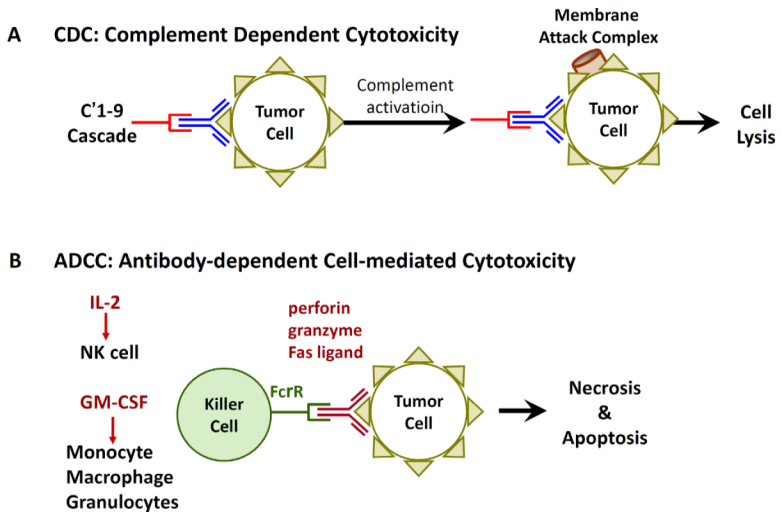

Figure 2.

Mechanism of GD2 antibody-targeted destruction of neuroblastoma by complement dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC)

A. CDC: Monoclonal antibody binds to the receptor and initiates the complement cascade, which results in the formation of a membrane attack complex that makes a hole within the cell membrane, causing cell lysis and death.

B. ADCC: The Fc fragment of the monoclonal antibody binds the Fc receptors on monocytes, macrophages, granulocytes and natural killer cells. These cells in turn engulf the bound tumor cell and destroy it. Natural killer cells secrete cytokines that lead to cell death.

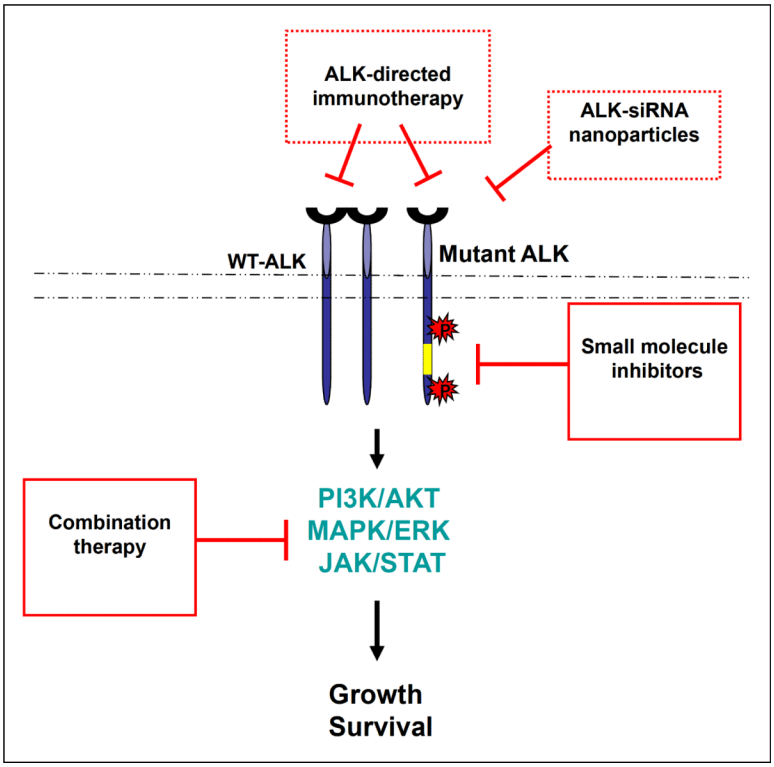

Figure 3.

Current and future prospects for targeting ALK in neuroblastoma. Mutated ALK could be targeted by small molecule inhibitors alone or in combination with inhibitors of downstream signaling pathways. ALK-directed immunotherapy such as anti-ALK antibodies could induce cytotoxicity by direct inhibition of the mutated receptor, or by provoking a cytotoxic immune response Nanoparticles carrying ALK siRNA, could result in inhibition of ALK activity, leading to cell death. WT, wild-type, siRNA, small interfering RNA; JAK, Janus kinase; STAT, Signal Transducers and Activators of Transcription; PI3K,, Phosphatidylinositol 3-kinase; AKT, Protein Kinase B; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase;

Table 1

Key clinical trials of therapies for neuroblastoma targeting hNET with MIBG, GD2 ganglioside with anti-GD2 antibody, and ALK aberrations with small molecule inhibitors*.

Trial	Phase	Patients (N)	Agent Dose	Results
A. MIBG *				
Hutchinson et al, 1992 (17)	Pilot	14	^{131}I-MIBG (50–220 mCi total);	3 MR; myelosuppression and nausea and transient LFT elevation
Lashford et al, 1992 (18)	1/2	25	^{131}I-MIBG 1,2, 2.5 Gy whole body dose	Adjusted by whole body radiation dose with MTD 2.5 Gy; Responses 8 PR
Matthay et al, 1998 (19)	1	30	^{131}I-MIBG 3–18 mCi/kg	Dose escalation to MTD of 18 mCi/kg; Responses 1 CR, 10 PR, 3 MR.
Matthay et al, 2007 (20)	2	164	^{131}I-MIBG 18 mCi/kg	Response rate (CR/PR) in refractory or relapsed disease of 37% with 18 mCi/kg; ASCT support given in 30% of patients
Matthay et al, 2012 (30)	1	15	No-carrier added ^{131}I-MIBG 12–18 mCi/kg	MTD with organ and tumor dosimetry of 18 mCi/kg; Responses 1 CR, 3 PR, 1 MR.
Sisson et al, 1991 (28)	1	7	^{125}I-MIBG 0.85–1.35 Gy whole body dose	Dose limited by thrombocytopenia
Howard et al, 2005 (26)	2	28	^{131}I-MIBG 18 mCi/kg	Multiple infusions (2–4) are feasible with 39% response; chronic thrombocytopenia in 46% after final infusion
Matthay et al, 2009 (25)	1	21	^{131}I-MIBG × 2 12–21 mCi/kg × 2	Rapid tandem infusion ^{131}I-MIBG with ASCT; MTD 36 mCi/kg; Responses 2PR, 8 MR
Johnson et al, 2011 (27)	2	41	^{131}I-MIBG	Two tandem infusions are feasible with 39% response; hematologic toxicity abrogated with PBSC after second infusion
DeKraker et al, 2008 (43)	Pilot	33	^{131}I-MIBG induction 300 mCi total	^{131}I-MIBG as induction therapy pre-surgery, 200 mCi followed by 100 mCi in newly diagnosed patients, 57% response
Schmidt et al, 2006 (44)	3	40	^{131}I-MIBG end induction	40/111 patients with Stage 4 at the end of induction and residual disease got ^{131}I-MIBG, but no impact on EFS in multivariate analysis compared to patients who did not receive MIBG
Gaze et al, 2005 (38)	Pilot	8	^{131}I-MIBG + topotecan 12 mCi/kg × 2	^{131}I-MIBG (12 mCi/kg × 2) with topotecan tandem therapy; targeted whole body dose of 4Gy, showed feasibility
DuBois et al, 2012(39)	1	24	^{131}I-MIBG + VCR/Irinotecan 8–18 mCi/kg	^{131}I-MIBG with vincristine and irinotecan dose escalation to MTD 18 mCi/kg; Responses 2 CR, 4 PR
Klingebiel et al, 1994 (33)	pilot	11	^{131}I-MIBG + CEM 15 mCi/kg	^{131}I-MIBG (15 mCi/kg) with carboplatin, etoposide, melphalan and ASCT; feasible
Miano et al, 2001 (34)	pilot	17	^{131}I-MIBG + Bu/MEL 4–11 mCi/kg	^{131}I-MIBG (4–11 mCi/kg) with Busulfan and Melphalan and ASCT; GI toxicity and pneumonitis
Yanik et al, 2002 (31)	pilot	12	^{131}I-MIBG + CEM 12 mCi/kg	^{131}I-MIBG (12 mCi/kg) with carboplatin, etoposide, melphalan and ASCT; feasible, good engraftment, mucositis main toxicity
Matthay et al, 2006 (35)	1	24	^{131}I-MIBG + CEM 12–18 mCi/kg	^{131}I-MIBG (12–18 mCi/kg) with carboplatin, etoposide and melphalan and

Trial	Phase	Patients (N)	Agent Dose	Results
				ASCT with MTD 12 mCI/kg; response rate 27% in refractory disease
B. Anti-GD2 Antibody**				
Cheung et al, 1987 (49)	1	17	3F8 (murine MAb) 5–100 mg/m^2	Dose escalation; response 7/17(CR/PR/MR); toxicities pain, hypertension, urticaria, and complement depletion
Huang CS, et al, 1992 (52)	1	15 (14)^	14G2A (murine MAb) 25–500 mg/m^2	Dose escalationNeuroblastoma responses 1 CR, 3 MR, toxicities controllable and reversible, pain, fever, tachycardia, hyponatremia, rash
Murrray et al, 1994 (51)	1	18 (5)^^	14G2A (murine) 50–200 mg/m^2	Dose escalation with MTD of 100 mg/m2; Neuroblastoma responses 2 PR; toxicities generalized pain, hyponatremia, fever, rash, paresthesias, weakness, and chronic refractory postural hypotension
Uttenreuther-Fischer et al, 1995 (62)	1	15	ch14.18 (chimeric) 10–200 mg/m^2	Dose escalation with pharmacokinetics showing t1/2 of 66±27.4 hr, which is significantly shorter than 181±73 hr reported in adult patients.
Handgretinger et al, 1995	1	9	ch14.18 (chimeric) 150–250 mg/m^2	Dose escalation (30–50 mg/m^2/d × 5 days); 5 responses (CR/PR/MR); toxicities pain and urticaria
Yu et al, 1998 (61)	1	11 (10)^	ch14.18 (chimeric) 10–200 mg/m^2	Dose escalation;Neuroblastoma responses 1 PR,4 MR. Toxicities of pain, tachycardia, hypertension, fever, urticaria
Yu et al, 1995 (64)	pilot	17	Ch14.18 + GM-CSF 200 mg/m^2	Ch14.18 50mg/m^2/d × 4 and GM-CSF 10 μg/kg/d × 14), 4 CR, 1 PR, 1 MR. Toxicities similar to ch14.18 alone, and transient thrombocytopenia
Yu et al, 1997 (65)	2	32	Ch14.18 + GM-CSF 200 mg/m^2	Ch14.18 50mg/m^2/d × 4 and GM-CSF 10 μg/kg/d × 14), 2 CR, 2 PR,1 MR; increase in ADCC activity correlated with clinical response.
Frost et al, 1997 (56)	1	33 (31)^	14G2A + IL-2 10–100 mg/m^2	Dose escalation 14G2A + IL-2; MTD 15 mg/m^2 per day; 1 PR in neuroblastoma and 1CR in osteosarcoma; toxicities pain and fever
Cheung et al, 1998 (57)	2	21/13 R1/R2	3F8 (murine) 100 mg/m^2	Stage 4 NB in 1st (R1) or 2nd (R2) remission after ASCT treated with 10 mg/m^2 × 5 days; responses by PCR bone marrow (7/12) or scintigraphy ^{131}I-3F8 (6/6)
Cheung et al, 2006 (58)	2	136	3F8 + GM-CSF 100 mg/m^2	3F8 in R1 or R2; 5-year EFS for R1 with favorable FCGR2A-131R/R was 52%, vs. 29% in the R/H or H/H group
Ozkaynak et al, 2000(67)	1	19	Ch14.18 + GM-CSF 80–200 mg/m^2	Ch14.18 with GM-CSF post ASCT; MTD 40 mg/m^2/d × 4 days. Toxicities: severe neuropathic pain, fever, nausea/vomiting, urticaria, hypotension, mild to moderate capillary leak syndrome, and neurotoxicity
Gilman et al, 2009(66)	1	25	Ch14.18 + GM-CSF + IL2 + isotretinoin 80–160 mg/m^2	MTD of Ch14.18 was 25 mg/m^2/d × 4 days) given post ASCT; feasible regimen with common toxicities: pain, fever, nausea, emesis, diarrhea, urticaria, mild elevation of hepatic transaminases, capillary leak syndrome, and hypotension

Trial	Phase	Patients (N)	Agent Dose	Results
Yu et al, 2010(5)	3	226	Ch14.18 + GM-CSF + IL2 + isotretinoin 100 mg/m^2	Randomized trial comparing isotretinoin post-ASCT to isotretinoin with immunotherapy (ch14.18 with GM-CSF and IL-2). Immunotherapy was superior to standard therapy by 2-year EFS(66+/−5% vs. 46+/−5%, P=0.01) and OS (86+/−4% vs. 75+/−5%, P=0.02)
Osenga et al, 2006(70)	1	28 (27)^^^	hu14.18-IL2 6–42 mg/m^2	MTD of the immunocytokine was 12 mg/m2/d × 3 days. Toxicities: hypotension, allergic reaction, blurred vision, neutropenia, thrombocytopenia, and leucopenia; anti-tumor activity noted in 3 neuroblastoma patient but no measurable responses
Shusterman et al, 2010(71)	2	36	hu14.18-IL2 36 mg/m^2	Responses to12 mg/m^2/d × 3 days in 5/23 patients with bone (MIBG) and/or bone marrow disease; responses in 0/13 with soft tissue disease.
Navid et al, 2011 (73)	1	32	Hu14.18K332A Ongoing escalation	Humanized anti-GD2 mAb with a single point mutation (K322A) reducing complement-dependent lysis); common toxicities: pain, fever and hyponatremia; well tolerated at higher doses than ch14.18.
Batova et al, 2004(75)	Pilot	31	mAb1A7 + QS21	Anti-idiotype anti-GD2 vaccine for patients in R1 or R2; 17/20 in R1 progression-free (median 47 months), 1/11 in R2 progression free.
C. ALK inhibitor				
Mosse study chair#	1/2	44+	PF-02341066 Ongoing escalation	ADVL0912: Oral Small Molecule Inhibitor ALK and C-Met; ongoing in phase 1 in COG.

Abbreviations: CR, complete response, PR, partial response, MR, minor response, R1, first remission; R2, second remission; ASCT, autologous stem cell transplant; MTD, maximum tolerated dose

*The trials here are only a representative sample of the development of this therapy in phase 1, 2 and combination trials

**Most of the anti-GD2 Phase I trials also included a few other GD2-postive malignancies, such as melanoma and osteosarcoma, although the majority of patients entered had a diagnosis of neuroblastoma. Responses are only reported for neuroblastoma.

^Number in parenthesis indicates the number of neuroblastoma patients enrolled, while the remaining patient had osteosarcoma

^^Number in parenthesis indicates the number of neuroblastoma patients enrolled, while the remaining 11 patients had Melanoma, 2 had osteosarcoma

^^^Number in parenthesis indicates the number of neuroblastoma patients enrolled, while the remaining 1 patient had Melanoma,

#There is no published pediatric trial in neuroblastoma of an ALK inhibitor, but this ongoing study in the COG Phase I consortium includes patients with relapsed solid tumors and anaplastic large cell lymphoma